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ICNPR 2024

INTERNATIONAL CONGRESS ON NATURAL PRODUCTS RESEARCH

Kraków, 2024, July 13-17

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Welcome Letter

Dear Participants, dear Colleagues,

on behalf of the Organizing Committee and the Scientific Committee it is our great pleasure to welcome you at the International Congress on Natural Products Research 2024 (ICNPR2024) which will be held in Kraków, Poland, July 13-17, 2024.

ICNPR2024 is co-organized by the Phytochemical Society of Europe (PSE), and our six sister societies representing the US, Europe and Asia, which include the Society for Medicinal Plant and Natural Products Research (GA), the Association Francophone pour l'Enseignement et la Recherche en Pharmacognosie (AFERP), the Italian Society of Phytochemistry and Sciences of Medicinal, Food and Perfume Plants (SIF), the American Society of Pharmacognosy (ASP), the Japanese Society of Pharmacognosy (JSP) and the Korean Society of Pharmacognosy (KSP). Every four years all of them meet together to share common interest in natural products research and exchange experience and simply unite together. It is always a unique possibility to experience this. ICNPR2024 is one of the largest gatherings of scientists involved in natural products and related fields from nearly every continent. The congress brings together experts and researchers from all over the world to share their latest findings and discuss the latest developments in the field of natural products research.

More than 1000 participants from 68 countries will deliver all together around 130 oral and almost 900 poster presentations. As a participant, you will have the opportunity to learn about cutting-edge research, network with other professionals in your field and getting involved in the extensive scientific program in many ways. Aside from that, you should also take the chance to explore the beautiful city of Kraków. It is one of the best recognizable cities in this part of Europe visited by about 10 million visitors annually. They are attracted by one-of-a-kind atmosphere and largest historical complex in Poland, unique on global scale. It is a city with a rich history and culture. The old town, with its beautiful architecture and narrow streets, is a UNESCO World Heritage site.

This congress is only possible with the collaboration of many people who were dedicated to do their best to make this event happen. First of all, we would like to thank the representatives of the seven organizing societies for their great support. Without the work of the members of the Scientific Committee it would not have been possible to handle more than 1000 abstracts and to create a scientific program of high quality, your efforts are very much appreciated. We also would like to thank plenary and keynote speakers for accepting our invitation to Krakow. We are grateful to the congress organizing secretariat, Symposium Cracoviense Sp. z o.o., for excellent cooperation in preparation of this event. Finally, we sincerely would like to acknowledge all financial support provided by the sponsors of this conference.

We hope that you will enjoy the time at ICNPR2024 and you feel not only the spirit of the city and place but also the touch of Polish culture and hospitality.

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Workshop ECRW

The natural diversity of acyltransferases reveals versatility and specificity in the synthesis of gene-encoded lipopeptides

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Lipopeptides comprise biochemical compounds that can be naturally produced or chemically synthesized. They exhibit diverse bioactive functions and are widely used in health care (Hamley, 2015). Despite their versatility, the production and diversification of lipopeptide compounds continue to be a challenge for state-of-the-art biological and chemical synthesis. In contrast, we offer a more streamlined and adaptable route to lipopeptide natural products via ribosomally synthesized and post-translationally modified peptides (RiPPs). Their primary structure is determined by a genetically encoded precursor protein, which is modified by maturases within the same biosynthetic gene cluster (Montalbán-López et al., 2021). The crucial lipidation reaction is catalyzed by a novel class of maturases of the GCN5-related *N*-acetyltransferase (GNAT) family, which introduce medium-chain fatty acids onto the sidechain amino group of lysines or arginine-derived ornithines (Hubrich et al., 2022) (Fig. 1).

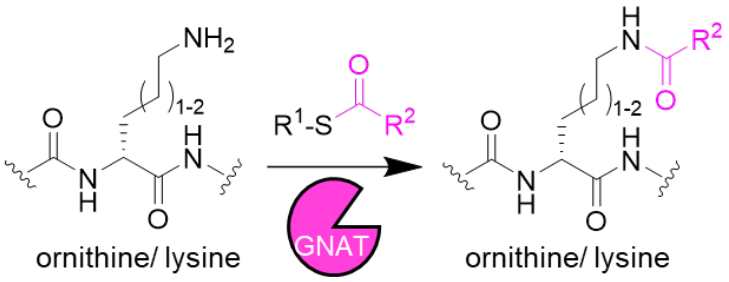

Fig. 1. Transfer of fatty acids to the sidechain of ornithines and lysines by RiPP GNATs.

Here we present the natural diversity of this GNAT family and their corresponding gene-encoded lipopeptides by characterizing the substrate scope of selected GNATs with their cognate precursor peptides. We could show that a variety of fatty acids can be introduced including chain lengths from C10 to C18. We are currently exploring this enzyme class to enable custom peptide engineering efforts towards generating bioactive lipopeptide compounds. We envision this platform to be used to facilitate the production of crucial lipopeptide compounds for human health and to quickly screen for antimicrobial lipopeptide compounds and non-ribosomal lipopeptide mimics.

Keywords: GNAT, RiPPs, lipopeptides, synthetic biology

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Anti-inflammatory biscembranoids from aquaculture-derived soft coral *Sarcophyton trocheliophorum*

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As the most abundant white blood cells in the human body, neutrophils are a key component of the immune system, playing a crucial role in defending against infections. However, excessive activation or improper regulation may lead to severe inflammatory diseases. Therefore, the development of drugs capable of inhibiting the activation of neutrophils holds the potential to effectively treat various inflammatory conditions. In our quest to discover bioactive biscembranoids sourced from cultured soft coral *Sarcophyton trocheliophorum*, we utilized molecular networking (MN) analysis. Our focus was on clusters within the *m*/*z* range of 650 to 750, as well as the clusters exhibiting a substantial inhibition rate (>70%) of superoxide anion generation. These clusters were prioritized for purification, leading to the isolation of ten biscembranoids, including four new compounds (sarcotrochelides A–D, **1**, **2**, **5**, and **6**) and six known analogues. Noteworthy, **5** and **6** exhibited an unprecedented 3-hydroperoxy-3-methylcyclohex-1-ene moiety. The structures of all isolated metabolites were elucidated through a series of spectral analyses, and their anti-inflammatory activities were assessed. Compound **8** demonstrated the highest inhibition against superoxide anion generation and elastase release in activated neutrophils, with IC_{50} values of 1.98 and 2.76 μM, respectively. The observed variations in bioactivity underscored the significance of lactone rings and double bond geometry within the biscembranoid skeleton. These results confirmed the potential of biscembranoids derived from cultured *S. trocheliophorum* as promising candidates for future development of anti-inflammatory agents tailored to target neutrophils.

Keywords: neutrophil, *Sarcophyton trocheliophorum*, biscembranoid, molecular networking, anti-inflammation

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Integration of *Caenorhabditis elegans* bioactivity assays for a sustainable identification of health promoting natural products

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The increasing life expectancy of the world's population has a profound impact on our society and healthcare system, resulting in an urgent need for innovative regiments to improve healthy aging. Additionally, great emphasis ought to be placed on sustainability of environmental and time constraints in early drug discovery studies. This project aims for the sustainable discovery of natural products (NPs) to decelerate the aging process and attenuate age-related diseases. The presented screening strategy is based on the 3Rs "robustness, reliability and resource conservation", which are inspired by both the 3Rs in animal studies and environmental protection. The screening approach uses the small model organism *Caenorhabditis elegans* as a simple phenotypic tool in different readouts (Kirchweger et al, 2023) to select the most promising starting materials for in depth phytochemical and pharmacological investigations including LC-MS/MS based dereplication, *in silico* prediction tools and target-oriented assays. Starting from *C. elegans* based screenings of >70 extracts from herbal and fungal origin, we applied a semi-automatic IR-based wormtracker and optimized methodological parameters in various *C. elegans* assays focusing on lifespan, health span, metabolic and neurodegenerative disorders. To best comply with the 3Rs, a workflow has been established, enabling to start with time- and cost saving high content assays to continuously scale down the number of bioactive NPs to be probed on more specific, but elaborate assays with genetically modified nematode strains (Redl et al, 2024). This procedure allows a stepwise increase of information regarding involved pathways, molecular mechanism, and involved chemistries.

Funding: The project is funded by the "Schwabe Phyto Innovation Challenge" by Dr. Willmar Schwabe GmbH & Co.KG.

Keywords: healthy ageing, *Caenorhabditis elegans*, natural product screening, sustainability

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Novel natural product inhibitors targeting oncogenic MAPK/ERK and PI3K/AKT signaling in melanoma: from large library screening to target identification

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Malignant melanoma is the deadliest type of skin cancer with unmatched mutation rates arising in both MAPK/ERK and PI3K/AKT signaling pathways. Although specific inhibitors of these critical pathways show spectacular initial results, most patients relapse within just a few months. Combination therapy can improve overall survival rates, but the currently available options are limited (Tanda et al., 2020). Therefore, novel inhibitors targeting oncogenic ERK and AKT signaling in melanoma are urgently needed. To tackle this issue, our in-house library of crude plant extracts was combined with an innovative high-content screen (HCS) that quantifies downstream inhibitory activity at ERK and AKT level. HPLC-based activity profiling of the active hits and subsequent targeted isolation of the bioactive constituents was performed (Dürr et al., 2022). To further explore the coverage of chemical space for such inhibitors, we also screened large pure compound libraries by accessing high-throughput screening pipelines through an EU-OPENSCREEN program. To this end, we screened 2,576 plant extracts and additional 25,696 pure natural and synthetic compounds. A total of 46 active compounds were confirmed as downstream inhibitors of ERK and/or AKT with IC_{50} values in the low micromolar range. The current challenge aims towards target identification of the most promising hits. Several key kinases of the ERK-AKT network were produced through different cloning techniques and heterologous expression systems. Our strategy further includes the assessment of physical binding as well as enzymatic inhibition. Ultimately, we envisage to develop these newly discovered inhibitors into lead compounds for future drug development.

Keywords: melanoma, high-content screening, EU-OPENSCREEN, MAPK/ERK and PI3K/AKT signaling, target identification

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Towards the engineering of the plant endoplasmic reticulum for sustainable production of specialized metabolites

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Plants are among the most creative survivors on the planet. They have optimized their own defence mechanisms against attackers, typically by producing a species-specific arsenal of specialized metabolites. These metabolites not only play vital roles within the plants themselves but also offer a multitude of applications as pharmaceuticals, fragrances etc. However, industrial production of these valuable compounds faces many bottlenecks and often requires intensive adaptations. To facilitate sustainable large-scale production of plant metabolites, we aim to develop a universal tool to boost plant-based production of specialized metabolites. Our strategy is based on recent findings in yeast, in which engineering of the endoplasmic reticulum (ER), which harbours a main part of the enzymatic machinery for many metabolite biosynthesis pathways, results in an enlarged ER size and an increased metabolite accumulation (Arendt et al., 2017). By interfering with the phospholipid biosynthesis pathway, a higher production of building blocks for the ER-membrane and ultimately an enlarged ER were achieved. Our project aims to translate the findings from yeast to plants. Thus far, we have obtained an increased accumulation of phospholipid building blocks in tomato (*Solanum lycopersicum)* roots, by knocking out the *PHOSPHATIDIC ACID PHOSPHOHYDROLASE* (*PAH*) genes. Currently, we are investigating whether this correlates with an increased production of bioactive specialized metabolites. Additionally, we are also exploring the potential of ER engineering by overexpressing other key phospholipid biosynthetic enzymes. With our obtained *in planta* results, we believe that ER engineering holds great promise for a more efficient and sustainable production of plant specialized metabolites.

Keywords: *Solanum lycopersicum*, endoplasmic reticulum, phospholipids, engineering, roots

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PKC-α activation with new semi-synthetic 7α-acetoxy-6β-hydroxyroyleanone derivatives for breast cancer therapeutics

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Breast cancer is the most prevalent cancer worldwide (WHO). PKC-α is an attractive anticancer target often associated with breast cancer (Tam et al., 2013). Species of the *Plectranthus* genus (Lamiaceae) are traditionally used in the treatment of several ailments, including cancer. The diterpenoid 7α-acetoxy-6βhydroxyroyleanone (**1**) is the major compound of *P. grandidentatus* Gürke. and demonstrated cytotoxic effects in several cancer cell lines (Ladeiras et al., 2019). Accordingly, the main goal of this study was to synthesize new derivatives of **1** and evaluate them as new antitumoral agents focus on breast cancer therapy. In the context of the study, it was possible to extract and isolated 1.0 g the lead compound **1** and subsequently, synthetize thirty new derivatives (**2** to **31**) through hydrogenation, photochemical and esterification reactions. The cytotoxic effects of all compounds (**1** to **31**) were evaluated on breast cancer cell lines. Notably, the analogues **6**, **7**, **18** and **21** revealed promising outcomes, displaying selectivity towards cancer cells and low IC_{50}, mainly against the aggressive triple negative breast cancer cell lines. Specifically, analogues **6**, **7**, **18** and **21** displayed IC_{50} values of 1.36, 1.92, 2.11, 6.90 μM in MDBA-MB-468 cells and 1.70, 3.63, 3.52, 9.44 μM in MDBA-MB-231 cells, respectively. Therefore, these derivatives were studied as PKC-α activators. Derivative **7** emerged as the most promising PKC-α activator, outperforming the control (PMA) and confirming its potential as antitumoral agent with application on breast cancer. These findings denote a significant progress in our attempts to develop new antitumor agents from natural sources.

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Keywords: *Plectranthus*, royleanone, semi-synthetic derivatives, antitumoral activity, PKC-α

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Development of a high throughput cell-based assay to highlight potential bioactive antiviral natural products against the respiratory syncytial virus (RSV)

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Respiratory syncytial virus (RSV) is a leading cause of bronchiolitis in children resulting in three million hospitalizations (Mazur et al., 2023). To address the need for effective RSV treatments, we present the development of a high-throughput fluorescence-based screening to identify antiviral activity in natural extracts (NEs). Our assay, which is based on a 2D cell model run in a 384-well plate, uses a mCherry-carrying recombinant virus to identify hits based on viral replication inhibition. The method was validated using 192 chemodiverse NEs from a plant collection of Pierre-Fabre Laboratoires (PFL) (Allard et al., 2023). All NEs were analyzed by UHPLC-HRMS^2 to partially assess their chemical composition. Dereplication strategies identified betulinic acid among several active NEs. This compound is known for such activity (Amiri et al., 2020) and its bioactivity profile obtained after targeted isolation indicates that our assay provides data comparable to literature under different screening conditions. It further demonstrates the efficacy of our dereplication pipeline to discard NEs containing betulinic acid. Based on this proof of concept, we have extended our screening to 800 NEs from the PFL. Hits showing >70% inhibition of viral replication and >70% cell viability represented 21% of NEs. Further data mining is currently underway using both the newly developed Experimental NP Knowledge Graph (Burdet et al., 2024) and prior knowledge of NP antivirals with the Lotus initiative (Rutz et al., 2022). This approach aims to identify compounds in NEs with efficacy against RSV, thereby contributing to the discovery of new RSV lead compounds.

Keywords: natural products, respiratory syncytial virus, antiviral activity, screening, UHPLC-HRMS^2

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In vitro model for predicting oral bioavailability of isoflavone C-glycosides, O-glycosides, and their aglycons

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Isoflavones are widely distributed in plants of the Fabaceae family mainly in legumes such as soy, clover and beans (Křížová et al. 2019). They recently received increased attention due to their putative health properties as a potential alternative therapy against cancer, cardiovascular diseases, and menopausal symptoms like osteoporosis (Gómez-Zorita et al. 2020). The potential biological activity of isoflavones in the human body highly depends on their absorption and metabolism in the small intestine and liver. Thus, in the scope of this project, an *in vitro* model for predicting oral bioavailability was developed. The approach consists of three stages (**Fig. 1**): the small-scale *in vitro* digestion model to study the gastrointestinal behaviour of the flavones, the Caco-2 cell model to analyse the absorption and metabolism in the small intestine, and the HepaRG™ cell model to investigate the hepatic metabolism.

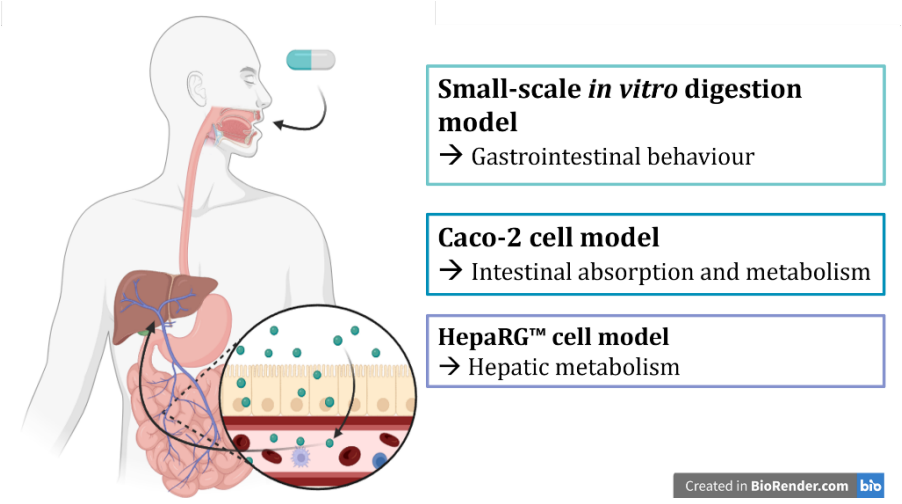

Fig. 1 Overview of the in vitro model for predicting oral bioavailability.

The investigations identified various structural motifs of isoflavones influencing their gastrointestinal stability and intestinal absorption. Based on the absorption in the Caco-2 model, the permeability coefficients of the compounds were determined. Moreover, numerous intestinal and hepatic metabolites were detected, and hepatic clearance was calculated. By calibrating the experiments with well-investigated model substances, these results could be correlated to the *in vivo* oral bioavailability. Therefore, the amount of isoflavones available for tissue distribution and thus for exertion of potential physiological effects can be estimated.

Keywords: Isoflavones, glycosides, bioavailability, hepatic clearance, intestinal absorption

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Dracaena cambodiana biflavonoid CB2 counteracts ferroptosis by directly inhibiting lipid peroxidation and inducing the key ferroptosis suppressor FSP1

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The pathophysiology of many degenerative diseases has recently been linked to ferroptosis, a form of programmed cell death dependent on iron-mediated phospholipid hydroperoxide formation (Dixon et al, 2012). Inhibition of ferroptosis therefore represents a promising therapeutic approach for degenerative diseases characterized by excessive cell death (Stockwell, 2022). Various ferroptosis inhibitors have been identified, but their clinical translation has been hampered (Koeberle et al, 2023). In our search for small molecules targeting ferroptosis, we screened an in-house library of over 200 structurally diverse natural products in human hepatocytes and identified the biflavonoid CB2 (EC_{50} = 150 nM) from *Dracaena cambodiana* as one of the most active biogenic ferroptosis inhibitors described so far. CB2 is efficient across different cell types (liver, brain, kidney and lung) and prevents ferroptosis irrespective of the triggering mechanism. CB2 efficiently scavenges radicals and suppresses lipid peroxidation in artificial membranes. At the cellular level, besides suppressing lipid peroxidation, it counteracts ferroptosis by inducing the expression of the key ferroptosis suppressor FSP1 without activating NRF2, the master regulator of antioxidant defense, or altering membrane unsaturation, which determines the susceptibility to ferroptosis. We conclude that CB2 prevents ferroptosis by inhibiting lipid peroxidation and promoting redox homeostasis by targeting a yet elusive factor in the control of FSP1.

Keywords: *Dracaena cambodiana,* ferroptosis, lipid peroxidation inhibition, FSP1

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Dietary molecules from *Glycyrrhiza foetida* and their modulation on metabolic syndrome pathways

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Glycyrrhiza is a genus of edible plants, commonly known as licorice, with a long use in folk medicine for their adaptogenic, anti-inflammatory, antioxidant effects, estrogenic activity and hepatoprotective properties. Among the less investigated species of this genus, *G. foetida* seems to be a new promising tool to treat metabolic syndrome (MetS). In fact, this plant has been pointed out as a source of amorfrutins, acetate-deriving polyphenols that explicate a potent insulin-sensitizer effect as PPARγ agonists that are quite rare in this genus (Weidner et al., 2012). The minor members of this class of compounds have not been in-depth characterized, thus, after a preliminary LC-UV-MS^2 dereplication step, we have carried out a NMR-based phytochemical screening of *G. foetida* aerial parts that led us to the characterization of 29 compounds, mainly polyphenols, including 11 previously undescribed amorfrutins and an unprecedented flavanone. The amorfrutins were tested on PPARγ and PPARα and, besides amorfrutin A, the most active compound on PPARγ, we have identified the new amorfrutin H as a selective PPARα agonist and the unprecedented amorfrutin E as a dual PPARγ/α agonist (Serino et al., 2023). Moreover, all the compounds were tested on other two MetS dysfunctional pathways, highlighting amorfrutin M and decarboxyamorfrutin A as mitochondrial stimulators, while amorfrutin 2 as a glucose uptake promoter. Thus, *G. foetida* is rich source of many bioactive ingredients that, through to the concomitant modulation of different pathways, can ameliorate MetS, emerging as a new potential functional food.

Keywords: *Glycyrrhyiza foetida,* metabolic syndrome, amorfrutins, mitochondrial activity, GLUT

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How to make “extreme” CPC greener: how to substitute alkanes in biphasic solvent systems using COSMO-RS predicting tools

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Centrifugal Partition Chromatography (CPC) is a support-free chromatographic technique involving the distribution and transfer of solutes between two immiscible liquid phases according to their partition coefficients, that avoids irreversible adsorption of the analytes, allows a total recovery of injected samples and enables separations of compounds within a large polarity range. To date, there are few alternatives to alkanes when it comes to purifying highly lipophilic compounds. However, most alkanes are classified as CMR or ecotoxic compounds. Replacing them with «greener» alternatives is an essential objective for the CPC development as an industrial-scale purification technique. To replace alkanes, a lot of alternatives are currently being studied (oils, ethers, esters, NaDES, ionic liquids, *etc*..), but testing all of them in different ternary or quaternary systems, at different compositions and on various extracts is an extremely time and money-consuming approach. COSMO-RS software is a based equilibrium thermodynamics method, placing compounds in a conductor-like environment and calculating a so-called « σ-profile », allowing to compare the similarity between two compounds (*i.e.*, their capacity as hydrogen donor or acceptor), and thus their miscibility. This software allowed the prediction of theoretical intermolecular properties and of ternary, quaternary and solubility diagrams of biphasic solvent systems containing “green” solvents such as CPME, MeTHF, Cyrene™, vegetable oils, *etc*. The predicted properties were compared with experimental data on hydrodynamic behavior with the visual-CPC tool, partitioning, selectivity, *etc*. As a proof of concept, the isolation of neutral triterpenes from *Pistacia lentiscus* has been achieved without using petroleum-based solvents.

Keywords: centrifugal partition chromatography, COSMO-RS, green chemistry, *Pistacius lentiscus*

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Investigating the anxiolytic activity of selected essential oils in zebrafish larvae and identification of active constituents using biochemometrics

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Anxiety disorders have become prevalent in modern day society mainly due to socioeconomic pressures and pandemics such as COVID-19. Current pharmaceutical interventions which include benzodiazepines have become undesirable due to unpleasant side effects (Hosein Farzaei et al., 2016). Traditional medicines on the other hand, have become popular over the years as they are deemed safe, inexpensive and have fewer side effects. Essential oils (EOs) are herein investigated as natural alternatives for the management of anxiety as they have a long history of traditional use for various central nervous system disorders. Commercially available essential oils (n = 63) reported to exhibit possible anxiolytic activity were sourced from Pranarôm International® (Belgium) and Scatters Oils® (South Africa). The oils were chemically profiled on an Agilent gas chromatograph, coupled to a quadruple mass spectrometer (MS) and a flame ionisation detector (FID) (GC-MS/FID). In order to assess anxiety-like behaviour in five days post fertilisation (5-dpf) zebrafish larvae, the light/dark transitions test was performed in a DanioVision observational chamber (Noldus) and locomotor activity was monitored. A biochemometrics approach was used to correlate essential oil profiles to anxiolytic activity data and subsequently identify bioactive compounds in the essential oils. Twenty nine out of the 63 EOs alleviated anxiety in the larvae to some extent, while *Boswellia carterii, Pogostemon cablin* and *Lavandula burnatii* essential oils displayed the best anxiolytic activity with significant reduction in locomotor activity ($p < 0.05$), as well as increased distance travelled, and time spent in the central arena during the dark phase (reverse-thigmotaxis behaviour). Biochemometric analysis identified α-pinene, *p*-cymene, camphene, camphor, eucalyptol, linalyl acetate, and *trans*-anethole as putative biomarkers, and experimental validation confirmed the anxiolytic activity of (+)-α-pinene in zebrafish larvae. The study provides scientific evidence to support the use of some essential oils to alleviate anxiety. Biochemometrics could successfully identify bioactive constituents in the essential oils.

Keywords: essential oils, anxiolytic activity, biochemometrics, zebrafish larvae

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Orals

Opening Lecture

Carbohydrate-based vaccines, therapeutics and diagnostics and medicines produced in flow

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Rapid preparation of polysaccharides is by automated glycan assembly (AGA) (Guberman et al., 2019) using a synthesizer (Hahm et al., 2017) provides access to diverse glycans as large as 151-mers (Joseph et al., 2020). I will describe a medicinal chemistry approach to the development of semi- and fully synthetic glycoconjugate vaccines against severe bacterial infections, including resistant hospital microorganisms (Seeberger et al., 2021). Synthetic glycans are key in combination with single molecule imaging, (Wu et al., 2020) to address fundamental questions of carbohydrate structure, folding and material science (Fittolani et al., 2023). Continuous flow systems are useful for automated reaction optimization (Chatterjee et al., 2018) and the modular assembly of active pharmaceutical ingredients (Ghislieri et al., 2015) have become increasingly interesting to practitioners of synthetic chemistry. The new "radial synthesis" concept enables the autonomous production of medications (Chatterjee et al., 2020). Production of the anti-malaria drug artemisinin and its use against different cancers will serve as an example for a drug produced in flow (Triemer et al., 2018).

Keywords: carbohydrate-based vaccines, bacterial infections

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Plenary Lectures

Innovative analytical methods for evaluation of efficacy, safety and quality of raw materials and final products in the phyto-area

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Analytical chemistry and cell biology have significantly advanced natural product research. Developments in chromatography have opened new avenues for the analysis and isolation of natural products, crucial due to the complexity of plant extracts, which contain hundreds to thousands of compounds at varying concentrations. Novel enrichment and purification methods (Huber et al., 2020), particularly advanced solid-phase extraction combined with high-resolution chromatographic separation and mass spectrometric detection (LC-MS), are essential for accurate phytochemical profiling and the quantification of specific metabolites (Kreidl et al., 2020). Combining separation science with spectroscopy, such as near- and mid-infrared (IR) spectroscopy, facilitates rapid, non-invasive qualitative and quantitative analyses of raw plants and extracts. The integration of modern drone technology with NIR spectroscopy expands applications in plant cultivation, enabling efficient determination of optimal harvest times. Traditional analytical methods focus on detecting low molecular weight compounds like phenolics, but the detection of DNA and proteins in plants is gaining importance (Mergner and Kuster, 2022). Plant proteins can serve as active ingredients or allergens, and their identification and quantification are critical for quality control, ensuring the effectiveness, quality, safety, and authenticity of plant products. The latest advancements in protein sequencing, specifically using Quantum-Si's Platinum® instrument for Next-generation protein sequencing™ (NGPS), allow for the direct sequencing of peptide barcodes with single-molecule resolution. This benchtop technology merges DNA-based method accessibility with the innovative capabilities of peptide-based barcoding, enabling the precise identification of unknown phyto-proteins and bioactive protein isoforms. These analytical methods not only enhance research and development but also improve quality control in phyto-analysis (Mair et al., 2023). Comprehensive biological studies complement these analytical approaches, identifying biological activities (Leitner et al., 2022) and conducting necessary safety assessments in phyto-pharmacy, phyto-cosmetics, and phyto-nutrition.

Keywords: natural compounds, analytical chemistry, phyto, NIR-spectroscopy, cell-biology

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Foundational principles can inspire bioactive natural products research

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Pharmacognosy approaches to the discovery of bioactive natural products (NP) can progress by two main mechanisms: improving interdisciplinary integration and advancing the basic sciences of the disciplines involved. Thus, the challenges in our field arise equally from the interdisciplinarity and basic science perspectives. Geoff Cordell's recent work, which can be acronymized as "L.O.V.E. for cyberecoethnopharmacolomics" (Cordell, 2019; 2024), addresses important public health needs for and the potential pathways toward better scientific integration. This presentation will focus on the flipside of the advancement challenge coin and discuss how foundational biological, chemical, physical, and mathematical principles affect today's integrated NP research methodology. The aim is to gain insights into how the application of overarching fundamental knowledge is a prerequisite for subsequent integration and can play a crucial role in advancing biomedicine collectively. To demonstrate how both long-existing and recently advanced foundational knowledge and methodology can inspire progress in applied NP research, we will examine a range of questions along the biology to mathematics continuum:

- What processes drive the discovery of bioactives and who produces them?
- What steps should be considered to help develop screening hits to clinical translation?
- Can bioactive NPs be "too good to be true"?
- What is a NP "compound" or "isolate"? How many structures does it have?
- What physiochemistry stands behind the purification of bioactive NPs?
- Is methodological innovation the first objective or a last resort?
- What is the difference between peak overlap and a multiplet? How can they be resolved?

To answer these questions as concisely as possible, the discussion will include experimental evidence from our own recent work in the discovery of antibiotics, dental biomodifiers, bioactive principles from medicinal and dietary plants, and methodology for pharmaceutical integrity analysis. The broader discussion and outlook part of the presentation will seek to reflect on the contemporary role of philosophy in Ph.D.-level NP research and the nature of experimental evidence in our framework of scientific understanding.

Keywords: bioactive natural products, discovery, advancement of research methodology

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Unusual enzyme reactions in natural product biosynthesis

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β-Nicotinamide adenine dinucleotide (β-NAD) is a pivotal metabolite for all living organisms and functions as a diffusible electron acceptor and carrier in the catabolic arms of metabolism. Furthermore, β-NAD is involved in diverse epigenetic, immunological, and stress-associated processes, where it is known to be sacrificially utilized as an ADP-ribosyl donor for protein and DNA modifications, or the generation of cell-signaling molecules. Here, we report the function of β-NAD in secondary metabolite biosynthetic pathways, in which the nicotinamide dinucleotide framework is heavily decorated and serves as a building block for the assembly of a novel class of natural products. The gatekeeping enzyme of the discovered pathway (SbzP) hereby catalyzes a sophisticated, pyridoxal phosphate (PLP)-dependent (3+2)-annulation reaction between β-NAD and *S*-adenosylmethionine (SAM), generating a 6-azatetrahydroindane scaffold. Members of this novel family of β-NAD-tailoring enzymes are widely distributed in the bacterial kingdom and encoded in diverse biosynthetic gene clusters. The findings of this work set the stage for the discovery and exploitation of β-NAD-derived natural products (Hu et al. 2019; Barra et al., 2021).

Keywords: β-NAD, S-adenosylmethionine, β-NAD-derived natural products, PLP-dependent enzyme

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AFERP Association francophone pour l'enseignement et la recherche en pharmacognosie

Medicinal plants in the fight against malaria: investigating the *Strychnos* genus, *Artemisia* sp. and ellagic acid derivatives.

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Malaria, a parasitic disease transmitted by *Anopheles* mosquitoes, remains a significant global health threat despite advancements in control efforts. In 2022, it accounted for 608,000 deaths and afflicted 249 million individuals worldwide, with 94% of fatalities occurring in Africa (WHO, 2023). The Pharmacognosy Laboratory in Liège, Belgium, has dedicated years to exploring medicinal plants for novel antimalarial compounds, particularly focusing on African flora. Over three decades, we have investigated more than a hundred plants, aiming to identify promising compounds or validate traditional remedies. Three projects of the laboratory will be shortly presented. The first project concerns bisindole alkaloids derived from *Strychnos* species, some showing potent antiplasmodial activity in both *in vitro* and animal studies, suggesting potential as lead compounds (Beaufays et al., 2018). Recently, molecular networking methods were applied to unveil new original antiplasmodial indole alkaloids (Bonnet et al., 2023). Another initiative began with screening Rwandan medicinal plants, leading to the discovery of ellagic acid as active compound (Muganga et al., 2014) and the development of semi-synthetic derivatives with antimalarial properties (Degotte et al., 2023). The third project is dedicated to *Artemisia annua* and *Artemisia afra*, well-known medicinal plants, focusing on standardization, metabolomics-based mode of action studies, and development of a clinical trial. These projects highlight the ongoing relevance of medicinal plants in malaria research. Despite modern pharmaceutical advancements, natural compounds continue to offer significant potential in the fight against this deadly disease.

Keywords: *Strychnos* sp., *Artemisia annua*, *Artemisia afra*, ellagic acid, malaria

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Pharmacognosy faces new challenges: proposals to combine chemometrics and annotations based on mass spectrometry and NMR for more accurate and faster identification of bioactive natural products in complex matrices

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With global warming, reduced biodiversity and the emergence of new pathogens for humans and their resources, researchers including natural products (NPs) chemists need to make the most of new regulatory, modelling and data-sharing tools to make faster and better progress regarding NPs valorization. In that context, as NMR spectrometers now provide useful data sets in a reasonable time frame, we made the MixONat software freely available for dereplication in 2020 (Bruguière et al. 2020, 2021, https://sourceforge.net/projects/mixonat). It allows the processing of ^{13}C chemical shifts (δ_C) as well as DEPT 135 and 90 data for a critical carbon type (*i.e.* CH_3, CH_2, CH and C) filtering. It requires experimental or predicted δ_C databases (DB) and displays interactive results that can be refined based on the user's phytochemical knowledge. To go further, this presentation proposes an integrated strategy combining tandem mass spectrometry (MS^2)-based molecular networking (MN), a partial least squares (PLS) chemometric model, as well as ^{13}C NMR-based dereplication using MixONat software. In addition, an advanced glycation end products (AGEs) assay was used for activity evaluation. The approach was implemented on a *Garcinia parvifolia* bark extract, which contains a high content of prenylated xanthones and had previously shown a remarkable inhibitory effect on AGE formation (Fig. 1) (Meunier et al. 2023).

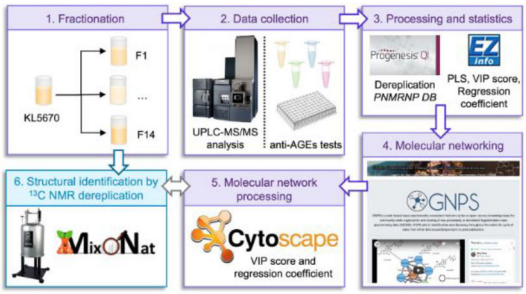

Fig. 1. Workflow for an activity-based molecular network approach that integrates statistical data (VIP score and RC) and annotation based on both ^{13}C NMR and MS^2-based dereplication for a higher level of confidence.

As a main result the proposed strategy permitted the identification of potentially active NPs within complex mixtures and their annotation with a higher level of confidence by both MS^2 and NMR data. Overall, this comprehensive approach provides a powerful and efficient solution for the targeting and annotating of active compounds in complex NPs mixtures.

Keywords: annotation, bioactivity-based molecular networking, Clusiaceae, dereplication, *Garcinia parvifolia*, in silico databases, level of confidence, MixONat, xanthones

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Empowering natural products research with machine learning and expert knowledge integration

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Landmark advances in artificial intelligence approaches and computational omics technologies, particularly in mass spectrometry (MS) and nuclear magnetic resonance (NMR), have recently enhanced the field of natural product (NP) research, putting today's practicing chemists in the enviable position of being able to efficiently speed up the NP discovery process (Mullowney et al., 2023). For some extent, the "art of NP isolation", shifted from the traditional "grind and find" model to the streamlined hypothesis driven targeting of NPs. This presentation is intended to describe through two selected cornerstone examples the new thinking in natural product chemistry resulting from the emergence of this new generation of sophisticated tools. The first part will be dedicated to the structure validation, of the first trimeric monoterpene indole alkaloids isolated from *Catharanthus roseus*, using a combination of ML-JDP4 (Tsai et al., 2022) approach and empirical computation (Szwarc et al., 2024). The second example will showcase how information-rich multicomponent reactions could be unravelled to extract reactivity patterns in line with Baldwin and Whitehead's manzamine alkaloids biosynthesis scenario (Leblond et al., 2024).

Keywords: metabolomics, structure elucidation, biosynthesis, alkaloids

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The evolution of botanical product quality testing

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Concerns about product quality and the means through which it is assessed has existed since the earliest development of commerce and trade. Both Pliny the Elder's (23–79 CE) Naturalis Historia and Dioscorides' (40–90 CE) Materia Medica described products based on their predominant characteristics and the methods through which adulteration could be detected. Despite scientific advances since the original publication of these books, our definition of quality has had to evolve to keep pace with an ever-changing environment. The development and appropriate use of analytical methods is dependent on many factors, including knowledge of analyte(s) and matrix, current state of the art, and nature of the analytical need. Some analytical challenges are more easily addressed, such as determination of target analytes in complex matrices, while reliable analytical solutions for qualitative analyses, such as authentication, remain more elusive. Given the complexity of botanical products and the lack of a direct link between traditional determinants of quality and efficacy, the concept of exploring plant chemistry by a more holistic means is attractive. Comprehensive characterizations can provide pathways to differentiating closely related species, detecting adulteration, and exploring intrinsic and extrinsic influences. Irrespective of the complexity of the analytical challenge or the innovation involved in application of new technologies, methods must be demonstrated to be fit for purpose, rugged, precise, and accurate. This award address will present an overview of experiences, ranging from method development on new analytical platforms to experimental approaches for demonstrating fitness for purpose for qualitative methods.

Keywords: Varro E. Tyler Award, botanical products, quality, authentication, analytical methods, validation, chemometrics

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Discovery and engineering of natural product molecules for drug development

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My talk will focus on various highlights from select research programs initiated and on-going since 1990. One project that has spanned this period relates to modular polyketide synthases, and our development of chemoenzymatic synthesis/cascade biocatalysis to generate new macrolide antibiotic systems. Our work has probed the mechanisms, and engineering of key catalytic domains, and we have linked our ability to generate new molecules with antibiotic activity and ribosome inhibition. A second area of long-standing interest has focused on fungal derived indole alkaloids and assembly of complex multi-ring systems through two classes of enzymes that catalyze intramolecular Diels-Alder reactions. Late-stage C-H oxidation and halogenation has also featured as an important aspect of macrolides and indole alkaloids as the precise modification of their core molecules ultimately enables potent biological activity. More recently, we have developed new semi-synthetic natural product molecules to advance an anti-HIV Nef series of lead compounds that provide a potential path to eradicate the virus. Pathway engineering has been essential for strain optimization to assure a sustainable supply of the two plecomacrolide source materials. I will also highlight studies to identify the endosymbiont producer of ET-743, and current programs to characterize cyanobacterial bloom-derived metabolites in the Great Lakes. Finally, our field collection programs have resulted in a growing marine/terrestrial-derived microbial strain collection and pure natural products/extract library. Centered at the Life Sciences Institute Natural Product Discovery Core, we have benefitted from an institutional Biosciences Initiative that has grown our capabilities significantly, and highlights will be described.

Keywords: natural products, polyketides, indole alkaloids, C-H functionalization, HIV Nef

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Session I: Marine organisms, fungi, microbes and plants as chemical factories

Topical Lecture

Efficient discovery of bioactive natural products by targeted metabologenomic approaches

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Natural products, which were considered as natural gifts, are small molecule material produced by living organisms. In the human history, they have been playing a key role in drug discovery. However, bioactive small molecules with new carbon backbones are still urgently required because drug-resistant diseases have become more widespread and the side effects of clinically-used drugs are problematic. In the past, discovery of new natural products was primarily based on serendipity. However, in the cotemporary genomic era, natural products are recognized as genetically-encoded small molecules. For logical and selective discovery of bioactive natural products, targeted metabologenomic methods were developed utilizing genomic and spectroscopic signatures of specific structural motifs. The application of the metabologenomic methods allowed for the efficient discovery of new macrolactams, piperazic acid-containing peptides, and terminal oxazole-bearing natural products from bacteria. Even though the integration of these metabologenomic techniques in natural product discovery is still in its early stages, it would lead a new paradigm from serendipity to logical discovery and eventually contribute to development of new drug leads.

Keywords: metabologenomic approach, bacteria, natural product, genomics, spectroscopy

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Unprecedented bicyclic and tricyclic non-canonical terpenoids via synthetic biology platforms: Isolation and structure elucidation of unique C_{16} and C_{17} terpenoids

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Terpenoids are the most diverse group of specialized metabolites having essential ecological roles, exhibiting diverse biological activities, and finding numerous applications in various industrial sectors. Their biosynthesis is based on the C_5 isoprene building block; thus, almost all terpenoids isolated are based on backbones that contain multiples of five carbon atoms. Nonetheless, a few terpenoids with non-canonical carbon numbers in their backbones have been reported. To explore if these unexpected isoprenoids are limited to the few examples identified so far, gene mining was combined with synthetic biology and natural products chemistry to investigate the extent and diversity of their biosynthesis. After mining available bacterial genomic information for putative non-canonical isoprenoid biosynthetic gene clusters, representative clusters were chosen and studied for their gene organization to reveal complex architectures of methyltransferase and terpene synthase genes. In the next step, the yeast *Saccharomyces cerevisiae* was used as a platform to functionally characterize the selected methyltransferases and terpene synthases. This allowed for the detection of several different non-canonical terpenoids. Copious chromatographic separations enabled the isolation and structure elucidation of a number of C_{16} (Ignea et al., 2022; Duan et al., 2023) and C_{17} metabolites, revealing intricate highly strained bi- and tricyclic backbones that are either rare or unprecedented in natural products. Our findings reveal the existence of an extensive class of non-canonical terpenoids in bacteria and prompt further investigation into their biosynthesis and potential pharmacological activity or ecological role, relevant for industrial applications.

Keywords: non-canonical terpenoids, isolation, structure elucidation, synthetic biology

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Peptide discovery and development – known and dark matter

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Interest in peptides for drug development is surging. This surge is propelled by the genomic revolution, enhanced comprehension of protein function, and advancements in peptide characterization techniques, driven by recent successful precedents. Furthermore, peptides give access to targets non tractable for small molecues, not the least in the modulation of protein-protein interactions. Importantly, techniques to study peptides are mature: genome sequencing to uncover ribosomally produced natural products; bioinformatic tools, e.g. AI, for structural determination and functional annotation; and synthetic and recombinant methods for their production. So, what contribution can pharmacognosy and natural product chemistry make? While bioinformatics and structural predictions are widely accessible, they inherently rely on existing knowledge. In this presentation, I will illustrate how natural product chemistry continue to contribute to the exploration of the unkown—the dark—peptide matter. We have been focusing on plant derived peptides, and our interest has long circled around the cyclotides (Burman et al., 2014). This family of peptides revealed the occurrence of naturally occurring head-to-tail cyclic peptides. I will show how we exploit this feature in the design of scavengers of antibodies in rheumatoid arthritis and Covid 19, and for development of antimicrobials againts gram negative bacteria (Gunasekera et al., 2018; 2020; Eriksson et al., 2023). But the remaining question is: are there other families of peptides to be discovered? The answer is "Yes", albeit if genomes are known or not, and I will demonstrate this with examples from both plants and animals (Jacobsson et al., 2018).

Keywords: peptide chemical biology, peptide discovery and design, antimicrobial, toxins

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Co-culture exploration of some selected species of *Fusarium*, *Pestalotiopsis* and *Colletotrichum* in different culture media

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Exploration of the axenic and co-cultures of phytopathogenic species of *Fusarium guttiforme* **F1**, *Pestalotiopsis diospyri* **F2**, *Colletotrichum horii* **F4** and *C. gloeosporioides* **F6** were carried out. The roles of these phytopathogens have been implicated in the infestation faced by protease-rich edible fruits and other agricultural produce. This results in huge economic losses every year (Savary et al., 2012). However, harnessing the co-cultivation of these fungi could be an avenue to understanding their specific needs for the biosynthesis of some specialized metabolites. Hence, informing a novel approach that is capable of affording metabolites with desired/anticipated potentials. The trio co- culture permutations [**CoF126** and **CoF246**] were carried out in rice and PDB (Potato Dextrose Broth) media to ascertain fungi-substrate effects. Although, in PDB fewer metabolites were produced due to **F1** generally, the rice medium favors the fungal exploration in terms of yields and diversity of metabolites. The EtOAc/Acetone extracts were subjected to chromatographic separation/purification and spectroscopic methods for the structure elucidation/identification of compounds. Comparison between ^{1}H NMR profiles of the co-culture with axenic revealed a significant increase in peaks signal in the former suggesting that certain biosynthetic pathways leading to enhanced production of metabolites were activated. Except in CoF126, where fusaric acid from **F1** slightly showed co- expression with the α-pyrone derivatives from **F2**, generally, there was an overexpression/predominance of these α-pyrones followed by chaetiacandin-type polyketide and colletotric acid from **F6**. This implies that the trio-fungal species co-existence was non-mutualistic but of cause beneficial to the search for antagonistic specialized compounds with antimicrobial and protease inhibitory activity.

Keywords: co-culture, *Fusarium guttiforme, Pestalotiopsis diospyri, Colletotrichum horii, Colletotrichum gloeosporioides,* metabolites

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Evaluation of biodiverse *Sorghum bicolor* and related genotypes for root exudation, sorgoleone production and potential carbon capture

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Many sorghum species express allelopathic properties resulting from the biosynthesis and release of long chain hydroquinone metabolites impacting the growth of plants and certain microbes (Weston et al. 2013). Earliest studies on the hydrophobic root exudates from the roots of *Sorghum bicolor* demonstrated their phytotoxicity to broadleaf crop and weed species, suggesting that root exudates play an important role in the growth inhibition of broadleaf weeds. Most recently, multiple long-chain hydroquinones have been identified in exudates, but sorgoleone, 2-hydroxy-5-methoxy-3-[(Z,Z)- 80,110,140-pentadecatriene]-p-benzoquinone, is the major constituent. Interestingly, sorgoleone affects multiple molecular targets (Weston et al., 2013) and has proven to be a potent inhibitor ofphotosystem II and respiration (Czarnota et al., 2001). Sorgoleone also strongly inhibits the enzyme HPPD (p-hydroxyphenylpyruvate dioxygenase), which is involved in the formation of plastoquinone and also acts as a respiratory inhibitor. Its phytoxic properties coupled with multiple target sites and the quantity of carbon released in the soil over time offer promise for sorgoleone as a natural product alternative to synthetic herbicides and also as a means to effectively capture carbon release into the surrounding soil environment. The biosynthesis of sorgoleone occurs in sorghum root hairs, and exudate is rapidly released from the root hair in the form of oily yellow droplets (Pan et al. 2016). Sorgoleone has been observed as osmiophilic globules in root hair cells, which are deposited between the plasmalemma and cell wall, and are associated with sorgoleone rhizosecretion (Czarnota et al., 2003). The biosynthetic pathway leading to the formation of sorgoleone was determined in recent years and was it later demonstrated that the biosynthesis of sorgoleone (Fig. 1) involves the production of an alkylresorcinolic intermediate (Weston et al. 2013). *Sorghum bicolour, S. Sudanese, S. halpense and S. leiocladum* have all been found to produce bioactive root exudates containing long chain hyroquinones.

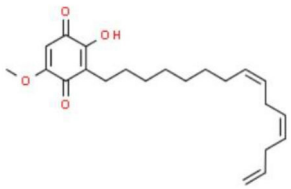

Fig. 1. Structure of sorgoleone **1**.

As a result of a Bayer Grants4Ag project, we are employing both metabolomics and genomics approaches to evaluate a biodiverse collection of *S. bicolor* plus other unusual native Australian sorghum genotypes for their ability to a) produce significant quantities of root exudates with a focus on carbon capture, b) generate consistent root hair density and extensive primary roots, c) produce bioactive root exudates containing long chain hydroquinones with bioherbicidal and antimicrobial activity influencing nutrient cycling processes and d) produce exudates with altered composition both qualitatively and quantitatively. We share our research results in the upcoming presentation.

Keywords: sorgoleone, *Sorghum* spp., phytotoxicity, root exudation, PSII inhibitor, mode of action, carbon capture

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'Two neutrons walk into a bar'. The power of the $^{35}Cl/^{37}Cl$ isotope effect in structure elucidation of natural products by NMR and mass spectrometry

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In the course of structure elucidation of chlorinated marine natural products (MNPs) from algae (*Rhodophyta*) and sponges (*Porifera*), we required a precise NMR method to locate Cl in the polyhalogenated molecular structures at the 'nanomole-scale'. Powerful 2D NMR pulse sequences were developed that allowed detection of both $^{1}J_{CH}$ and $^{n}J_{CH}$ (n = 2,3) cross peaks at C-Cl bonds that were 'split' by the $^{35}Cl/^{37}Cl$ isotope effect. Here, we show extension of the method, with complementary isotope-rationing mass spectrometry (IRMS) measurements, that reveal $^{35}Cl/^{37}Cl$ isotopic fractionation during the biosynthesis of chlorinated MNPs; a pattern that correlates with different modes of biosynthetic halogenation that create C-Cl bonds.

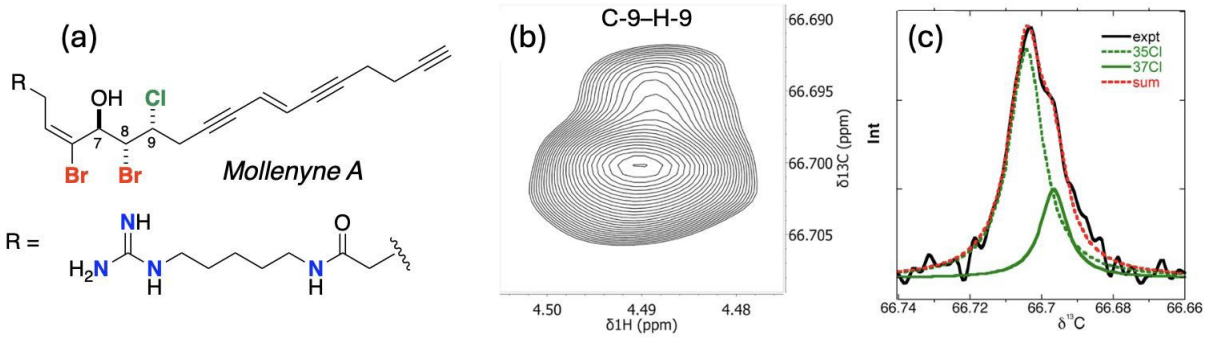

Fig. 1. (a) Mollenyne A. (b) bsHSQC $^{35}Cl/^{37}Cl$ isotope shifted C-9–H9- cross-peak (c) slice through (b) and (c) deconvolution of ^{35}Cl and ^{37}Cl components.

Keywords: marine natural product, chlorine, trichloroleucine, NMR, mass spectrometry, *Dysidea herbacea*, *Porifera*, *Rhodophyta*

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Charting the chemical content of toxic and lethal French fungi through a multiply annotated molecular networking-based approach

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The chemical features underlying the toxic effects of mushrooms remain understudied and greatly vary from a species to another. While some syndromes (e.g. phalloidian syndrome) are chemically well characterized (Walton et al., 2018). Others, such as the resinoid, the paxillian or the rhabdomyolytic syndrome, are still poorly documented from a chemical standpoint. Often, the true nature of the toxic species is still debated (Rzymski et al., 2018). The difficulties raised by mycotoxicologists often stem from a lack of chemical knowledge about macromycetes. A collaboration with the CEFE unit in Montpellier allowed us to raise a collection of more than 3,700 DNA-barcoded entries. This collection will serve as a basis for small-scale extractions of the main fungi responsible for the intoxication syndromes recorded in Europe, prior to acquiring a reference set of LC-MS/MS data. To help us choose the most relevant fungal species to include in a toxicological context, we relied on statistics from the Mycoliste (Bourgeois et al., 2017), that allows French poison control centers to centralize cases of fungal intoxication. We plan to generate molecular networks annotated with a syndromic tag using the GNPS platform (Wang et al., 2016). Merging metabolomics data with toxicological knowledge will enable to visualize the chemical space occupied by fungal toxins and hopefully contribute to answer some long-lasting questions. Besides, disseminating the MS/MS datasets should facilitate the detection of fungal-derived metabolites, either in a context of natural product chemistry targeting new analogues or in a context of analytical toxicology/forensics (Allard et al., 2018).

Keywords: macromycetes, toxins, analytical toxicology, molecular networks

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Discovery of novel biopesticides from an agricultural disease nursery – a proof of concept study

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This presentation will discuss results from a proof-of-concept study suggesting that the combination of in-situ isolation using an ichip, and soil subjected to high disease pressure, results in the isolation of novel bacterial species that are promising biopesticide candidates and sources of novel natural product leads. This work revealed that a *Fusarium graminearum* disease nursery in PEI, Canada, which has been maintained at artificially high disease-levels to test resistant crops, harbours a significant number of microbes able to combat fungal pathogens including *F. graminearum*. Half of the isolates recovered were novel strains or species of bacteria and the majority exhibited antagonism against fungal pathogens as summarized in **Fig. 1.** Previously, ichip devices have been used to isolate novel microorganisms under physiological stress such as contaminated soils (Polrot et. al., 2022), or extreme temperatures (Zhao et al., 2023). To our knowledge environments under biotic stress have not been explored in such ways provide compelling evidence that soils experiencing high disease pressure are a valuable source of novel biopesticidal bacteria and antimicrobial compounds. Results from the isolation, genome sequencing, activity assays, and natural products screening experiments of ichip isolated bacteria will be discussed.

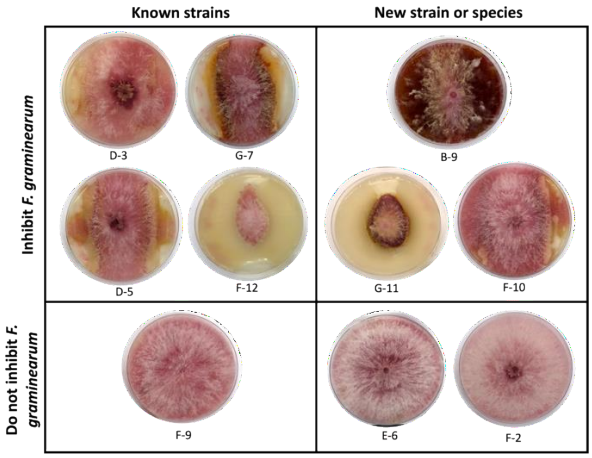

Figure 1. Plate antagonism assay isolates recovered from a *F. graminearum* nursery using an ichip where half of the isolates are novel strains or species, and the majority exhibit antagonism against F. graminearum.

Keywords: *Fusarium graminearum,* ichip, biocontrol, biopesticides, soil microbiota

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Deep ocean biodiscovery – diving into abyss

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The extreme variety of deep-sea habitats, encompassing ancient abyssal plains some of which date to over 180mya, to high-temperature hydrothermal vents, makes the deep-sea the perfect evolutionary test-lab for natural product research. Often, we think of deep-sea as void of life, but a recent study has uncovered 5,000 new species in Pacific deep-sea mining regions (Rabone et al., 2023) and we know that understanding diversity-ecosystem function is of primary importance in the face of biodiversity loss. These ecosystems have remained largely unexplored in terms of biodiversity and biomedical potential, yet over recent years they have gained significant interest due to the potential of deep-sea mining for metals such as cobalt and nickel. Here we aim to profile the biodiversity and biomedical (antibiotic) potential of deep-sea microorganisms. Microbial drug discovery in the 'omics era relies on three key datasets, biosynthetic, chemical, and biological (activity). Yet, to integrate and interrogate these large and complex datasets remains a challenge and results in the low-throughput prioritization of only a few strains based on observed antibiotic activity. Despite this wealth of genomic and metabolomic data, linking metabolites to the BGC responsible for their production and to observed bioactivity is limited, slow (manual) and challenging. Here, approaches to combine data sets consisting of bacterial genomes (and their predicted BGCs), the chemical products of these same strains and their bioactivity profiles will be discussed. Several datasets of Actinobacteria genomes have been mined for BGCs and these strains cultured to generate metabolite extracts for comparative metabolomics (high resolution tandem mass spectrometry / molecular networking) and antibiotic screening (against a panel of clinically relevant pathogens). Driven by biological questions, tools have been developed to establish patterns across strains and learn relationships between BGC, spectral features and bioactivity – here we will discuss experimental data considerations and how 'omics information is informing biodiscovery from the deep-sea.

Keywords: *Streptomyces*, antibiotics, metabolomics, genomics, deep-sea

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Specialized metabolites of the herptile gut fungus, *Basidiobolus*

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The zygomycete fungus *Basidiobolus* is often a prominent member in the herptile (amphibians and reptiles) gut microbiome. Draft genomes of *Basidiobolus* show evidence of horizontal gene transfer (HGT) of nonribosomal peptide synthetases (NRPS), some of which are predicted to originate in co- occurring gut bacteria (Tabima et al., 2020). It is suspected that the peptidic metabolites encoded play a role in modulation of the herptile gut microbiome. Here, untargeted LCMS^2-based metabolomics using computational tools (GNPS, Sirius5, and CANOPUS) for structural class prediction revealed that 10 unique *Basidiobolus* isolates (derived from field collected herptile fecal pellets) produce cyclic peptides belonging to the same molecular family in laboratory culture (Vargas-Gastélum et al., 2024). An analysis of the antimicrobial activity of extracts from a gecko isolate of *Basidiobolus*, using the Bioactive Molecular Networks Project protocol, highlighted 16 mass features that correlated with the observed antimicrobial activity against a panel of human pathogens. Analysis of tandem mass spectra assigned these mass features as peptides and depsipeptides and isolation and structure elucidation efforts are currently underway. Furthermore, a laboratory model was developed that can establish stable infections of specific *Basidiobolus* isolates in adult wood frogs (*Lithobates sylvaticus*). This allows monitoring effects *Basidioblous* has on microbiome community structure and metabolism. These experiments support the hypothesis that cyclic peptides from *Basidiobolus* can influence microbial growth and lay the groundwork for further study of the implications *Basidiobolus* has on herptile health.

Keywords: *Basidiobolus,* herptile microbiome, cyclicpeptides, horizontal gene transfer, antimicrobial

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Session II: Natural products in the era of emerging infectious diseases

Topical Lecture

From microbes to medicine: leveraging nature's arsenal for innovative anti-infectives

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The global rise of antimicrobial resistance, mainly due to the mis- and overuse of antibiotics, is one of the most pressing issues of our time. To counteract this development, novel resistance-breaking antibiotics are urgently needed. Historically, the vast majority of antibiotics have been derived from microbial natural products. As compared to traditional bacterial producers, such as actinomycetes and bacilii, myxobacteria have been studied less extensively and thus harbor a large potential for the discovery of entirely new natural product scaffolds exhibiting promising bioactivities. Comparisons of myxobacterial metabolite profiles with the number of underlying biosynthetic gene clusters encoded in their large genomes show, that many compounds still remain unknown. Further, recent studies indicate that the order of myxobacteria likely comprises many more biodiverse representatives than previously assumed. According to metagenomics analyses, myxobacteria (including many underexplored representatives) are highly abundant in the soil microbiome, where they play a crucial role in soil nutrient and carbon cycling. Taken together with our recent genomic analyses, these findings suggest that the biosynthetic potential of myxobacteria is a long way from being exhausted.

We recently demonstrated that chemical diversity correlates with taxonomic distance in myxobacteria. Accordingly, we are more likely to isolate novel compound classes from strains which are phylogenetically distant from previously characterized strains as compared to closely related strains. This knowledge is applied to prioritize strains for natural product discovery, thus increasing the chance of discovering compound classes with yet unknown chemical structures and biological activities.

I will discuss recent results from our laboratory regarding the identification of novel bioactive NPs from myxobacteria based on different approaches, and show recent advances in the preclinical development of selected compound classes.

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Observations and insights into the prolific and varied metabolites of the fascinating plant genus *Dalea* (Fabaceae)

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Work on the phytochemistry of *Dalea* spp. continues to afford new phenolic metabolites with no sign of diminishing returns after two decades. Metabolites of *D. jamesii* and *D. nana* (Belofsky et al., 2023 and 2024), provide insights into in vitro structure-activity relationships of isoflavanoids toward methicillin-resistant *Staphylococcus aureus*, and other human pathogens. The prenylated isoflavans isolated exhibit MICs ≤9 mg/mL (aq. DMSO) that appear correlated to the number and type of these lipophilic groups (e.g., **1** and **2**, **Fig. 1**).

Prior work on pawhuskins A-C (Belofsky et al. 2004), k-opioid antagonists from *D. purpurea*, was unique among all *Dalea* spp. examined until current work on *D. candida* var. *oligophylla* afforded a new member of this class, pawhuskin D (**3**). The continued work on extractives of this species will be presented.

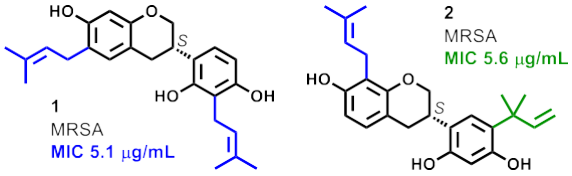

Fig. 1. Structures of antimicrobial isoflavans **1** and **2** and of pawhuskin D **3**.

Cumulative results clearly support the ecological observation that more structurally diverse metabolites, with more potent antimicrobial activities, are concentrated in the roots rather than aerial portions of *Dalea* spp. Details will be presented of our exclusive use of open-column chromatography, that makes such research accessible to students at a primarily undergraduate institution.

Keywords: *Dalea* spp., Fabaceae, isoflavanoid, pawhuskin, MRSA

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Target identification of 3-(phenethylamino)demethyl(oxy)aaptamine (PDOA) as an anti-dormant Mycobacterial substance.

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Tuberculosis is an infectious disease caused by infection with *Mycobacterium tuberculosis*, resulting in approximately 1.3 million deaths annually. The presence of dormant *M. tuberculosis*, which is tolerance to anti-tuberculosis drugs, is considered a reason for requiring prolonged chemotherapy through multi-drug combinations. There is a need to discover new medicinal seeds effective against dormant *M. tuberculosis*. 3-(phenethylamino)demethyl(oxy)aaptamine (PDOA, **1**), isolated from Indonesian marine sponge *Aaptos* sp., was effective against dormant *Mycobacterium* species (Sumii et al., 2020). However, its target molecule is not yet identified. In this study, we synthesized the probe molecule of **1** and validated its utility as a probe molecule, as well as attempted to identify binding protein from the bacterial cell lysate. From the result of structure-activity relationships study using synthetic analogs of **1** (Sumii et al., 2023), PDOA probe (**2**) was designed and synthesized (Fig. 1). The functional evaluation of **2** was conducted using BSA. As a result, **2** bound to BSA only when subjected to UV irradiation. The Huisgen reaction between **2** and a biotin-labeled PEG linker with an azide group proceeded in an aqueous solvent. Furthermore, **2** having biotin was found to be capable of pulling down BSA using streptavidin beads. Therefore, we further attempted to identify the binding protein of **1** using **2** from cell lysate of *M. bovis* BCG. As a result, we succeeded in finding some proteins on SDS-PAGE gel that were not detected in the absence of **2** or when **1** was added to the reaction system as a competitor, but were selectively detected in the presence of **2**.

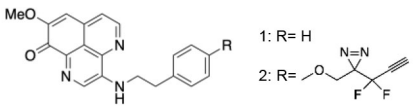

Fig. 1. Structures of PDOA (**1**) and PDOA probe (**2**)

Keywords: *Aaptos* sp., PDOA, anti-microbial, tuberculosis, target molecule

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Revealing anti-SARS-CoV-2 natural products from traditional remedies using molecular networking

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Natural remedies have a long-standing tradition in the treatment of respiratory diseases. This ethnopharmacological knowledge provides a remarkable feature of natural product research, forshadowing potential new bioactivities. In this study, 179 extracts derived from 154 natural remedies traditionally used against respiratory infections and inflammation were tested for their anti- SARS-CoV-2 activity in a Caco-2 cell model (Wasilewicz et al., 2024). 13 extracts exhibited distinct anti-SARS-CoV-2 activity with IC_{50} values below 50 µg/mL; notably, five extracts demonstrated IC_{50} values below 5 µg/mL. In the search for constituents within the complex and highly biodiverse extract set, non-toxic anti-SARS-CoV-2 active extracts and some inactive extracts were dereplicated using a molecular network (MN) approach. For this purpose, MS^2 spectra of both active and inactive extracts were recorded and used for the generation of a feature-based MN (Nothias et al., 2020). The obtained MN analysis enabled the determination of compounds classes characteristic of active extracts. Thereby, in particular some flavonoid glycosides, triterpene saponins, sesquiterpenes and acetogenins were unravelled as putative bioactive metabolites from the screened extracts.

To summarize, the herein applied bioactivity-featured MN approach allowed to identify the most promising constituents from the hit extracts, thereby providing crucial chemical insights into known as well as novel structural features with anti-SARS-CoV-2 activity. Thus, this work serves as a basis for the targeted isolation of novel or known natural compounds with yet unexplored anti-SARS-CoV- 2 activities.

Keywords: SARS-CoV-2, ethnopharmacology, molecular networking

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Acknowledgments

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Identification and SAR studies of indole-functionalized polyamines as antibiotic enhancers

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Antibiotics have long been pivotal in modern medicine, saving countless lives by effectively combating infectious diseases (Lewis, 2020). However, the rise of drug-resistant strains of bacteria coupled with a dearth of new antibiotic development presents a pressing need for novel approaches (Singh, 2014). One promising strategy involves identifying compounds that can augment the efficacy of existing antibiotics, particularly those losing effectiveness against resistant bacteria (Douafer et al., 2019).

Initial screening of compounds sourced from our in-house collection of purified marine natural products and their synthetic derivatives, identified a promising lead: an indole-functionalized polyamine (Fig. 1). This compound not only restored the activity of the antibiotic doxycycline against the drug-resistant Gram-negative bacterium *Pseudomonas aeruginosa* but also demonstrated intrinsic antibacterial properties. Subsequent Structure-Activity Relationship (SAR) studies yielded a range of novel analogues, many of which displayed potent enhancing effects on both doxycycline and erythromycin against *P. aeruginosa* and *Escherichia coli*. The forthcoming presentation will detail the results of these studies, shedding light on the mechanisms of action of the most potent compounds and their capacity to augment the efficacy of diverse antibiotics against drug-resistant bacterial strains.

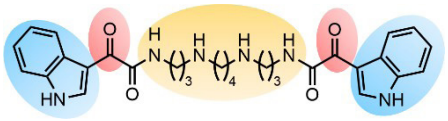

Fig. 1. Representative structure of indole-functionalized polyamine

Keywords: Indole, polyamine, antimicrobial, adjuvant, SAR

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Hope in hop

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Hop (*Humulus lupulus* L., Cannabaceae), a plant of economic and cultural interest, is cultivated for its female inflorescences, called hops or cones, used by the brewing industry and for their medicinal properties. Hops produce both an essential oil, mainly represented by non-oxygenated monoterpenes and sesquiterpenes, and original prenylated compounds, including chalcones and acylphloroglucinols (α and β acids) (Bocquet et al., 2018). The composition of the essential oil and the α acid content are particularly sought after by brewers. The evolution of the beer market, in particular the growing interest in craft beers with a trend toward strongly hopped beers, is impacting hop production and varietal selection (Paguet et al., 2022). Hops are also studied for their many pharmacological activities, including sedative, estrogenic, antioxidant, anti-inflammatory and antimicrobial activities (Bocquet et al., 2018). In this context, we focused our research on the chemical and genetic diversity of wild hops harvested in the North of France in order to underline their interest both for potential varietal selection and for the identification of new bioactive specialized metabolites. This presentation will also highlight the various work carried out by our teams on the antimicrobial potential of hop metabolites and their co-products for human and plant health.

Keywords: *Humulus lupulus* L., prenylated phenolic compounds, antimicrobial agents, co-products, wild hops

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Acknowledgments

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Hops *(Humulus lupulus* L.*)* compounds against two tropical infectious diseases: *Chikungunya* and *Oropouche* virus (Bunyavirales)

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Humulus lupulus L. (Cannabaceae), commonly named hop, is widely grown around the world for its use in the brewing industry. Its female inflorescences (hops) are particularly prized by brewers because they produce some secondary metabolites that confer bitterness, aromas, and antiseptic properties to the beer. These sought-after metabolites as prenylated phenolic compounds, mainly acylphloroglucinols (bitter acids) have shown numerous biological activities. We investigated whether purified hop components α- and β- acids and prenylflavonoids (xanthohumol) exhibit antiviral activity against Chikungunya virus (CHIKV) and Oropouche virus (OROV), two arboviruses that cause serious health problems in tropical and subtropical regions. The bioactive compounds were purified by the countercurrent separation method. For CHIKV, the antiviral activity was determined by the plaque reduction test. Almost all hop compounds demonstrated a promising post-treatment viral inhibition. β-acids in the highest tested concentration of 125 µg/mL expressed the strongest virucidal activity (EC_{50} = 15.21 µg/mL), in a drug-addition experiment on Vero cells. Hypotheses for the mechanism of action concerning protein kinase C (PKC) transduction cascades were proposed. Same bioactive compounds from hops were also tested against OROV. The evaluation of the inhibitory potential on the viral cycle of OROV was performed through two complementary approaches. The first approach applied cell-based assay post-inoculation experiments to explore the inhibitory potential on the latest steps of the viral cycle, such as genome translation, replication, virion assembly, and virion release from the cells. The second part covered *in silico* methods evaluating the ability of these compounds to inhibit the activity of the endonuclease domain, which is essential for the transcription, binding, and cleaving of RNA. In conclusion, the β-acids beta acids showed strongest inhibitory potential in the post-treatment assay (EC_{50} = 26.7 µg/mL). The results observed from the docking and MM/PBSA analysis confirmed the affinity and among the three tested ligands, Lys92 and Arg33 exhibited the highest affinity with the protein.

Keywords: tropical infectious diseases, *Chikungunya, Oropouche,* hops compounds, countercurrent chromatography

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The phototherapeutic potential of fungal pigments

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Mushrooms are an underestimated source of natural photosensitizers, especially those that produce polyketide pigments. Since fungi share their ecological niche with other microorganisms, we hypothesized that some fungal pigments are photoantimicrobials. To test our hypothesis, fungal species of the genus *Cortinarius* producing colored fruiting bodies were explored (e.g., *Cortinarius sanguineus* or *C. holoxanthus*). The extracts were assessed among others against the yeast *Candida albicans* as well as the bacteria *Escherichia coli* and *Staphylococcus aureus* under different irradiation conditions (λ = 428, 478, or 528 nm, H = 30 J cm^{-2}) and in the dark. A significant photoantimicrobial effect was discovered for several extracts (c < 25 µg/mL). Targeted isolation of the responsible pigments from *C. sanguineus* led to the identification of two new potent photoantimicrobials. One, dermocybin, was active against *S. aureus* and *C. albicans* under green light irradiation, while it was ineffective in the dark and non-toxic to cell lines. The other, emodin, was also active against *E. coli* in the low micromolar range. Inspired by the activity of these anthraquinones, we tested the potential of related polyhydroxy anthraquinones and showed that besides aloe-emodin and chrysophanol, trihydroxy anthraquinones are also potent photoantimicrobials.

In sum, here we will show the remarkable potential of fungal pigments against the gram-positive and gram-negative bacteria as well as several yeasts, causing unpleasant skin infections in humans.

Keywords: dermocyboid *Cortinarii*, photoantimicrobials, anthraquinones

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Villosane A, an allene containing bisabolane sesquiterpene with potent antiplasmodial activity isolated from cultures of the marine derived basidiomycete *Halocyphina villosa*

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The chemotherapeutic armamentarium to fight parasitic diseases such as malaria and Chagas disease needs novel bioactive molecules with potential for development into effective drugs. Sustained efforts in antiparasitic high throughput screening of a collection of microbial extracts (Annang et al. 2015; Pérez-Moreno et al. 2016) coupled with bioassay-guided compound isolation led to the discovery of a new family of potent antiparasitic bisabolane sesquiterpenoids containing a hydroxamide functionality, villosanes A-C, from an extract of the marine derived basidiomycete *Halocyphina villosa* CF-090066. (+)-ESI-TOF mass spectrometry, nuclear magnetic resonance spectroscopy and X-ray diffraction were used to establish their structures and absolute configurations.

Villosane A, featuring a rare allene moiety in its structure was the most potent and most selective of the three compounds, exhibiting EC_{50} values of 0.0055 and 6.2 μM against *Plasmodium falciparum* 3D7 and *Trypanosoma cruzi* Tulahuen C4 parasites, respectively. Villosanes B and C exhibited weaker EC_{50} values of 0.062 and 4.83 μM, respectively, against *P. falciparum* and were both inactive against *T. cruzi* at 25 μM. Preclinical profiling of villosane A revealed non-inhibition of hERG, Nav

1.4 and Cav 1.5 ion channels at 50 μM, adequate in vitro metabolic stability, weak potential for drug- drug interaction and no in vivo toxicity at a dosage of 50 mg/kg in mice. Hence, villosane A, the major component of the extract, presents a promising potential for development into an effective antimalaria drug.

Data on the production, isolation, structures and antiparasitic properties of the villosane family of natural products will be presented in this communication.

Keywords: *Halocyphina villosa,* bisabolene sesquiterpenoids, allenes, bioassay-guided isolation, antiparasitic

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Naphthylisoquinoline alkaloids streamlining mycetoma drug discover by circumventing differential diagnosis

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Mycetoma is a chronic, slowly developing, devastating, and progressive neglected tropical infection of the subcutaneous tissues caused by either bacteria (actinomycetoma) or fungi (eumycetoma), leading to life-long disabilities and placing a significant burden on the poorest members of the society. The lack of reliable, safe, fast, and affordable diagnostic methods for identifying the causative agent is a considerable problem that hampers prescribing appropriate medication. Current therapy of eumycetoma causative agents, such as *Madurella mycetomatis*, consists of long-term azole antifungal agents coupled with surgery, yet with unsatisfactory clinical outcomes. Actinomycetoma is more responsive to treatment with co-trimoxazole and amikacin (Relhan et al., 2017). Hence, there is a pressing need to discover novel broad-spectrum antimicrobial agents to circumvent the time- consuming and costly diagnosis. A series of 23 naphthylisoquinoline (NIQ) alkaloids and related naphthoquinones were subjected to *in vitro* screening against three fungal strains of *M. mycetomatis*, and two bacterial strains of *Actinomadura madurae*, and *A. syzygii* (Abd Algaffar et al., 2021, 2022). Seven NIQs, mostly dimers, showed promising *in vitro* activities against at least one strain, while the naphthoquinones did not inhibit any of the tested strains. A synthetic NIQ dimer, 8,8'''-*O*,*O*-dimethylmichellamine A **1**, inhibited all tested fungal and bacterial strains (IC50 = 2.81-12.07 µg/mL). Michellamine B **2**, an *in vitro* active dimeric NIQ inhibited a strain of *M. mycetomatis* and significantly enhanced the survival rate of *M. mycetomatis* infected-*Galleria mellonella* larvae at concentrations of 1 and 4 µg/mL, without being toxic to the uninfected *Galleria* larvae (Konings et al., 2021).

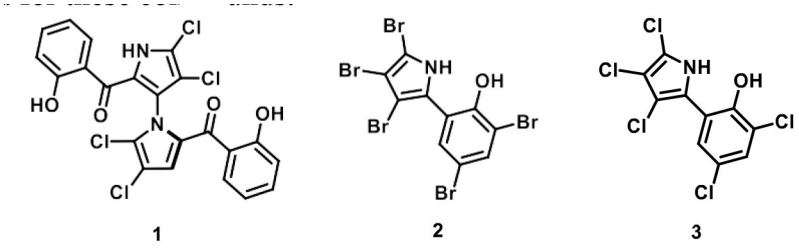

Fig. 1. Structure of 8,8'''-*O*,*O*-dimethylmichellamine A **1** and michellamine B **2**

Keywords: eumycetoma, actinomycetoma, broad-spectrum agents, naphthylisoquinoline alkaloids

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Antibacterial marinopyrroles and pseudilins act as protonophores

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Elucidating the mechanism of action (MoA) of antibacterial natural products is crucial to evaluating their potential as novel antibiotics. Marinopyrrole (**1**), pentachloropseudilin (**2**), and pentabromopseudilin (**3**) are densely halogenated, hybrid pyrrole-phenol natural products with potent activity against Gram-positive bacterial pathogens like *Staphylococcus aureus* (Fig. 1) (Hughes et al., 2008). However, the exact way they exert this antibacterial activity has not been established. In this study, we explore their structure–activity relationship, determine their spatial location in bacterial cells, and investigate their MoA (Castro-Falcón et al., 2024). We show that the natural products share a common MoA based on membrane depolarization and dissipation of the proton motive force (PMF) that is essential for cell viability. The compounds show potent protonophore activity but do not appear to destroy the integrity of the cytoplasmic membrane via the formation of larger pores or interfere with the stability of the peptidoglycan sacculus. Thus, our current model for the antibacterial MoA of marinopyrrole, pentachloropseudilin, and pentabromopseudilin stipulates that the acidic compounds insert into the membrane and transport protons inside the cell. This MoA may explain many of the deleterious biological effects in mammalian cells, plants, phytoplankton, viruses, and protozoans that have been reported for these compounds.

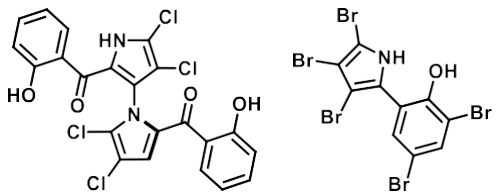

Fig. 1. Structure of marinopyrrole (**1**), pentachloropseudilin (**2**), pentrabromopseudilin (**3**)

Keywords: halogens, antibiotics, protonophore, fluorescent microscopy, *Staphylococcus aureus*

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Session III: Biotransformation and biosynthesis of natural products

Topical Lecture

Flavoenzyme-driven structural diversification of bacterial polyketide and tropone natural products

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Bacterial aromatic polyketides as well as tropone natural products are structurally diverse and often play crucial roles in mutualistic and antagonistic symbiotic interactions with eukaryotic hosts. The biosynthesis of their backbones relies on dedicated polyketide synthases and accessory enzymes in the case of the aromatic polyketides and on an unusual intertwining of primary and secondary metabolism for the tropones, in which the initial steps depend on enzymes from phenylacetic acid catabolism that give rise to a central tropone precursor. The structural diversification of these backbones are then mediated by tailoring enzymes that are specific to each type of natural product and producer strain. In my talk, I will highlight how structurally and functionally distinct flavoenzymes are employed during late-stage biosynthesis for the functionalization of both pentangular rubromycin-type polyketides as well as tropone scaffolds and thereby generate potent protein inhibitors, virulence factors and antibiotics.

Keywords: bacterial aromatic polyketides, tropone natural products, flavoenzymes

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Streamlined chemoenzymatic synthesis of cyclic peptides by non-ribosomal peptide cyclases

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Macrocyclization of peptide improves their pharmaceutical properties such as cell-membrane permeability, target specificity and metabolic stability. However, regio- and chemoselective intramolecular cyclization remain challenging. Inq the biosynthesis of non-ribosomal cyclic peptides, macrocyclases such as thioesterases (NRPS-TEs) efficiently catalyze the intramolecular peptide cyclization in a regiospecific manner without the use of protecting groups. Thus, NRPS-TEs are promising biocatalysts for the efficient synthesis of macrocyclic peptides. NRPS-TEs generally require the thioester leaving groups on its substrate, which are usually installed by solution-phase coupling reactions during substrate synthesis. However, this step often generates epimerized products which are necessitates the purification of desired peptide from diastereomeric mixture, which is notoriously difficult and time-consuming. In this study, we have developed a streamlined chemoenzymatic approach to synthesize cyclic peptides that bypasses the need for leaving group installation in solution phase (Kobayashi et al., 2023). Linear peptides with diol ester functionalities on C-terminus were synthesized on a solid support to circumvent the solution-phase installation of leaving groups. Cleavage of the resin-bound peptides yielded the diol esters with sufficient purity. The diol-activated peptides were efficiently cyclized in a head-to-tail manner by SurE, a representative penicillin-binding protein-type thioesterase which we previously discovered in biosynthesis of non-ribosomal peptide, surugamides (Kuranga et al., 2018; Matsuda et al., 2019; 2020). Explorations of homologous wild type enzymes as well as rational protein engineering, have broadened the scope of the enzymatic macrolactamization (Kobayasi et al., 2023). This method will potentially accelerate the exploitation of NRPS-TEs as biocatalysts.

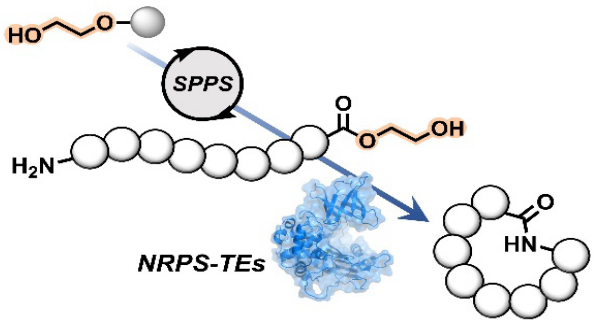

Fig. 1. Diol-activated peptides were synthesized by SPPS and cyclized by NRP cyclases.

Keywords: NRPS, cyclase, cyclic peptide, thioesterase, SPPS

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Enzymatic synthesis of human phase II metabolites of flavonoids and other (poly)phenols

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Standards of Phase II metabolites of flavonoids and other (poly)phenols, needed as standards to assess their bioavailability and biological activity, are generally not commercially available. Enzymatic biotransformation is a promising route for the synthesis of such metabolites, it offers advantages in terms of efficiency, selectivity and sustainability compared to conventional chemical methods. This contribution explores the applications of enzymes in the production of phase II metabolites including challenges such as substrate specificity, scalability, and optimization with particular focus on aryl sulfotransferases (ASTs). AST from *Desulfitobacterium hafniense* has been successfully used for the sulfation of various (poly)phenols (Valentová 2017, Valentová 2018, Káňová 2020, Kolaříková 2022, Petrásková 2022). New potential recombinant ASTs were then selected, produced in *E. coli*, purified, and characterized including kinetic parameters. Substrate specificity was tested using *p*-nitrophenyl sulfate and 4-methylumbelliferyl sulfate as donors and various flavonoids as acceptors, which differed mainly in the amount and positions of the -OH groups (chrysin, apigenin, luteolin, kaempferol, myricetin, quercetin and silydianin). The reactions were monitored using optimized HPLC methods. Enzymatic sulfation of polyphenols with bacterial ASTs is an effective method for the production of authentic metabolites.

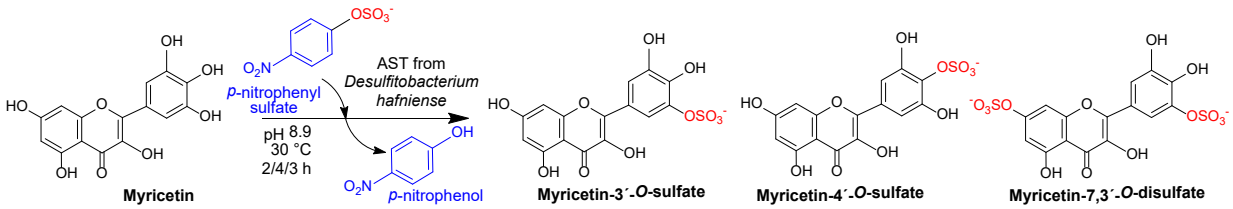

Fig. 1. Sulfation of myricetin

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Keywords: aryl sulfotransferase, sulfation, flavonoid, phenolic, metabolite

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Fungi – important biocatalysts in production of natural pigments and fragrances

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Due to the awareness of consumers regarding the origin of food additives, as well as the growing demand in the industry for natural pigments and fragrances, the development of an alternative method for their synthesis is currently explored (Maurya et al., 2022). Biotechnological methods constitute the most attractive solution as they are more sustainable and environmentally friendly processes with an emphasis on the prevention of waste generation and avoidance of hazardous compounds. Fungi, among the microbial populations, could be used as the most efficient biotechnology agents for the production of colorant and aroma compounds due to their tolerance, flexibility, and cheap requirements of growth. Microbial pigments, in contrary to plant-based pigments, show several advantages including the ease of scaling up and harvest as well as not subjected to the nature vagaries. Herein, the application of endophytic fungi as an efficient production platform for natural pigments will be discussed. A promising strain isolated from leaves of *Origanum majorana* was identified as *Monascus ruber* SRZ112 produced several types of pigments, mainly rubropunctamine, monascin, ankaflavin, rubropunctatin, and monascorubrin (El-Sayed et al., 2022). In the area of flavors and fragrances different biosynthesis and biotransformation methods involving whole fungal cells will be presented: filamentous fungi transformation of ferulic acid to vanillin using different agro-industrial by-products; Basidiomycete-mediated synthesis of anisaldehyde, vanillin, veratraldehyde and piperonal (Serra et al., 2024). Our research provide results with both high knowledge value and applicability of the developed fungal-based transformations in the synthesis of natural pigments and flavors.

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Keywords: fungi, *Monascus*, piperonal, vanillin, agro-industrial wastes

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First insights in the metabolic faith of paraconic acids from *Cetraria islandica*

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Cetraria islandica or Icelandic moss is a lichen employed in traditional European medicine to treat mainly respiratory diseases, but also some gastrointestinal complications such as gastric and duodenal ulcers, indigestion, diarrhoea, and dysentery. The activity is mainly related to the contained polysaccharides, but several other compounds can be detected in decoctions of the lichen thalli, representing a recommended formulation. Among depsides and depsidones, also paraconic acids (PA), with known *in vitro* pharmacological properties on the arachidonic acid pathway, can be detected up to 2.7 w/% of used lichen material, when quantified by LC-MS. Up to now, several PA have been identified from *C. islandica* including dihydroprotolichesterinic acid, roccellaric acid, protolichesterinic acid, lichesterinic acid **1** (Fig 1), and nephrosterinic acid (González et al., 2023; Fernandes et al., 2020).

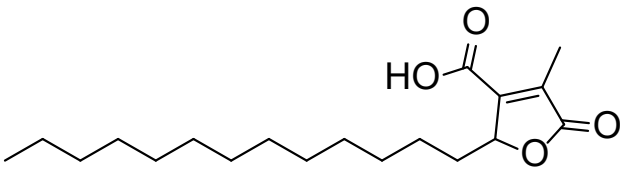

Fig. 1. Structure of lichesterinic acid **1**.

Thus, this project aimed to analyse if PAs could potentially penetrate into the gut tissue by analysing the permeation and absorption of lichesterinic acid through and into a Caco-2 monolayer. A weak to moderate absorption profile was indicated by analysis of the Caco-2 monolayer model results (time points 0, 6, 24, and 48 hours) using two concentrations (50 and 100 µM) of **1**, which prompted a thorough examination utilizing an *in vitro* gastrointestinal digesting model (GIDM). Due to the described antibacterial effects of PAs we also monitored the colon microbiota viability in the presence of PAs via quantification of colonies formed.

Keywords: paraconic acids, *C. islandica,* caco-2 monolayer, *in-vitro* gastrointestinal digestion model

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Biotransformation of favelines from *Cnidoscolus quercifolius* into a new bis-nor-diterpene with anti-melanoma potential

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C. quercifolius is a Brazilian medicinal plant, rich in bis-nor-diterpenes known as favelines. These molecules have a tricyclic benzocycloheptenone basic skeleton and showed significant cytotoxic activity against chemoresistant human melanoma cells (A2058 cell line). Phyllacanthone, its major compound, exhibited pro-apoptotic activity associated with tubulin depolymerization, resulting in cytoskeleton disruption (Oliveira-Junior et al., 2022). To design new anti-melanoma favelines, we performed biotransformation assays on *C. quercifolius* crude extract using 12 fungi and bacterial strains from the microorganism collection of NMNH (Paris, France). All biotransformation reactions were carried out in citrate buffer (pH 5.0), and the formation of new products was monitored by HPLC-DAD and/or UHPLC-DAD-HRMS/MS every 24 hours. A combinatorial approach allowed the selection of *Mortierella isabellina* as the active strain, resulting in a major biotransformation product after 24 hours of reaction with the crude extract. This compound was obtained from the supernatant by liquid-liquid extraction and from the biomass by ultrasound-assisted extraction, and then purified by preparative HPLC. After HRMS and 1D and 2D NMR experiments, the new compound was identified as a phyllacanthone derivative hydroxylated at C-12 and C-14. Analytical biotransformation assays with the major favelines isolated from the crude extract (phyllacanthone and isofavelol) led to the same biotransformation compound, indicating that they probably served as substrates for *M. isabellina* (Fig. 1). Finally, the cytotoxic activity of the new faveline was assessed on A2058 cells using the MTT method, resulting in an IC_{50} = 65.8 µM.

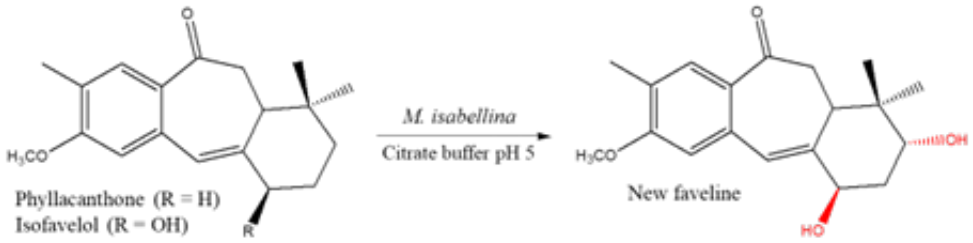

Fig. 1. Biotransformation of phyllacanthone by the fungus *M. isabellina* NRRL 1757.

Keywords: bioconversion, diterpene, Euphorbiaceae, melanoma, skin cancer

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Enterolactone and enterodiol produced by fermentation of Valerian root extract with human fecal samples

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Valeriana officinalis L. is commonly used to alleviate mild mental stress and sleep disorders. However, like in many herbal extracts, there remains a lack of scientific consensus regarding its active ingredients (Shinjyo et al., 2020). With the aim to investigate its potential gut microbiome-mediated biotransformation, we subjected valerian root extract to fermentation with human fecal microbiota from three healthy donors (24 h, anoxic, 37°C) (Pérez-Burillo et al., 2021). Annotation of the constituents present in the native preparation and of the metabolites formed during anaerobic fermentation was accomplished by UHPLC-HRMS. In the extract, lignans and caffeic acid derivatives were detectable as major constituents and valerenic acids, valepotriates and baldrinals as minor constituents. During fermentation with fecal samples, the phenylpropanoid derivatives were found to be readily catabolized by gut microbiota. The mammalian lignans enterolactone and enterodiol were found as the major metabolites in all donor samples. It is noteworthy that enterolactone was recently associated with lower prevalence of depressive symptoms (Cui et al., 2022) and sleep disorders (Sun et al., 2020). This indicates that the metabolic activation of *V. officinalis* constituents by the gut microbiota, leading to newly produced mammalian lignans, could contribute to the observed therapeutic effects of Valerian root preparations.

Conflicts of interest: The investigations and MEG have been funded by, and OK is fully employed by Steigerwald Arzneimittelwerk, Bayer Consumer Health.

Keywords: *Valeriana officinalis*, gut microbiota, biotransformation, enterodiol, enterolactone

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Biosynthesis of bicyclo[3.3.1]nonane derivatives in *Hypericum sampsonii*

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Polycyclic polyprenylated acylphloroglucinols (PPAPs) with bicyclo[3.3.1]nonane cores are medicinally intriguing compounds, which exist in two constitutions (Yang et al., 2018). Prominent examples are hyperforin (type A) and garcinol (type B). Both PPAP types are present in *Hypericum sampsonii* (Hypericaceae). This presentation will focus on the identification and characterization of a pair of bifunctional prenyltransferases (*Hs*CPTa, *Hs*CPTb), which catalyze regiodivergent prenylative cyclizations to yield the PPAP regiomers 7-*epi*-nemorosone and 7-*epi*-clusianone (Fig. 1; Ernst et al., revision submitted).

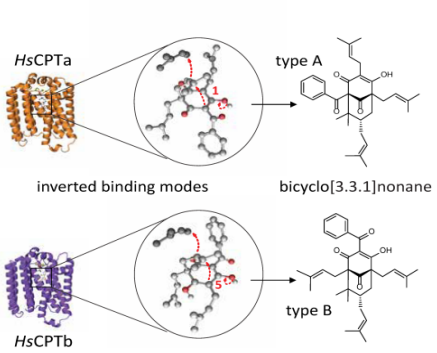

Fig. 1. Inverted substrate binding modes in *Hs*CPTa and *Hs*CPTb, yielding type A and B bicyclo[3.3.1]nonane scaffolds

Molecular modelling and docking experiments predicted inverted substrate binding modes in the active site cavities of the two enzymes. Reciprocal mutagenesis demonstrated that the regiospecific binding modes are interconvertible by ninefold mutation. Detailed structural elucidation of the enzymatic products confirmed *endo* configurations of the transferred C-7 prenyl groups, required for biosynthesis of caged adamantane-type PPAPs characteristic of *H. sampsonii* (Hu et al., 2000).

Keywords: biosynthesis, acylphloroglucinols, *Hypericum sampsonii,* prenyltransferases

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Unravelling the biosynthesis of phenylphenalenones: insights from metabolites and enzymes in *Musella lasiocarpa*

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Phenylphenalenones (PPs) are complex polycyclic phytoalexins found in banana and plantain (Musaceae) plants. Previous studies have linked the disease susceptibility of commercial banana cultivars to low levels of PPs compared to disease-resistant wild bananas (Krishnamurthy et al., 2023). However, how plants synthesize PPs is still poorly understood.

Fig. 1. Characterized transformations to phenylphenalenone in *Musella lasiocarpa* (Musaceae).

We find that PPs are the major secondary metabolites of mature seeds of *M. lasiocarpa* and that seeds can be a model system to study PP biosynthesis (Lyu et al., 2023). Using transcriptomic data from *M. lasiocarpa* seeds at different developmental stages, we identified three biosynthetic genes involved in the formation of dihydrobisdemethoxycurcumin (Lyu et al., under review). Furthermore, through multiple rounds of feeding potential intermediates to *M. lasiocarpa* root protein extracts, we demonstrate the stepwise conversion of dihydrobisde-methoxycurcumin to the PP 4'hydroxylachnanthocarpone (Fig. 1). The discovery of a monocyclic diarylheptanoid as an unexpected PP intermediate opens a two-step cyclization route to the phenylphenalenone scaffold which contrasts the commonly hypothesized Diels–Alder mechanism.

Keywords: biosynthesis, phenylphenalenones, diarylheptanoids, Musaceae, natural products

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A synthetic biology platform for refactoring and combinatorial biosynthesis of anthracyclines

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Aromatic polyketides produced predominantly by actinobacteria typically exhibit antimicrobial, anticancer, and immunosuppressant activities (Hulst et al., 2022). Aromatic polyketides, such as antitumor compound doxorubicin, are biosynthesized by type II polyketide synthases (PKS), which carry out the iterative condensation of acyl-CoA precursors. Doxorubicin is a broad-spectrum anticancer agent used in the clinic. However, its use is diminished by cardiotoxicity. New investigations into cardiotoxicity-free doxorubicins, which do not induce DNA strand breakage, have reinvigorated interest in this class of natural products (Hulst et al., 2022). We have employed a biosynthetic approach to complement the synthetic studies recently reported. Developing standardized genetic tools to facilitate true "plug and play" experimentation could further enhance the combinatorial biosynthesis of these pathways. Here we present a metabolic engineering platform that allows rapid generation of anthracyclines for bioactivity testing [3]. Our modular BioBricks-based design architecture for (i) aglycone biosynthesis, (ii) tailoring steps, (iii) TDP-carbohydrate biosynthesis and (iv) resistance and carbohydrate transfer allows facile construction of any desired combination from existing genetic parts. Simultaneous development of a stable host chasses *S. coelicolor* M1152*ΔmatAB* and a multi-plasmid gene expression system has facilitated robust production of the natural products. Current progress on the iterative engineering of anthracycline pathways will be discussed (Nguyen et al., 2022, Wang et al., 2022, Tirkkonen et al., 2023).

Keywords: synthetic biology, actinomycetes, BioBricks, polyketide synthase, biosynthesis

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Discovery of new bacterial sesterterpene synthases and their products

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While terpenes remain the largest family of natural products, their structural diversity is what sets them apart from other families of natural products and confers a vast array of different biological activities. Sesterterpenes, made up of C25 products, remains a relatively understudied subfamily that accounts for approximately 1,000 of the over 80,000 known terpenes, and even more staggering is that less than 25 of these compounds were isolated from bacteria (Rudolf et al., 2021). Here we describe a terpene production system in *E. coli* that we have developed to screen bacterial sesterterpene synthases and the isolation of their C25 products. Our work here will help to characterize new bacterial sesterterpene synthases, isolate and elucidate the structures of new sesterterpenes.

Keywords: sesterterpenes, terpenoids, terpene synthases, biosynthesis, enzymes

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Colletofragarone A2 from the fungus *Colletotrichum* sp. promoted the degradation and aggregation of mutant p53

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The tumour suppressor p53 regulates the transcription of genes for apoptosis, cell cycle arrest, and senescence. p53 are mutated in more than 50% of tumours, and mutant p53 aggregates in cells to promote cancer progression and lead to the loss of its tumour suppressor function. Therefore, compounds targeting mutant p53 are promising for cancer therapy and we search for compounds that show functional recovery of mutant p53 to wild-type like p53. Recently, we isolated colletofragarone A2 (1) and three new compounds colletoins A (2)–C (Fig. 1) from an endophytic fungus *Colletotrichum* sp. (Sadahiro et al., 2021). Compound 1 showed cytotoxicity with an IC_{50} value of 0.18 *m*M against SK-BR-3 ($p53^{R175H}$) cells and decreased tumour cell growth *in vivo* using xenograft model mice with HeCCT1 ($p53^{R175H}$) cells (Sadahiro et al., 2022). Compound 1 decreased mutant p53 levels in the cells, induced the degradation of mutant p53 by the proteasome, and promoted the accumulation of aggregated mutant p53 in the cells.

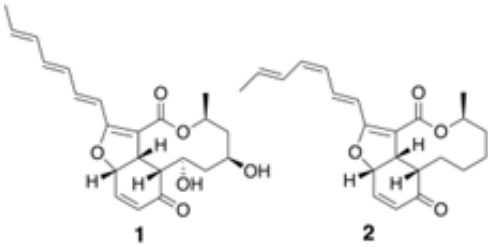

Fig. 1. Structures of colletofragarone A2 (1) and colletoin A (2).

Keywords: *Colletotrichum* sp., mutant p53, cancer

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Novel thiosugar sulfoniums, salacinol and neokotalanol, with antidiabetic activity obtained from plants of genus *Salacia*

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During our studies characterizing functional substances from food resources for the prevention and treatment of lifestyle diseases, we isolated the active constituents, salacinol (1) and neokotalanol (2), and related thiosugar sulfoniums, from the roots and stems of the genus *Salacia* plants (Hippocrateaceae) such as *Salacia reticulata* Wight, *S. oblonga* Wall., and *S. chinensis* L., and observed their antidiabetic effects (Fig. 1) (Yoshikawa et al., 2002; Muraoka et al., 2011). These plant materials have been used traditionally in Ayurvedic medicine as a specific remedy at the early stage of diabetes, and have been extensively consumed in Japan, the United States, and other countries as a food supplement for the prevention of obesity and diabetes (Morikawa et al., 2021). Here, I would like to present our studies on the antidiabetic effects of plants from the genus *Salacia*, from basic chemical and pharmacological research to their application and development as new functional food ingredients (Morikawa, 2023).

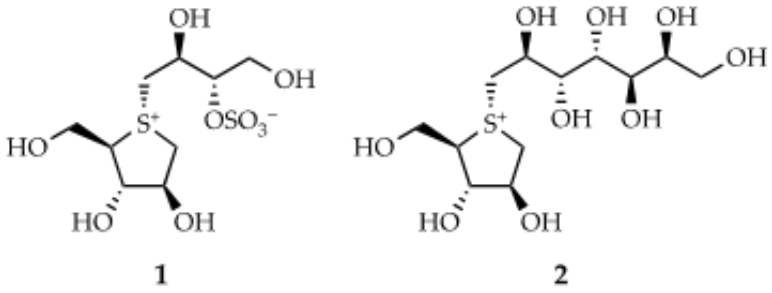

Fig. 1. Structures of salacinol (1) and neokotalanol (2).

Keywords: *Salacia,* salacinol, neokotalanol, a-glucosidase inhibitor, diabetes

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3-epi-18β-Glycyrrhetinic acid and its 30-O-glucuronide involves the development of licorice-induced pseudoaldosteronism in humans with individual differences

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Licorice is a crude drug used in traditional Japanese Kampo medicine and is also used as a sweetener. It occasionally causes pseudoaldosteronism (PsA) as a side effect. Its major symptoms are hypokalemia, hypertension, edema, and low plasma aldosterone levels. PsA is thought to be caused by some metabolites of glycyrrhizic acid (GL), a component of licorice. The development of PsA varies greatly among individuals, but the factors that determine the individual differences are still unknown. Overall, 78 patients who had taken Kampo medicines containing licorice were enrolled, and we obtained laboratory data including their serum potassium levels, plasma aldosterone concentrations, and residual blood and/or urine samples. Concentrations of GL metabolites in serum and urine samples were measured by LC-MS/MS. In the serum samples of the 78 participants, 18β-glycyrrhetinic acid (GA) was detected in 65 cases, 3-epi-GA in 47 cases, 3-oxo-GA in 63 cases, 18β-glycyrrhetinyl-30-O-glucuronide (GA30G) in 62 cases, and 3-epi-GA30G in 3 cases. Among 29 urine samples obtained, GA30G in 27 cases, and 3-epi-GA30G in 19 cases. 3-epi-GA30G was a new GL metabolite, and the finding of 3-epi-GA, 3-oxo-GA, and 3-epi-GA30G in human samples was the first time. The appearances of 3-epi-GA in serum and 3-epi-GA30G in urine had high individual differences, and their concentrations significantly correlated with serum PsA markers. 3-epi-GA, 3-oxo-GA, GA30G, and 3-epi-GA30G inhibited human 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) almost similar titers. These findings suggest that 3-epi-GA and/or 3-epi-GA30G would be associated with the cause of individual differences in the development of pseudoaldosteronism.

Keywords: licorice, pseudoaldosteronism, 3-epi-18β-glycyrrhetinic acid

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Amway-Nutrilite

Roses and buds, in the current state of botanical dietary supplements – a global perspective

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This oral session is dedicated to the 90th anniversary of the Nutrilite Dietary Supplement Brand from Amway. Nutrilite™ is one of the leaders in the dietary supplement industry in the world. This session will cover topics from pharmacognosy, and emerging technology related to consumers' health and wellbeing in the supplement industry. The session panel comprises of leaders in industry from Asia, Europe, and United States. ROSES represents the strengths and all the value-added developments that are currently happening and where this sector is headed in the future. BUDS are the emerging areas that can be seen as opportunities to improve or build on to convert into roses (evolution or continuous improvement). Topics such as botanical quality, efficacy, botanical authenticity, future of traditional medicine, AI-linked precision nutrition, sustainability, traceability, integrity, and impact of extreme weather will be covered in detail. A message to cross-pollinate the science of pharmacognosy with botanical ingredients and phytonutrients in commercial supplement products will be emphasized. For ICNPR (attendees and organization) it will be a unique opportunity to take a leadership role in industry and partner with industry cohorts for the enhancement of quality, safety, and efficacy of botanical products on a global stage. This session will have the speaker panel deliver individual talks followed by a moderated panel discussion and ending with an interactive discussion between panel and audience.

Keywords: botanicals, ai-nutrition, sustainability, ESCOP, traditional herbals, safety, quality, east and west

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Society Session: GA

Welcome address

Planta Medica Best Paper Award and PM Special Issue „Celebrating Austrian Pharmacognosy"

W. Schwabe Young Talent Price and Willmar Schwabe Award

Dr Cherry Lifeng Li, Baptist University, Hong Kong

GA Synergy – Award

Prof. Andreas Bender, University of Cambridge, United Kingdom

E. Stahl Bronze – Award

Dr Valentina Parisi, University of Salerno, Italy

Session I: Medicinal natural products: from bench to bedside

Topical Lecture

Will artificial intelligence become a game changer in natural product drug discovery?

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Nature has historically proven to deliver the smartest chemical motifs for drug discovery. Despite tremendous advances in instrumentation technologies, the exploration of natural sources is challenging. It requires not only deep knowledge and sophisticated technical equipment for getting natural compounds isolated, identified, and pharmacologically characterized; also aspects of sustainable supply and property rights of the underlying natural material have to be considered. On the other hand, already millions of data on physicochemical and biological properties of natural as well as synthetic compounds are in the public domain, which can be utilized by AI to streamline the experimental efforts for identifying underexplored natural hit compounds. Special care must be taken on the reliability of data and the AI method most suitable for the underlying research question. Here, I will present and compare some recently developed AI strategies implemented in natural product drug discovery workflows, which can be used to harness the advances in computational techniques for taking advantage from the huge amount of information available in the public domain. This ranges from virtual screening for hit identification, machine learning tools, molecular networking to a previously developed HNMR-MS-based biochemometric approach (Grienke, 2019). The effectiveness, strengths and limitations of the presented strategies will be demonstrated on most recent application examples for the identification of novel natural lead structures (Kirchweger, 2022; Langeder, 2023; Wasilewicz, 2023, 2024; Zwirchmayr 2023).

Funding: Wilhelm-Doerenkamp-Foundation, Switzerland (NATVANTAGE Grant 2018); Austrian Science Fund (FWF P34028)

Keywords: cheminformatics, biochemometry, molecular networking, SARS-CoV-2, traditional medicine

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Exploring lipidomic changes: the impact of St. John's wort extract ze 117 and escitalopram in a rat model of depression induced by chronic stress

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Chronic stress plays a pivotal role in depression development, triggering hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis and subsequent elevation of glucocorticoids (GC) (Kim et al., 2022). Persistently heightened GC levels disrupt neuroplasticity and impact brain lipid metabolism (Pinto et al., 2022). This study utilized repeated corticosterone injections to induce depression-like behavior in male Sprague-Dawley rats. Rats were subjected to corticosterone injections (40 mg/kg, s.c.) for 22 consecutive days, co-administered with varying doses of the St. John's wort extract Ze 117 (30, 90, or 180 mg/kg, p.o.) or escitalopram (10 mg/kg, p.o.). Behavioral changes were assessed using a modified forced-swim test (FST) (Johnson et al., 2006). Corticosterone injections significantly reduced the latency to first immobility. Co-treatment with Ze 117 dose-dependently and significantly increased this latency to first immobility compared to corticosterone-only treated rats ($p<0.001$). The lipidome of plasma and hippocampus samples was analyzed by shotgun mass spectrometry to explore the biochemical effects of corticosterone-induced stress and potential counter-regulation by antidepressants. Corticosterone-induced stress altered key lipid metabolites in plasma, while hippocampal samples exhibited changes primarily in glycerophospholipids, such as dose-dependent increases in lysophosphatidylethanolamines (LPEs) induced by Ze 117. These changes correlated significantly with the latency to first immobility. In conclusion, this study unveils significant behavioural and lipidome-modulating effects of Ze 117 and escitalopram in rat plasma and hippocampus, offering novel targets for clinical diagnosis and antidepressant intervention.

Keywords: *Hypericum perforatum,* escitalopram, chronic stress, forced swim test, lipidomics

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Proton spin network fingerprints for dereplication, pharmacophore identification and standardization of the botanical supplement *Centella asiatica*

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Centella asiatica (Apiaceae) is a botanical dietary supplement that is used in Asia, Madagascar, and the United States to provide resilience to age related cognitive decline. This property has been supported by preclinical evidence including our work on NRF2 activation in HepG2-ARE cells and increased expression of the antioxidant response gene Nrf2 *in vivo*. Similar to many botanical dietary supplements, lack of consistency of *Centella asiatica* (CA) formulations limits the validation of its medicinal benefits in clinical studies. Efforts have been made to develop liquid chromatography coupled with mass spectrometry (LC-MS) techniques to detect, dereplicate and characterize metabolites in botanicals using different refinement of ionization techniques and mass libraries. For *C. asiatica*, comprehensive identification of chemical constituents present in active samples is still necessary to complement standardization and quality control efforts by populating mass libraries. Bioactivity has previously been associated with CA's triterpene and caffeoylquinic acid (CQA) and new CQAs are being identified. Our laboratory focuses on development of a method that helps identify proton spin network fingerprint (SNF) of CA metabolites that bear pharmacophores. After being identified, encrypted, and stored in an in-house database, proton SNFs are coupled with mass spectrometric data to accurately identify isomeric metabolites such as CQAs. This presentation will focus on the process of identification of pharmacophore containing chemical markers of *C. asiatica* provided by BENFRA center, by SNF coupled with two-dimensional NMR experiment as well as LC-MS spectrometric analyses. Application of SNF-coupled LC-MS in standardization and quality control will be also discussed.

Keywords: dietary supplement, *Centella asiatica*, proton spin network, LC-MS, standardization

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Drug discovery pipeline reveals ellagic acid to reduce forgetting in *Caenorhabditis elegans* through specific Musashi inhibition

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Memory maintenance and forgetting are fundamental processes in our lives. However, increased forgetting due to aging or neurodegenerative conditions (e.g. Alzheimer`s disease) dramatically impairs the life quality of millions of people worldwide. A recently discovered protein called Musashi (MSI) has emerged as a crucial player in promoting forgetting (Hadziselimovic et al., 2014). The fact that no efficient treatment for age- or disease-related forgetfulness is available, prompted us to search for new natural product-based MSI inhibitors. Our drug discovery pipeline included the screening of an in-house plant extract library (2576 extracts) with a newly developed MSI inhibitor assay, followed by HPLC-based activity profiling to localize the active compounds in these extracts. Targeted isolation of the active constituents of the *Freziera candicans* Tul. (Pentaphylacaceae) extract has elucidated flavonol glycosides and ellagitannins. While flavonols inhibited MSI at low micromolar concentrations, the series of ellagitannins demonstrated a strong structure-activity relationship, revealing ellagic acid (EA) as by far the most potent MSI inhibitor in the low nanomolar range. EA was further tested in an associative learning and memory experiment in *Caenorhabditis elegans* (*C. elegans*) and remarkably led to a concentration-dependent reduction of forgetting in short- and long-term associative memory tests. Further *in vivo* experiments confirmed EA to act specifically via MSI inhibition. EA was more potent *in vitro* and *in vivo* than the previously published (-)-gossypol (Mastrandreas et al., 2023). Additionally, results on the role of EA in the rescue of Alzheimer symptoms in a *C. elegans* model mediated through MSI inhibition will be presented.

Keywords: memory & forgetting, ellagic acid, Musashi inhibitors, *C. elegans*, Alzheimer disease

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Anti-inflammatory and cytoprotective polypharmacology of BNO 2103 reveals targeting of IKK-NF-κB and p38-MK2-RIPK1 axes

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Urinary tract infections (UTIs) are among the most frequent infections, affecting approximately 150 million patients per year worldwide. Associated inflammation and tissue damage contribute significantly to symptoms, e.g., dysuria and urge. Canephron, an herbal medicinal product with anti-inflammatory (Künstle at al., 2013, Nausch et al., 2019), spasmolytic (Brenneis et al., 2012), anti-adhesive (Künstle at al., 2013), and anti-nociceptive (Nausch et al., 2019) activities, is approved for treatment of uncomplicated UTIs. To investigate cytoprotective and anti-inflammatory properties of the active component of Canephron N (BNO 2013: mixture of pulverized rosemary leaves, centaury herb, and lovage root), and underlying signaling mechanisms, a newly established NF-kB-reporter kidney epithelial (HK-2) cell line, luminescent assays (NF-kB activity), Western blots (signaling pathways and protein phosphorylation), and multiplex-immunoassays (Luminex® xMAP® technology, cytokine release) were used. BNO 2103 repressed NF-kB activation (IC_{50} = 241.1 µg/mL) by *lipopolysaccharide* (LPS) and tumor necrosis factor alpha (TNFα), inhibiting the phosphorylation and degradation of IkB±. BNO 2103 also suppressed inflammation-specific S536 phosphorylation of p65 (NF-kB protein) and transcription of pro-inflammatory cytokine genes. In contrast to other, specific NF-kB inhibitors, BNO 2103 revealed a strong cytoprotective effect against TNF±-induced cytotoxicity. Our data indicate that BNO 2103 acts through the p38 MAPK/MK2 axis, promoting RIPK1 phosphorylation at S320 while repressing S166 autophosphorylation and subsequent activation of RIPK1, which is required for apoptotic and necroptotic responses to TNFα. According to our findings, Canephron exhibits anti-inflammatory and cytoprotective effects mediated by a well-defined and highly specific mechanism.

Keywords: urinary tract, inflammation, NF-κB, cytoprotection, herbal medicinal product

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Verification of the medicinal value of Patriniae Herba for colon cancer using preclinical models

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Patriniae Herba, referring to the perennial herbs such as *Patrinia villosa* (Thunb.) Juss. and *Patrinia scabiosaefolia* Link (family Valerianaceae), is a folk medicine for treating gastrointestinal disorders over thousands of years in China. With the anti-tumor activities of *P. villosa* (PV) previously reported (He et al., 2017), our recent studies aimed to elucidate the underlying mechanism of the anti-tumor effects of PV aqueous extract in colon cancer using preclinical models. In order to obtain the genuine herbal material of PV, our research team established a systematic authentication procedure which includes morphology, chemical characterization and DNA markers (Wong et al., 2024). The PV aqueous extract (PVW) was prepared, chemically characterised and used for biological evaluation in colon cancer cell-based and animal studies. Our results showed that PVW exhibited cytotoxicity, apoptosis, cell cycle arrest and inhibitory effects on cell motility and migration in human and murine colon cancer cells via regulating ErbB, focal adhesion, and TGF-β signaling pathways. Furthermore, PVW suppressed tumor growth and metastasis in tumor-bearing mice via modulating the levels of immune cells (cytotoxic T cells and MDSCs) (Yang et al., 2023a). Besides, the compound 8,9-didehydro-7- hydroxydolichodial was firstly proven to be present in PVW and was shown to suppress colon cancer cells migration via increasing expression of tumor suppressor p53 (Yang et al., 2023b), thus it could be considered as an active component.

In conclusion, our findings scientifically verified the therapeutic value of the herb *Patrinia villosa* and provided evidences to support the clinical use in colon cancer patients.

Keywords: *Patrinia villosa,* Valerianaceae, Patriniae herba, authentication, colon cancer

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Symphytum genus: novel insights into its phytochemistry and bioactivity

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With around 34 recognized species, the *Symphytum* genus (comfrey), Boraginaceae, has been traditionally used for its anti-inflammatory properties in treating musculoskeletal disorders (Trifan et al., 2020). This multi-methodological study aimed to i) map comfrey species' metabolic complexity using state-of-the-art spectro-chromatographic techniques; ii) isolate main phenolic markers by liquid-liquid chromatography (LLC); iii) assess their anti-inflammatory potential in lipopolysaccharide (LPS)-stimulated human neutrophils; iv) remediate pyrrolizidine alkaloids (PAs) from comfrey extracts using a polymer-based resin. LC-HRMS/MS-based metabolite profiling of roots and aerial parts of underutilized *Symphytum* species identified numerous phenolic acids, flavonoids, PAs, and organic and fatty acids (Luca et al., 2024). Four caffeic acid oligomers (rosmarinic acid, rabdosiin, globoidans A and B) were isolated from *S. officinale* (comfrey) roots following a LLC approach. The four compounds significantly inhibited IL-1β release in LPS-stimulated neutrophils, contributing to comfrey's overall anti-inflammatory activity (Trifan et al., 2020). Additionally, a survey across European countries identified these phenolics as phytochemical markers in comfrey roots (Trifan et al., 2021). Lastly, in light of PAs-induced toxicity, a polymer-based resin depletion procedure was employed for their removal from comfrey extracts. PA depletion did not alter the profile of the main phenolic markers while reducing PAs to below 2 ppm. Moreover, PA removal did not affect the inhibitory effects of comfrey extracts on the release of IL-1β, IL-8, and TNF-α in LPS-stimulated neutrophils (Trifan et al., 2023). Thus, the *Symphytum* genus shows promise for drug development, with PA-depleted extracts being considered for cosmetic and pharmaceutical products.

Keywords: comfrey, Boraginaceae, pyrrolizidine alkaloids, anti-inflammatory

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Insight into the mechanism of anti-migraine activity of butterbur (*Petasites hybridus* L.)

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Petasites hybridus (L.) Gaertn., B. Mey. & Scherb (Fig. 1) is a plant of Asteraceae genus. It is the most common butterbur species, which is used in migraine treatment as a clinically approved drug (Petadolex®) (Kulinowski et al., 2022). This presentation is intended to shed a light on mechanism of action of *P. hybridus* extracts and pure compounds in the treatment of migraine. The effectiveness of *P. hybridus* rootstocks extracts is proved in many clinical trials, but the side and mode of action are not fully understood. We will cover the recent reports regarding mode of anti-migraine activity of petasins, which are considering the active components of butterbur extracts.

Fig. 1. *Petasites hybridus* (L.) Gaertn., B. Mey. & Scherb

We will also discuss the pathophysiology and theories of the development of migraine pain, including vascular, neural, trigeminovascular, and the newest molecular theories. We will then consider the effects of butterbur and petasins in the context of those theories, according to recent research on its effects on e.g. TRPA1 and TRPV1 ion channels (Benemei et al., 2017), calcitonin-gene related peptide (CGRP) activity (Kleeberg-Hartmann et al., 2021), and inflammatory mediators (Arnold et al., 2015; Fiebich et al., 2005). In the end we will identify gaps and set the stage for the future research of the activity of butterbur in migraine.

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Keywords: *Petasites hybridus,* butterbur, petasins, anti-migraine, TRP channels, CGRP

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Study of the prebiotic potential of *Althaea officinalis* L. root extract using the high throughput *ex vivo* technology SIFR®

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The extract of *Althaea officinalis* L. root is used in irritations of the pharyngeal mucosa and resulting dry cough, but also in gastrointestinal complaints. While the underlying mucosa protective and anti-inflammatory effects have been studied thoroughly over the last years, data on ist effect on the gut microbiota are missing. Given that the extract constituents can be assumed to be not fully absorbed in the small intestine, its influence on the gut microbiota in the large intestine was intended to be studied. The impact of an extract (STW 42, AR) on the gut microbiome was investigated using the high-throughput, *ex vivo* SIFR® technology (Cyptobiotix, Ghent, Belgium). Considering six subjects individually covered the broad spectrum of microbial compositions that occurs *in vivo*, in line with the so-called enterotypes, thus ensuring representative findings. Simulating a single intake, AR was dosed at 1.875 g/d and inulin (IN; used as positive control) was dosed at equivalent of 2 g/day. AR significantly increased total SCFA and significantly decreased pH. While the extent of the effects was lower than with IN, health-related propionate/butyrate was significantly increased, showing a specific effect on butyrate production, which suggests a product-specific impact on the microbiome. Potent treatment effects were noted at higher phylogenetic resolution (OTU level): AR exerted effects including the consistent stimulation of an OTU related to Ruminiclostridium cellulolyticum. Overall, the SIFR® study demonstrated the prebiotic potential of marshmallow root extract (AR) by its influence on microbial metabolite production and microbial composition.

Keywords: *Althaea officinalis,* gut microbiota, cough and cold, gastrointestinal complaints

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Development of bio-based active materials for textiles and high-traffic objects

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Infectious diseases pose a significant threat to both human health and the global economy, they account for more than 20% of global mortality and viruses are responsible for about one-third of these deaths. To date, we know of about 200 infectious diseases and about 80% of infectious diseases are transmitted by unclean hands touching contaminated surfaces. SUSAAN project is focused on development of new sustainable antiviral and antimicrobial coatings for textiles and high traffic objects made of plastics and metal, involving textile, bathrooms and switches manufacture industries. Rhubarb and horseradish (root and leaves) were both studied within this project due to their rich composition in anthraquinones and isothiocyanate, respectively. Produced extracts were purified and characterized by chromatography (e.g. UPLC-DAD-MS for anthraquinones, GC-MS for isothiocyanates) and their antimicrobial activity was studied against Gram-negative *Escherichia coli* bacteria and Gram-positive *Staphylococcus aureus* bacteria. The obtained antibacterial activities showed that the bio-based extracts exhibit good bacteriostatic activity but are not bactericidal. Therefore, these plant extracts have the potential to be used for the development of bio-based active nanomaterials.

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Keywords: Active biomaterial, natural products, infectious diseases, antimicrobial efficiency

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Macroalgae derived natural product scavengers of reactive aldehyde and carbonyl species and their implications in slowing the progression of prevalent chronic diseases

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Reactive Aldehyde and Carbonyl Species (RACS) arise from the degradation of essential primary metabolites—sugars and lipids. The resulting stress, derived from their ability to spontaneously react with proteins, DNA, and monoamine neurotransmitters, is a fundamental biological pressure that has driven the evolution of diverse mechanisms for minimizing its impact on organismal health. Relevant to human health, the complications that accompany metabolic disorders, such as diabetes, illustrate what happens when RACS stress exceeds the body's capacity to prevent excessive damage. These include elevated risks for cardiovascular disease, liver disease, kidney disease, neurodegeneration, and cancer, which are accompanied by a heightened vulnerability to infections—illustrating the broad need for targeted interventions. Presented here is a novel quantitative NMR assay for evaluating and screening natural products to discover scavengers of a prominent RACS, methylglyoxal. This tool was employed to explore the chemical diversity of marine macroalgae, with a focus on species that have a history in the diets of the World's healthiest populations and are subject to production by aquaculture. The results revealed the exceptional RACS scavenging capacity and AGE formation inhibition of phlorotannins, a class of molecules abundantly produced by Phaeophytes. Likewise, the guanylureas an unusual class of alkaloids found in Rhodophytes, were found to possess similar activity to the diabetes front line therapy, metformin—evoking questions about their impact on the longevity of populations that regularly consume them. Both illustrate the potential for macroalgal natural products to displace or outright replace widely used drugs and supplements.

Keywords: macroalgae, aquaculture, degenerative disease, aldehydes, diabetes

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Mitochondrial modulators: the defenders

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Mitochondria, commonly known as the cell's energy core, are increasingly becoming attractive targets in the search for potent therapeutics against neurodegenerative diseases. Impaired mitochondrial function has been extensively cited as a common hallmark in the pathogenesis of several neurodegenerative diseases, especially Parkinson's disease (PD) (Annesley and Fisher, 2019; Makinde et al. 2023). Given the limitations associated with available treatments for mitochondrial dysfunction-related ailments, the search for new, potent alternative therapeutics has become imperative. In this work, we present the screening of 4224 fractions obtained from 384 Australian marine organisms and plant samples using a modified lead-like fractionation protocol to search for natural products with mitoprotective properties. Primary screening of the fractions using PD patient-sourced olfactory neurosphere-derived (hONS) cells with rotenone as a mitochondria stressor revealed 108 hits from 11 different biota. The potency of these hits was validated in another round of primary screening using human neuroblastoma (SH-SY5Y) cells with 6-hydroxydopamine as a mitochondria stressor. This was followed by a mitochondrial membrane potential assay, resulting in 35 active fractions from 8 biotas. The most active fractions were subjected to LC-Orbitrap MS analysis to identify constituent natural products. A total of 56 natural products were identified, 12 of which have been previously reported to have significant mitoprotective activity. Finally, we also report the isolation, characterization, and testing of metabolites from the top 3 active biotas, leading to the isolation of a new dimeric aaptamine compound **1** (Fig. 1)

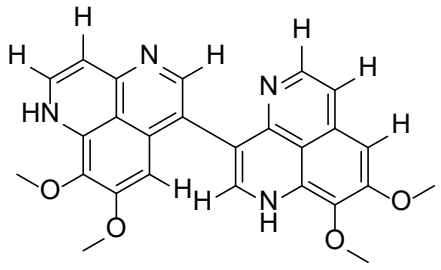

Fig. 1. Structure of a new dimeric aaptamine compound

Keywords: mitochondria, *Aaptos,* aaptamine, Parkinson, Neurodegenerative

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Bioassay-guided fractionation, identification and docking experiments of α-glucosidase inhibitors from *Geum kokanicum* root

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Geum kokanicum Regel & Schmalh. ex Regel (Rosaceae) is a perennial herb used as a natural remedy for gastric dysfunctions such as diarrhea, dysentery, dyspepsia, and gastroenteritis. Despite some studies reporting the anti-diabetic activity of this plant, bioactive compounds have not been well identified. The present study was designed to isolate bioactive constituents from the root of *Geum kokanicum* with anti-diabetic properties (Farzaneh et al, 2022). The α-glucosidase inhibitory effect of various extracts (chloroform, ethyl-acetate, methanol) were investigated. Regarding a bio-assay guided procedure, the active compounds were isolated using different chromatography methods. The structure of the pure compounds were elucidated by analytical techniques and their IC_{50} values were determined for α-glucosidase inhibition. In addition, a molecular docking study was used to evaluate the candidates for *α*-glucosidase inhibitory activity. Furthermore, 3, 3′, 4′-tri-O-methylellagic acid **(1),** daucosterol (**2**) from the chloroform and (+) - catechin (**3**), casuarinin (**4**) and ellagic acid (**5**) from the ethyl-acetate fraction were identified. Evaluation of the compounds **1**–**5** toward α-glucosidase revealed that compounds **4**, **5** and **1** were the most potent and selective inhibitors of α-glucosidase enzyme with the IC_{50} values of, 1.01 ± 0.30, 30.00 ± 1.10 and 94.00 ± 0.21 μM, respectively. Docking study was in harmony with *in-vitro* α-glucosidase inhibitory also revealed that compound **4** presented the lowest docking binding energy (-12.4 kcal/mol) by forming several strong hydrogen bonds in the active site of the α-glucosidase. The results of this study demonstrated the role of phenolic compounds on anti-dabetic activity of *Geum kokanicum* root.

Keywords: anti-diabetes, α-glucosidase, *Geum kokanicum*, casuarinin, ellagic acid

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Session II: Natural product research and the challenges of climate change – ecological and environmental solutions

Topical Lecture

Marine chemical ecology and natural products research in our changing ocean

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Macroalgae and benthic cyanobacteria are becoming increasingly abundant on coral reefs worldwide. The die-off of the sea urchin *Diadema antillarum* in Caribbean waters in the 1980s coincided with a dramatic increase in macroalgal biomass on coral reefs. More recently, marine heat waves are also contributing to increased macroalgal and cyanobacterial abundance on reefs. Algal natural products play important roles as chemical defenses against grazers such as sea urchins and herbivorous fishes. These compounds may also play a role in algal competition with corals and other benthic organisms. Experiments were designed to test interactions between chemically defended species of algae and benthic cyanobacteria and different life history stages of corals. Some extracts and isolated compounds from macroalgae and cyanobacteria negatively influenced the settlement of coral larvae. On reefs experiencing increased abundance of chemically defended macroalgae and benthic cyanobacteria, the rebuilding of coral populations may be inhibited by algal natural products. These chemical interactions are taking place under changing ocean conditions where increases in temperature and carbon dioxide concentrations are leading to changes in pH (ocean acidification) and warming. In contrast, some marine red algae (crustose coralline algae) and bacteria can induce settlement and metamorphosis of coral larvae. Studies of these settlement cues may lead to the development of compounds that are useful in coral restoration. Changing ocean conditions have also contributed to increased incidence of marine diseases, coral bleaching and hypoxia. How compounds in microbes and macroorganisms respond to these changing ocean conditions is an area of ongoing research interest.

Keywords: chemical defense, black band disease, stony coral tissue loss disease, coral settlement, climate change

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Search for anti-phytopathogenic compounds by studying biotic interactions between *Fusarium* sp. and the endophytic fungi of the palm tree model *Astrocoryum sciophilum*

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Biopesticides from living microorganisms or compounds from natural substances are used for biological control as alternative pest management. In order to find microorganisms that naturally possess weapons against phytopathogens, we investigate the biological and chemical diversity of plant leaf endophytes. Endophytes are microorganisms living in association with plants and can improve host plants resistance against biotic stresses, especially phytopathogens aggressions (Arnold et al. 2003). The palm *Astrocaryum sciophilum* is the host plant model chosen in this work. Due to the longevity and the easy dating of its leaves (Charles-Dominique at el., 2003), we expect to find a highly competitive and diverse microbial community. 197 endophytes have been isolated from eight palm specimens. Firstly, we selected a population of 42 fungi species from the *Colletotrichum* genus due to their extracts exhibiting biological activities relevant to their role within our macroscopic host. Strains were classified using MALDI-ToF-MS lipid and protein fingerprinting along with chemotaxonomic network analysis. Using protein extracts, rapid chemotaxonomic classification correlated distinct chemotypes with observed biological activities. The molecules accountable for the observed activities across different chemotypes were annotated, isolated, and characterized (Barthélemy et al., 2020). Secondly, we focus on the chemical study of a *Xylaria* sp. strain, whose extract exhibited activity against *F. oxysporum*. Through comparison of the LC-MS/MS profiles of the extracts and employing molecular networking, we identified metabolites from both strains whose production is induced by competition. Additionally, we observed the biosynthesis of these compounds over time during competition using MS imaging (Barthélemy et al., 2021).

Keywords: endophyte, competition, phytopathogen, metabolites

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Using natural, greener, biodegradable and recyclable solvents to enhance lignin extraction from Acacia wood

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The increasing interest in sustainable materials has motivated the adoption of a circular economy approach, where residues are treated as useful resources to produce new materials of added value. In this respect, lignin has gained significant attention as an alternative renewable feedstock due to its abundant availability and broad range of interest and applications. However, its extraction from biomass is a challenging task given its complex structure. Among the different extraction methods available, deep eutectic solvents (DES) have shown promising results. Enhanced selectivity and extraction performance have been reported when using ternary mixtures with tuned properties (Fernandes et al., 2021). These mixtures present high solubility towards lignin and low cellulose dissolution making them highly selective and yielding high purity lignin. In addition, DES can be prepared with natural ingredients and be easily recovered at the end of the process with further reuse without compromising the extraction efficiency. New binary (lactic acid:choline chloride) and ternary DES mixtures (lactic acid:citric acid:choline chloride) for lignin extraction from Acacia wood were tested and are covered in this presentation. Not only DES physicochemical properties but also their extraction capacity was greatly affected by their composition and extraction conditions. As for DES recovery and reuse, it was observed that it did not negatively affect its efficiency or selectivity, up to 3 cycles of re-use, leading only to a small decrease in lignin's purity (~80%) (Figure 1). Therefore, the use of a natural-based and recyclable DES makes this process highly appealing for biomass fractionation with low environmental impact.

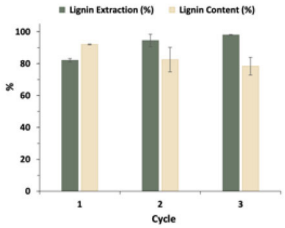

Fig. 1. Extraction yield and purity of freshly synthesized and reused DES.

Keywords: natural DES, greener wood extraction, recycling and reuse

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Development of biobased films from 'Annurca' apple cellulose dopped with phenolic compounds with novel characteristics

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The circular biomanufacturing of biodegradable and bioactive materials using agro-food byproducts is being promoted in food sector applications. Herein, the use of 'Annurca' apple waste is considered for the first time to introduce cellulose-based blended films using polyphenolic compounds to improve their bioactive functionality. The current strategy demonstrates ultrasound-assisted ethanolic extraction of bioactive compounds followed by the extraction of crystalline cellulose from the leftover biomass. The conversion of 'Annurca' cellulose into carboxymethyl cellulose (CMC) was conducted and processed for the development of films using casting technique. For this purpose, a combination of plasticizer (both glycerol and sorbitol, with 40% (*w/w*) individual contents) was used and heated for 1 h at 40–50°C. Polyphenolic extract (0.02%, *w/w*) was used in the casting solution to improve the functional properties of resulting films. Further, a control film was prepared without the addition of polyphenolic compounds. Both films were analyzed using Fourier transform infrared spectroscopy (FTIR). For visual appearance stereomicroscopic images was used, while the advanced surface morphological analysis was performed by scanning electron microscopy (SEM). Morphological analyses indicated crystalline nature of cellulose and CMC, while the resulting films were transparent and smooth with a uniform distribution of polyphenolic compounds. FTIR findings further supported that polyphenolic compounds are well dispersed in 'Annurca' CMC matrix, and no chemical reactions occurred during the film formation. Further, the newly developed film incorporating bioactive compounds showed excellent antioxidant and antimicrobial characteristics against both gram-positive and gram-negative bacteria. In conclusion, the present study describes a facile method to prepare 'smart' multifunctional biobased materials for different bio-sector applications.

Keywords: circular biomanufacturing, biobased materials, antioxidant, antimicrobial, waste utilization, bioactive compounds

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Potential and barriers of triterpenoid saponins as tomorrow´s plant based bio-pesticides

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Synthetic pesticides have increased yields, but come with a high cost for the environment, human health, and biodiversity. At the same time, climate change is leading to increased food insecurity, making crops more susceptible to fungal diseases and agricultural insect pest. Plant pests and diseases account for a reduction of between 20 and 40% of global crop yields per year. This calls for novel sustainable alternatives to the current use of synthetic pesticides for both conventional and organic farming. There are only a few biocontrol crop protections available on the market, with less than 5% of plant protection products sold worldwide being biocontrol agents. We can work with, and learn from, nature and find bio-solutions. Plants have co-evolved with biotic stress and therefore have the ability to defend themselves, typically by producing bioactive compounds. We have been focusing on how some triterpenoid saponins convey resistance to insect pest and others. Saponins are amphipathic molecular present in more than 100 plant families. While toxicity to humans is regarded as low, they have an untapped potential against plant pests. We are conducting mode-of-action studies to understand their toxicity at the molecular and at the ecosystems level, by combining 2D and 3D structure analysis with bioactivity assays to uncover structure-activity-relationships. Finally, we determine targeted vs non-target organism's responses to explore the potential of saponins as tomorrow´s biocontrol agents.

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Purification of derivatives of 2-amino-3H-phenoxazin-3-one using CPC

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Aminophenoxazinones represent natural products, originating from a plant produced precursor (benzoxaziones), produced by members of the Poaceae family, which are further metabolized by microorganisms to form the additional aromatic ring. Among all identified downstream metabolites, this compound class has shown multiple biological activities, like a pronounced phytotoxic activity (Macías et al., 2005), via a novel mode of action, identified as HDAC inhibition. Additionally, they act as anti-inflammatories, anticancer agents, antibiotics, and immunologic regulators, to mention some (Zorrilla et al., 2021). To improve their physio-chemical properties and bioactivities, we synthesized derivatives of the natural product 2-amino-3*H*-phenoxazin-3-one (Figure 1), introducing different heteroatoms and functional groups to the scaffold at the carbons C-1, C-9, C-7, or C-8.

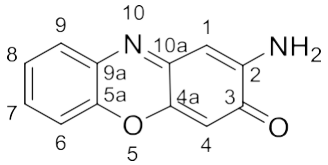

Fig. 1. Aminophenoxazinone scaffold.

Despite successful synthesis, purification of the reaction mixtures was challenging due to strong compound interactions with solid phases. We addressed this using Centrifugal Partition Chromatography (CPC). CPC (Pauli et al., 2008) is a support-free liquid–liquid chromatographic technique with mobile and stationary phases consisting of two immiscible liquids forming a two- phase solvent system, where compounds are distributed and transferred between the two phases according to their corresponding partition coefficients. The stationary phase is maintained in the rotor by centrifugal forces while the mobile phase is pushed through it via a pump. Because of the absence of solid support, CPC allows the full recovery of the sample. With this technique, we were able to purify different derivatives.

Keywords: allelopathy, natural products, CPC, aminophenoxazinones

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Leveraging phytochemical variation in *Piper scintillans* Trel. to discover novel flavanone meroterpenes

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Ecology-driven approaches to natural products discovery leverage phytochemical variation resulting from environmental and genetic factors to uncover structurally distinct novel molecules. The *Piper* genus produces phytochemicals from a diverse array of chemical classes that have varied biological activities and traditional uses (Salehi et al., 2019), and is a model genus for studying chemically mediated ecological interactions (Dyer and Palmer, 2004). A genomics and metabolomics survey of 100 *Piper scintillans* Trel. parent-offspring sets led to the discovery of a small group of genetically related individuals that produce a novel dioxane-linked flavonoid meroterpene, raduladioxanolide (**1**).

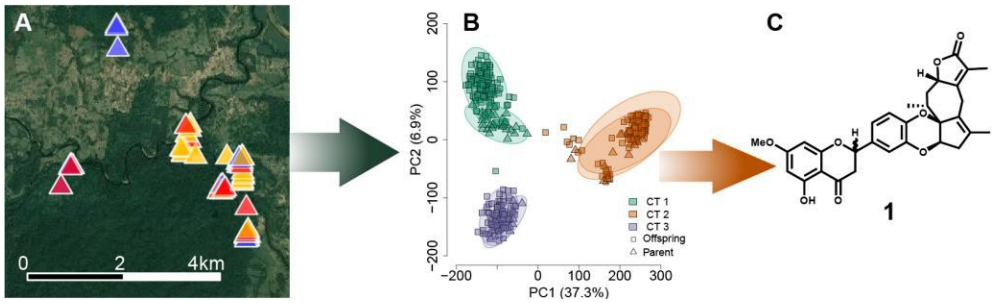

Fig. 1. Discovery of flavanone meroterpene raduladioxanolide from population-level phytochemical survey of *P. scintillans*.

Leaf cuttings and seeds were collected from parent plants within La Selva Biological Station, Costa Rica (Fig. 1A) and offspring were propagated from seed in a common garden. Foliar tissue from parent plants and their adult offspring were analyzed by UPLC-IM-QTOF and crude ^{1}H NMR. Cluster analysis revealed three distinct chemotypes (Fig. 1B), one of which (Fig. 1B, CT 2) contained a subset of parent-offspring "families" that produced a set of flavanone meroterpenoids including **1** (Fig. 1C). Absolute stereochemistry at flavanone C2 of **1** remains unknown but IM data suggest a 2:1 diastereomeric ratio (CCS = 215 Å^2, 240 Å^2). We propose that raduladioxanolide results from the oxidative cyclization of sternbin and americanolide G as both were solely isolated from individuals that produce **1**. We have demonstrated how leveraging phytochemical variation within a plant species can reveal unexpected and structurally unique molecules, highlighting new avenues for the discovery of novel natural product architectures.

Keywords: piper, meroterpenes, flavonoids, metabolomics, chemical ecology

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Invasive weeds: a treasure chest of medicinal chemicals from Palmer amaranth *(Amaranthus palmeri)*

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This presentation addresses the surprising finding that abundant chemicals of the weed Palmer Amaranth (*Amaranthus palmeri* L.) have medicinal utility. Palmer amaranth is an annual weed that threatens the yield of many crops in the USA, a plant native to the southwest and Mexico (Sauer, 1957). This study originally targeted herbicide development. It was discovered that under specific growth conditions and environmental challenges associated with global change, Palmer amaranth produces numerous chemicals, especially phenylpropanoids, useful for medicinal purposes, with some similar chemical types of our prior work in broccoli (Gurgul et al. 2023). With recent global change, Palmer amaranth has become a significant threat to crop yield, especially soybean. Metabolomics and lipidomics studies were conducted on specific growth stages of Palmer amaranth under stress to look for any chemicals of interest. Dozens of identified structures (reported in other unrelated plants) are in abundance that show promise for drug and nutraceutical development. Data indicate a living drug factory, despite global change, where controlled growth may be possible to benefit humankind.

Keywords: phenylpropanoids, Palmer amaranth, medicinal, weeds, stress

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Ecotoxicity studies of natural sesquiterpene lactones on *C. elegans* as soil model organism for potential bioherbicides

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Sesquiterpene lactones (SLs) and their derivatives are recognized as potential bioherbicides for controlling weed growth in crop plantations intended for human consumption (Macías et al., 2019). This approach, rooted in natural products, offers an alternative to conventional herbicides, which often comprise persistent soil pollutants detrimental to water ecosystems. Various SLs have demonstrated efficacy as pre- and post-emergent bioherbicides against a range of monocot and dicot weeds (Macías et al., 2020). Additionally, their effectiveness against parasitic plants has been documented. However, research regarding their impacts on soil fauna remains limited.

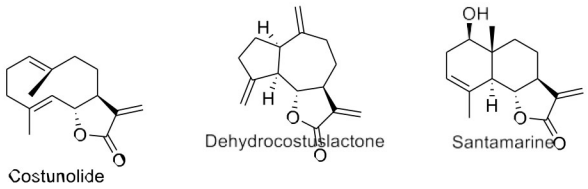

Fig. 1. Relevant phytotoxic sesquiterpene lactones

Among numerous model organisms, *C. elegans* stands out as a particularly compelling nematode for analysis due to its ubiquitous presence in temperate soils, transparency, and approximate length of 1000 µm (Brenner, 1974). This presentation will delve into toxicity studies of various SLs families— eudesmane, guaiane, germanacrane, and dimers—on the *C. elegans* N2 ancestor strain. Evaluation will encompass toxicity assessments on both adult worms and eggs, alongside examination of degradation products post-bioassay to identify optimal candidates for bioherbicide applications. Costunolide, dehydrocostuslactone, and santamarine exhibited significant inhibition of *C. elegans* motility at 1000 µM, while showing no discernible effects at 300 µM, an advantageous trait considering their bioherbicidal properties, where they inhibit wheat coleoptile elongation by approximately 90% compared to the negative control at 100 M. Moreover, these compounds undergo rapid degradation within 24 hours, boasting a half-life of approximately 12 hours in culture media, thus mitigating environmental pollution risks.

Keywords: sesquiterpene lactones, *C. elegans,* ecotoxicology, phytotoxicity, agrochemistry

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Session III: Regulation for the use of natural products in food and drugs

Topical Lecture

Three-pronged strategy of TCM quality control: deep research, holistic standard and precision testing

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It is of a tremendous challenge for the quality control of traditional Chinese medicines due to its extreme complexity largely caused by its multicomponent feature. TCM contains hundreds or even thousands of chemical components in a single herb, not to mention the compound formulas composed of a handful of even tens of herbs. This generates gigantic hurdle to clarify its chemical composition, which is the fundamental basis for the research of quality control, mechanism, pharmacological and toxicological actions, PK/PD, clinical trial, new drug discovery, etc. As to these challenges, we developed a three-pronged strategy dubbed "Deep Research, Holistic Standard and Precision Testing" to have intended a delivery of an ultimate solution. By implementing a quality-oriented deep research, mainly a integrative approach with chemical, metabolic and biological analysis namely "trinity analysis", the quality markers will be clarified to facilitate the ensuing quality standard elaboration. Based on the comprehensive analysis, a holistic quality control model for herbal quality was developed and practiced in herbal drugs and their products therefrom such as dispensing granules, classic formulas and finished combination products. This type of research cemented for elaboration of quality standards. Due to the deficiencies of the developed quality standards, it is of great necessity to develop the cutting-edge precision testing techniques to compensate the inadequacy of quality standards to fully control the quality. In the past several decades, our research team has devoted to the herbal quality control by practicing the three-pronged approach. A set of herbal drugs, granules and the finished products were researched in depth, which will be exemplified in the lecture.

Keywords: Traditional Chinese medicines, quality control, trinity analysis, three-pronged strategy

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Herbal Health Products in Europe – an overview on challenges and chances

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Herbal Health Products (HHP) account for more than 20% of the OTC market. HHP can be assigned to different product categories like medicinal products, botanicals, substance-based medical devices, cosmetics. Concurrently all these categories have to comply with a different legal base. The ongoing revision of the EU pharmaceutical legislation as well as the dissensus of European Commission and European Parliament not to implement Regulation (EC) No 1924/2006 on nutrition and health claims made on foods might have great impact on the market. On 20 February this year GA hosted an EU Summit on Herbal Health Products (HHP) in Brussels, bringing politics, industry and academia together to exchange minds on how a level playing field for these kind of products might be restored to guarantee reliable information and safe use to consumers and patients. This talk will highlight relevant issues and summarize essentials.

Keywords: (traditional) herbal medicinal products, food supplements, botanicals, EU Summit on Herbal Health Products

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Regulation (EC) No 1925/2006 of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods.

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Understanding psilocybin mushrooms and aiding in setting standards for a burgeoning industry in Jamaica

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Psychedelic fungi are experiencing a surge in interest in recent years, particularly among patients under treatment for a variety of mental health issues. These include depression, anxiety disorder, addiction, post-traumatic stress disorder, and various psychiatric treatments within group or individualized therapy sessions. 'Magic mushrooms' biosynthesize the alkaloid psilocybin, which undergoes dephosphorylation following ingestion to its bioactive counterpart, psilocin. The expression of the tryptamine content (determined using HPLC) in 13 psilocybin containing species (within 3 genera) were explored in this study, under numerous growth conditions, including solid/liquid media, varying light/dark conditions, with and without additives, along with optimum conditions determined for drying and storage, following examinations of growth curves. Optimized growth conditions for *Psilocybe cubensis*, the most commonly cultivated species, confirms that an additive layer of gypsum and casing layer provides maximum yield (896.6 g/kg of dried substrate), with a biological efficiency of 89.6%, while also maintaining high total tryptamine expressions (>0.95%). Emanating fruiting bodies from this unique, legal, EU-GMP certified lab, has subsequently been encapsulated and tested (for mycotoxins, microbes, moisture, heavy metals, pesticides and stability) for use by medical practitioners in Jamaica. This presentation will detail our journey and further outline on-going biological activity investigations including the impact on the activities of heterologously expressed human microsomal Cytochromes P450 enzymes.

Keywords: psilocybin, psychoactive, cytochromes P450, mental health, drug interactions

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Lavandula angustifolia Mill. based soft gel capsules: authenticity and quality assessment

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Lavandula species, including their hybrids and approximately 400 varieties, are extensively used as phytopharmaceuticals, food, and cosmetics (1). *Lavandula angustifolia* Mill., for instance, has a long established application in alleviating restlessness, agitation, insomnia, and gastrointestinal discomfort associated with nervousness (1). Silexan®, a proprietary high-quality lavender oil, is clinically proven and authorised as a medicine for the treatment of anxiety disorders in numerous countries. Following our previous work (2), using GC-FID, here we evaluated the composition of lavender oil in over 35 soft gel capsules available on the global market, promoted for several indications, including restlessness, anxiety disorders and improving sleep quality in adults and children. The GC-FID analysis showed a significant difference between products in terms of their chemical composition as well as the concentration of metabolites. Marked differences were seen at the approximate time periods of 46.5 and 46.8 minutes corresponding to main pharmacologically active metabolites found in *L. angustifolia*, linalool, and linalyl acetate. However, this variability between products was also noticed with regard to the eight other marker compounds particularly for 1,8-cineole and camphor at time periods of 20.7 and 44.5 minutes which are more indicative and prominent markers in terms of concentration for other lavender species e.g., *Lavandula latifolia* Medik (Fig. 1).

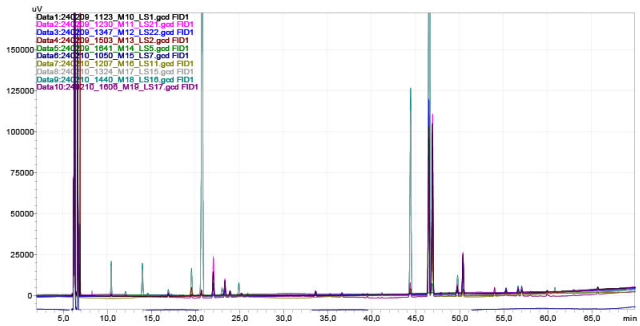

Fig. 1. GC-FID analysis of *Lavandula augustifolia* Mill. based soft gel capsules.

This work will have implications for the generalizability of the efficacy and safety data of phytopharmaceuticals tested in clinical trials such as Silexan® (3) as well as for the general public and healthcare professionals who are using or recommending these products.

Keywords: *Lavandula angustifolia,* soft gel capsules, GC analysis, oral dosage forms, lavender oil

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Session: Animal Health Care (VET)

Topical Lecture

Nature's Pharmacy" in the context of insects – is there anything to be learned?

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Secondary natural products, plant secondary metabolites (PSMs) and those synthesised by animals, play an extraordinary role not only in all ecosystems, but also directly for humans. For millennia, we have been using many of these non-nutritional chemicals from what is often called "Nature's Pharmacy". When individuals suffering from health problems (e.g., parasites, infectious diseases) ingest compounds with medicinal effects through a specific behavioural activity which is independent of their daily needs and primary metabolism, and serves to cure or prevent infections or attacks by parasites or support well-being, it is usually referred to as "self-medication". This is distinct from "compensatory feeding", which involves nutritive substances and minerals.

Other animals also take advantage of Nature's Pharmacy by deliberately gathering compounds from particular natural sources with a specific behaviour that is distinct from the general activities of the species, independent of its nutritional needs and not essential for living. This behaviour may be related to a health problem, but it may also be independent of health status and serve to increase chances of survival and biological fitness in various ways. It is sometimes referred to as "pharmacophagy".

This presentation explores the commonalities and differences in the use of Nature's Pharmacy for self-medication *s.l. versus* pharmacophagy by humans, vertebrate wildlife, and especially insects. By adding ecological contexts to the discussion (including population ecology and sensory physiology), it attempts to clarify conceptual and terminological issues, identify gaps in general understanding, and suggest key aspects of future research.

Keywords: herbal medicine, self-medication, pharmacophagy, physical fitness, biological fitness, chemical defense

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Gut-health histomorphometry in fattening lambs fed with hazelnut skin and linseed

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Agro-industrial byproducts in animal nutrition offer animal feed that is both economical and environmentally friendly (Vastolo et al., 2022). In particular, hazelnut skin (*Corylus avellana*), due to the phenolic compounds and oleic acid content, may affect the rumen microbiota and polyunsaturated fatty acids (PUFA) biohydrogenation, potentially modifying the rumen fermentation and morphology (Priolo et al., 2021). Moreover linseed (*Linum-usitatissimum*) has been demonstrated to be capable of increased accumulation of PUFA in meat (Andrés et al., 2014). To evaluate the potential effects of these natural products, 40 male lambs were randomly assigned to four dietary treatments: conventional (C), 8% linseed (LS), 15% hazelnut skin (HS), and a combination (7.5% HS + 4% LS) as a partial replacement of maize in the diet. The lambs (n=20) were assessed for growth performance, and ruminal/gut histomorphometry after a 60- day feeding period. The growth performance did not significantly differ among the groups. Ruminal ventral sac papillae were lengthy (0.508 ± 0.143 cm, $p < 0.05$) and exhibited strong positive association (0.610**, $p<0.04$) with HS+LS. The HS showed somewhat higher intestinal inflammation scores (3.275 ± 0.568, $p < 0.05$) than the control group. These results would be fortified by the on-going rumen microbiota and VFA analysis. The pro-inflammatory effects by HS need further investigations to identify their mechanism. In summary, HS+LS/HS seem to have an impact on gut health and particularly on rumen histomorphometry. These findings emphasize the need for further assessment of new feed components to improve animal health outcomes, to maximize animal well-being.

Keywords: hazelnut/linseed, lambs, gut-health, VFA, histomorphometry

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Malassezia pachydermatis sensitivity to plant extracts from the Brazilian Amazon Rain Forest

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The present work covers the search for new active plant extracts to be used in the treatment of infections caused by *Malassezia pachydermatis*, one of the most important pathogenic agents related to canine otitis and dermatitis. The microorganism can be cross-species transmitted from pets to tutors, and its control shows epidemiologic importance. Our group screened more than 2,300 Brazilian plant extracts against *M. pachydermatis*, a pathogenic yeast that has both human and veterinarian importance, using sensitivity tests (Suffredini et al., 2023) such as disk diffusion (DDA) and microdilution broth (MBA) assays and bioautography to identify antifungal extracts, compared to Amphotericin B (AmB) solution. The extract obtained from the leaves of *Guatteria* sp. (Annonaceae) showed antifungal activity in the DDA (12.31 ± 0.32 mm; 100 mg/mL), compared to AmB (18.76 ± 0.65 mm); in the MDB (minimal inhibitory concentration, MIC, of 5 mg/mL; AmB – MIC of 78 µg/mL). Bioautography showed that fractions eluted to Rf = 0.5 showed antifungal activity. Analytical thin-layer chromatography (TLC) was performed and indicated the presence of alkaloids and phenolic compounds; preparative TLC was performed to assess gas-chromatography mass spectrometry analyses. Results indicated the presence of gamma-sitosterol **1**, 3',5'-dimethoxyacetophenone **2**, and palmitic acid **3** in the fractions (Fig 1).

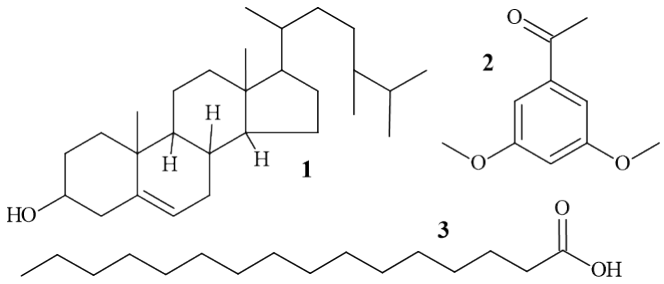

Fig. 1. Structure of gamma-sitosterol **1**, 3',5'-dimethoxyacetophenone **2**, and palmitic acid **3** The extract obtained from the Annonaceae species showed antifungal activity, which may be related to the alkaloids that are present in the active fractions, combined or not with **1, 2,** and **3**.

Keywords: Antarctic Continent, apoptosis, Brazilian Rain Forests, cytotoxicity, wound healing

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Orangutan herbal remedies: lessons for human health

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Both human and animal health are continually threatened by infectious diseases. The "One Health concept" has highlighted the strong interrelation between human and animal health in recent years. Understanding factors affecting individual health is crucial for determining the underlying health and resilience of the environment. The health of both individuals and ecosystems depends on homeostasis, the balancing of dynamic processes through continuous interaction and feedback within the integrated system.

Parasites play a significant role in individual, species, and ecosystem health. Given the phylogenetic similarities between humans and orangutans, studying orangutan feeding behavior is relevant, particularly regarding specific plant foods consumed to combat parasitic infections. Orangutans, the only ape species in Southeast Asia, face critical endangerment, listed as such by the IUCN.

Self-medication in primates sheds new light into the complex interactions of the animal, plant and parasite. While primates consume a variety of non-nutritional plant compounds and nutrient-poor bark, little is understood about the potential medicinal benefits of such ingestion. Our recent research confirms self-medication in orangutans (*Pongo pygmaeus*) for the first time, based on pharmacological analyses.

Several plants in the orangutan diet hold potential medicinal value, some used in traditional human medicine. This presentation will explore plants within the orangutan diet that demonstrate effectiveness against parasites through *in vitro* testing, such as *Piper betle, Archidendron fagifollium, Mimosa sp., Knema laurina.* Our findings, along with descriptions of plant species exhibiting novel antiparasitic effects, may inform drug development efforts to combat infectious diseases in both human and animal populations.

Keywords: self-medication, anti-parasitic activity, orangutan, *in vitro* tests

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Willow *(Salix acmophylla)* leaf and twig extracts inhibit sporulation of coccidia *(Eimeria spp.)* in goats

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Willow (*Salix acmophylla*) trees, found worldwide, contain secondary metabolites that are valuable as dietary supplements for animal feed and as anti-parasites (Awwad et al., 2021). This study investigated the ethanol extracts from leaves and branches of three willow genotypes for their secondary metabolite composition and their potential to inhibit *Eimeria* sp. sporulation, a major concern in ruminant health. The extracts were characterized for phenolic compounds, flavonoids, and salicylic acid using HPLC. The total phenolic content of willow leaves and branches was similar in the three different genotypes. The total flavonoid content of the branches was significantly higher than that of leaves of the same genotype; however, the salicylic acid content was significantly higher in leaves than in branches. Importantly, all extracts exhibited significant inhibition of *Eimeria* sporulation, where over 70% inhibition was obtained at concentrations as low as 750 ppm. The sporulation inhibition of branch or leaf extracts exceeded 80% for leaves and 90% for branches at concentrations above 1250 ppm. HPLC analysis identified the flavonoids rutin and hyperoside in both leaves and branches, but their concentrations, along with those of salicin were higher in leaves. Conversely, p-coumaric acid was detected in branches but not in leaves. The study highlights the potential of using *Salix* extracts as bioactive compounds for biological control of coccidiosis in ruminants and emphasizes the importance of genotype-specific variations in secondary metabolite profiles. We conclude that all parts of *S. acmophylla* can provide secondary metabolites that act as a coccidiostat to treat *Eimeria* in goats.

Keywords: Ethanol extracts; Plant secondary metabolites; Coccids sporulation; *Eimeria* sp.; Willow genotypes

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Hot Pepper (Capsicum annuum) extract: not only a flavor enhancer, but also a useful fish feed additive

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The present study investigated the effects of hot pepper (*Capsicum annuum*) extract (CAO) supplementation on growth performance, serum biochemical parameters, immune responses, histology, gene expression levels, gut microbiome, and sensory characteristics of rainbow trout. Five isonitrogenous and isoenergetic diets were formulated to contain hot pepper extract at levels of 0.7% (C7), 1.4% (C14), 2.1% (C21), and 2.8% (C28). Fish were fed experimental diets for 30 days (experiment one) and 45 days (experiment two). It was determined that significant improvements were achieved in growth performance, serum biochemical parameters and expression levels of genes responsible for immunity along with some humoral immune responses. Additionally, dietary CAO induced changes in the intestinal histological structure, as evidenced by an increased number of goblet cells in the CAO-supplied groups.

The sensory attributes of cooked trout fillets demonstrated that capsicum's pungent odor and taste were evident in the C14 group and subsequently increased with higher dietary CAO levels. Importantly, our results indicate, for the first time, that dietary CAO at levels of 7–14 g/kg can enhance the growth of rainbow trout without adversely affecting sensory characteristics or gut health.

Keywords: Rainbow trout, Oncorhynchus mykiss, immune responses, gene expression, biochemistry

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Session IV: A.I.M.S. Cluster: Prospecting aquatic and terrestrial natural biological resources for biologically active compounds

Oral contributions

Enhancing bio-based compound diversity through advanced biotechnological approaches: The SECRETed project

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SECRETed, an EU-H2020 funded initiative, leads in aquatic biotechnology, targeting innovation in agrochemical, pharmaceutical, cosmetic, and chemical sectors. This multidisciplinary consortium utilizes the genetic diversity of biosurfactants and siderophores from marine and extremophilic microorganisms to create novel hybrid molecules with tailored properties. By leveraging existing microbial collections and advanced Machine Learning algorithms, SECRETed has developed a comprehensive microbial amphiphilic compound database, decoding genetic blueprints to enhance chemical diversity and sustainable industrial applications. Significant progress in data collection and analysis has been achieved through natural language processing and graph convolutional neural networks (Qin et al., 2021), enriching the database's depth. A top-down approach using novel QSAR/QSPR models that identified over 3700 molecules from natural product databases, based on their chelating or surfactant abilities. In addition, a bottom-up approach updated the MIBiG database (Terlouw et al., 2023) with retrosynthetic details on 473 compounds linked to 417 biosynthetic gene clusters (BGCs), significantly advancing our understanding of microbial biosynthesis. In parallel, screening of natural producers has been performed towards identification of appropriate microbial hosts, which were combined taking advantage of metabolic engineering methods to achieve growth coupled production of custom industry-driven biosurfactants and siderophores. Fermentation and downstream processing of selected microbial hosts has been scaled up, while their target metabolites (biosurfactants and siderophores) are currently being tested on end-user applications. The applications include encapsulation of enzyme Q10 (cosmetics and nutraceuticals) and essential oils (agrochemicals), siderophore-based antibiotics production, pharmaceuticals with cytotoxic effects and RNAi-based drug delivery formulations for eye diseases.

Keywords: biosufactants, siderophores, green chemistry, systems and synthetic biology

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Marine biodiversity as sustainable resource of disease-suppressive microbes and bioprotectants for aquaculture and crop diseases "MARBLES"

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The marine environment remains a largely untapped and poorly understood source of natural products with potential for application in the pharmaceutical and food industries. Challenges include finding and identifying the bacteria and their molecules, extracting their active ingredients in a sustainable, effective way, and the commercial and regulatory difficulties of bringing a discovery from the lab to market. Currently there is an urgent need to sustainably harness new compounds with pharmaceutical and nutritional applications. MARBLES project addresses this need by exploring and exploiting the microbiomes of marine ecosystems including sponges, microalgae and fish, which rely on their microbiomes and microbial natural products for disease resistance. MARBLES is obtaining novel bioactive compounds and microbes that act as bioprotectants in aquaculture, agriculture and pharmaceuticals. MARBLES ecology-based bioprospecting strategy focuses on unique host-microbe interactions in marine environments. Partners' existing microbial collections and new ones generated during MARBLES are being harnessed for the discovery of novel natural products and microbial communities. For this, MARBLES is using a systems-wide genomics approach to uncover the bioactive agents in disease-suppressive microbiomes. Also, MARBLES is exploring host- and microbe-derived chemicals that elicit production of bioactive compounds to revitalise drug screening. The expected outcomes will be sustainable production of bioprotectants (microbes and consortia and bioactive natural products, their derivatives and elicitors) with potential to fight infectious diseases in the agrochemical, aquaculture and healthcare industries. MARBLES contributes to UN SDG 2, 3, 12, 13 and 14, as well as to current UN processes (BBNJ, DSI, SynBio).

Keywords: marine bioprospecting, microbiome, sustainable aquaculture and agriculture, bioactive natural products, healthy ecosystems

Acknowledge: This work has been funded by H2020-FNR-11-2020: MARBLES (Grant number: 101000392).

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Sustainable skincare ingredients from plant cells with scientifically proven efficacy

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The global cosmetics market was valued at $375.3 billion in 2022 and is expected to reach $560.5 billion by 2030. Manufacturers are focusing on natural ingredients to meet consumer demands for bioactive and sustainable cosmetic products. However, the sector is facing technical and economic challenges due to the lack of raw materials and insufficient scientific evidence of efficacy. *InnCoCells* is an EU-funded project aiming to address these issues by revolutionizing the way cosmetic ingredients are manufactured from underutilized plant resources, leading to sustainable products with scientifically proven effects. There are 17 partners in the *InnCoCells* consortium, bringing together academic and industry researchers from nine EU member states as well as the UK and Norway. The project is coordinated by VTT (Finland) and has a budget of €7.9 million. *InnCoCells* is developing pilot-scale production platforms based on plant cell suspension cultures, hairy roots, whole plants and agrifood side-streams, as well as innovative downstream processing methods to generate bioactive extracts. This includes a cascade biorefinery concept that uses by-products and biowaste to extract value-added ingredients. The safety and efficacy of the extracts is tested using a broad and diverse panel of enzymatic and cell-based assays, and the active ingredients are identified by detailed metabolic profiling. The most promising candidates will be tested in human volunteers and will ultimately be marketed by our industrial partners. As a case study, we discuss chili (*Capsicum* spp.) cell cultures for the production of bioactive metabolites with antimicrobial and antioxidant activities that are suitable as cosmetic ingredients.

Keywords: plants and plant cell cultures, bioassays, cosmetic ingredients, sustainability, underutilized plants resources

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New trends in pharmacognosy and cosmeceuticals

Pharmacognosy in the digital era: exciting opportunities offered by metabolomics and accessible digitised data

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With the advent of omics, digital sciences, and artificial intelligence (AI), a real revolution has taken place in life sciences and is also affecting pharmacognosy. Today, detailed metabolome data of various organisms can be readily obtained, accelerating our understanding and offering a more comprehensive perspective on Earth's chemodiversity (Rutz et al. 2022). In metabolomics, the challenges remain significant, and several questions need to be addressed: How reliable are metabolite annotations based on LC-MS profiling of natural extracts? Massive qualitative information is obtained, how is it related to amounts of active ingredients? Is it possible to deduce 3D structure, key to bioactivity, from metabolite profiling data? As part of our research, we obtained metabolomic information on thousands of highly biodiverse extracts of both plant and fungal origin (Allard et al. 2022). We are trying to understand how the massive amount of information collected and the taxonomic links can be used to answer questions about confidence/redundance in MS annotation (Rutz et al. 2023). On the other hand, the resulting management of such massive data represents a further challenge. With the development of knowledge graphs (KG) based on semantic web tools (Gaudry et al. 2024) it is possible to organize this data and make it searchable by advanced queries (Gaudry et al. 2022). We will address the practical challenges associated with automating the assessment of extract chemical composition. We will discuss the potential of KG and AI in analyzing metabolomics data within the context of pharmacognosy findings, aiming to foster new discoveries.

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Keywords: metabolomics, LC-MS, knowledge graph, metabolite annotation, semantic web

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Nanophytocosmetics – a new trend in cosmeceuticals

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Cosmeceuticals are a subclass term of cosmetics with vast product miscellany and increased share in the world cosmetic market. Natural and/or organic cosmetics are receiving more attention from consumers all over the world concerning their safety and efficacy. Nanocosmetics with herbal substances refer to cosmetic products that incorporate nanoparticles along with natural herbal ingredients. These nanoparticles are typically on the nanometer scale, which allows for enhanced delivery of active herbal compounds into the skin or hair follicles, leading to improved efficacy and targeted delivery. The herbal substances used in these nanocosmetics can include extracts from plants, essential oils, and pure natural compounds as active ingredients. These products are often marketed as offering benefits such as increased hydration, antioxidant protection, anti-inflammatory properties, and overall skin health improvement. However, further research is needed to fully understand the safety and efficacy of nanocosmetics with herbal substances, as well as their potential long-term effects on human health and the environment. Consequently, the research and development of novel phyto-based cosmeceuticals *via* extensive screening studies on plant extracts and pure natural substances have been conducted by our group using *in vitro* (enzyme inhibition, *etc.*), *in silico* (molecular docking and toxicity screening), and cell-based assays. Some active ingredients identified and formulated for skin bleaching, anti-wrinkle, and anti-aging purposes by us up to date have been as the extracts from *Cotinus coggygryia*, *Geranium glaberrimum*, *Garcinia mangostana*, *etc.*, in the forms of niosome, nanofiber or nanoemulsion formulations. In this connection, an anti-acne formulation based on a number of plant extracts tested against *Propionibacterium acnes* has also been developed by our group, which led to several patent applications. For wound healing, we have been studying nanofiber formulations loaded with some plant extracts. Our continuing analyses on discovering novel, effective, and safe natural ingredients with possible cosmeceutical use have so far yielded 3 patents and 5 patent applications. In this talk, our current upshots on phyto-based nanocosmeceuticals will be underlined.

Keywords: nanocosmetics, phytocosmetics, natural products, skin bleaching, anti-aging

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Nitrogenous flavonoid derivatives production by reacting with amino acids

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A class of nitrogenous derivatives of flavonoids was detected using *in vitro* cell culture medium and *in vivo* mice produced without enzymatic catalysis have been consistently overlooked in literature. In this study, 39 flavonoids were incubated in Dulbecco's modified eagle's medium (DMEM) at 37° for 2 h to explore the reaction mechanism behind nitrogenous derivatives from flavonoids. Baicalein, scutellarein, DMY, GC, EGC, and EGCG were found to produce corresponding nitrogenous derivatives in both DMEM and mixed amino acid solution. The nitrogen source of these 6 flavonoid nitrogenous derivatives was revealed to be amino acid. The reaction site with amines in these flavonoid nitrogenous derivatives was identified as OH of the pyrogallol moiety in flavonoids *via* LC-MS/MS and NMR. This pyrogallol group was a key motif being first oxidized into quinone, further, to react with Strecker degradation of amino acids to yield N-flavonoids and corresponding aldehydes. Reaction optimization revealed that a slightly alkaline environment accelerates flavonoid nitrogenous derivatives formation by promoting the formation of flavonoid quinone. These results provide the first mechanistic evidence for the *in vitro* generation of flavonoid nitrogenous derivatives yet to be tested using *in vivo* assay.

Keyword: Nitrogenous flavonoids; baicalein; dihydromyricetin; catechin; pyrogallol; amino acids

Acknowledgement

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SIF Italian Society of Phytochemistry and Sciences of Medicinal, Food and Perfume Plants

Investigating structural features and bioactivity of natural products by NMR and computational techniques

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Marine and terrestrial species provide an inexhaustible source of active secondary metabolites (Atanasov, Zotchev et al. 2021). These entities feature a vast array of 3D structures, particular biochemical properties, and other molecular characteristics, which make them a reliable starting point for the identification and development of potential lead compounds in the field of drug development. The correct elucidation of the structural features of already reported Natural Products ("old NPs") and unprecedented ("new NPs") ones represents the primary critical step for accelerating their complete biochemical and pharmacological characterization. Considering the complex structural architecture which often characterizes these NPs (especially if compared to synthetic molecules), the elucidation of their 2D structure and the precise 3D arrangements is required to provide essential insights into ligand-target interaction, which can drive a rational lead optimization process. Combining NMR spectroscopy with modern computational techniques (Chini, Urbani et al. 2020, De Vita, Terracciano et al. 2020) is one of the most effective approaches to achieve this task. In this context, the DFT/NMR integrated method is a valid support for clarifying the configuration and conformational profile of active secondary metabolites, shedding light on their molecular basis of mechanism of action. Also, the robust and efficient Inverse Virtual Screening (IVS) approach, developed and optimized by us (Chini, Lauro et al. 2021), can be helpful in achieving the target identification task (De Vita, Chini et al. 2020, De Vita, Chini et al. 2023), guiding subsequent biological investigations and pharmacological tests and for speeding-up, when possible, the drug repurposing. Moreover, the complete comprehension of NPs structural determinants responsible for their biological activity could be used for designing and synthesizing novel molecular platforms through computer-aided modifications of their original active skeletons (De Vita, Chini et al. 2020). This could lead to semi-synthetic derivatives with enhanced potency and selectivity towards specific targets involved in pathological events, such as inflammation and cancer.

Keywords: natural products, bioactive compounds, structural elucidation, NMR, computational tools

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The epidemic of antibiotic-resistant: a call to research and development of new natural antimicrobial molecules

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Presently, the world is in the midst of an emerging crisis of antibiotic resistance for microbial pathogens (Akram et al.,2023). The global spread of this phenomena is associated with misuse and overuse of existing antimicrobials in humans, animals and plants, which is accelerating the development and spread of antimicrobial resistance. Scientists and pharmaceutical companies must urgently focus on a common mission: the search for new antibiotics compounds possibly acting through unexploited mechanisms and molecular target(s). Plants represent a promising source of bioactive compounds (Rajput et al., 2020). Based on these considerations, a study aimed at shedding light on the effects of manool, a labdane diterpene from *Salvia officinalis* L. (Lamiaceae) with full blown antimicrobial activity, on the dental pathogen *Streptococcus mutans* (MIC 6 µg/ mL), which is considered a new Gram-positive model organism, was undertaken. Drug Affinity Responsive Target Stability (DARTS) (Lomenick et al.,2011) allowed the identification of transporters ATP-binding cassette (ABC), a large superfamily of integral membrane proteins involved in the absorption of nutrients, as manool target. To validate the inhibition of ABC proteins, AA and sugar uptake assays were performed by MS and NMR analyses. The obtained results offer two possible scenarios: the direct or indirect binding of manool to the ATP site, common to the majority of identified proteins. Furthermore, expression proteomics and metabolomic studies were also conducted in *S. mutans* treated or not with manool, allowing to define the pathways primary affected by diterpene treatment.

Keywords: bioactive compounds, antibiotic resistant, plants, drug affinity affinity responsive target stability

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Metabolomic patterns in response to abiotic stimuli of *Cladonia* lichens from dry grasslands

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Lichens are complex organisms, defined as a self-sustaining ecosystem generated from the symbiosis between photobionts and mycobionts (Hawksworth and Martin, 2020). This study focuses on 4 species of genus *Cladonia* (*C. foliacea, C. polycarpoides, C. rangiformis*, *C. rei*), endowed with numerous bioactivities (Adenubi et al, 2022). Each species was sampled monthly for one year, in 5 different sites alongside dry grasslands of the Ticino River valley (Italy). The objectives were: 1) to acquire and elucidate (for the first time) the ^{1}H NMR profile of these species, 2) to picture intraspecies metabolomic differences, and 3) interspecies metabolomic variation due to environmental factors. By chemometrics were obtained validated models, and the species were clustered not only based on their biomarkers (i.e. usnic, protocetaric, fumarprotocetaric, rangiformic, norrangiformic, sekikaic and homosekikaic acids), but also for the enrichment in primary metabolites. For instance, fumarate and sucrose were more concentrated in *C. polycarpoides*. *C. rangiformis* had the highest concentration of arabinitol, acetate and alanine, while glucose and trehalose were more abundant in *C. rei*. Several intraspecies differences, both related to time-point and harvesting site, were also detected. Moreover, the metabolome of all species resulted significantly affected by the temperature gradient. Arabinitol emerged as one of the most important metabolites produced in response to chilling. The overall results offer a metabolomics-based discrimination method for *Cladonia* species, bringing insights both on when to harvest these lichens in view of a specific medicinal use, and on how they might adapt to environmental changes.

Keywords: *Cladonia,* lichens, ^{1}H NMR-profiling, metabolomics, lichen-climate adaptation

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Natural products as active substances and structure components of delivery systems: the case of nanovesicles

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We report the development of nanovesicles made of natural active substances used as bilayer architecture components, replacing the conventional bilayer fluidity modulators such as cholesterol or acting as co-modulators. These nanovesicles can be easily loaded with other actives to improve their biopharmaceutical properties. Different cases will be presented. Escinosomes are deformable nanovesicles made of phosphatidylcholine and escin, a triterpene saponin performing both biologically active components and bilayer modulator of fluidity, successfully reported for the delivery of natural drugs, i.e., berberine chloride for cutaneous administration (Vanti et al., 2019) and andrographolide for subcutaneous route (Vanti et al., 2022). Ascosomes represent a further innovative nanocarrier made of phosphatidylcholine and derivates of L-ascorbic acid, i.e., ascorbyl octanoate and ascorbyl decanoate, investigated as bioactive constituents of the vesicle bilayer (Risaliti et al., 2020). They were also studied as potential nanocarriers for the skin delivery of khellin, a natural furanochromone with various applications in skin pathologies, and selected as a lipophilic drug model. Moreover, escinosomes and ascosomes were gelled using cellulose derivates. The obtained nanovesicles-loaded hydrogels combined the benefits of a controlled release and improved transdermal permeability of loaded active ingredients thanks to the nanovesicle components, with optimized viscosity properties thanks to the polysaccharide matrix. Both nanosystems demonstrated a good safety profile in rats. A further study developed nanovesicles based on resins of *Cistus creticus* L. and *Pistacia lentiscus* L. These nanovesicles were loaded with the essential oils of *Origanum dictamnus* L. and *Salvia fruticosa* Mill. All these species are from the Balkan Peninsula, selected because of their ethnomedical uses related to skin diseases. However, formulating essential oils and resins in stable dosage forms is mandatory to overcome their low chemical stability and improve their therapeutic use.

Keywords: nanovesicle, escin, ascorbyl derivates, resin, *Pistacia lentiscus*, berberine, andrographolide, khellin, *Origanum dictamnus, Salvia fruticosa,* essential oil

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Session I: Cutting edge technologies in natural product drug discovery, formulation and development

Topical Lecture

Mass spectrometry in natural products from Brazil: from understanding chemical signaling to pre-clinical studies

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Plants, animals and microrganisms emit, detect, and respond to an amazing number of chemical signals. Advances in mass spectrometry have enabled natural products analyses at several levels, including complex metabolomic analysis. In this talk we will describes the most recent analytical developments in metabolomics applying examples of the Brazilian biodiversity. We also present studies with plants, frogs, and microorganism, which have allowed us to elucidate different biological activities and to advance in the understanding of the eco-physiological function of secondary metabolites. Finally, we describe the selected substances subjected to pre-clinical studies where MS imaging techniques allowed new alternatives for innovation processes.

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Oral contributions

Ion channels: beyond native mass spectrometry

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Ion channels represent a significant class of drug targets. The inwardly rectifying potassium Kir4.2 channel plays a crucial role in regulating membrane potentials and maintaining potassium homeostasis. Kir4.2 has been implicated in various physiological processes, including insulin secretion, gastric acid regulation, and the pathogenesis of central nervous system diseases. Transient receptor potential melastatin 2 (TRPM2) is a calcium-permeable, nonselective cation channel. It is involved in many pathological and physiological processes, making it a potential therapeutic target for various diseases, including Alzheimer's disease, Parkinson's disease, and cancers.

Native mass spectrometry has been established as a robust platform for investigating membrane protein-lipid interactions (Laganowsky et al., 2014). Native MS has been proven capable of revealing thermodynamics and allostery of individual lipid binding events (Cong et al., 2016). Nativeomics is an approach integrating native MS with small molecule fragmentation, to identify bound ligands released after native MS. The ligand is fragmented for identification through database searching (Gault et al., 2020).

We developed a mass spectrometry approach to directly detect the ligand after capture of the protein-ligand complex (Fig. 1). We firstly demonstrate the detection of the known ligands, polymyxin B and clotrimazole, respectively. Screening of a natural product library comprising 2000 compounds, with each pool containing 100 compounds, each compound at a concentration of 10 μM, was screened against Kir4.2 and TRPM2 to identify ginsenoside Rg1 and ginsenoside F3, respectively.

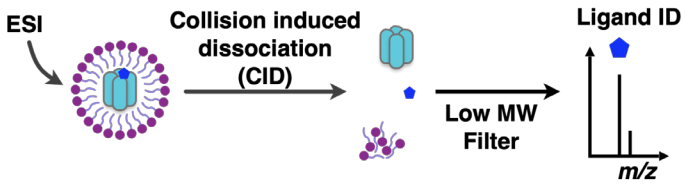

Fig. 1. Collision-induced affinity selection mass spectrometry (CIAS-MS) can be utilized to directly identify ligands binding to ion channels.

CIAS-MS circumvents the need for high mass detection typically associated with mass spectrometry of large membrane proteins.

Keywords: CIAS-MS, TRPM2, Kir4.2, ion channels, collisional induced activation

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High-throughput cell phenotype screening coupled with ion mobility spectrometry of American *Aconitum*

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Aconitum plants have been widely used as traditional medicine in Asian countries for centuries. However, there are few records about how American *Aconitum* has been used medicinally, despite its phylogenetic closeness to medicinally important Asian species (Luo et al., 2005). This study aims to discover the bioactive compounds in American *Aconitum* based on a systematic molecular network strategy integrating both mass spectrometric data and a high-throughput phenotype screening assay, cell painting. The chemical profile in different parts of two American *Aconitum* species was obtained by ion mobility spectrometry technique and compared with non-American *Aconitum* species. Image analysis and feature extraction were performed on four different concentrations of *Aconitum* extracts assayed in cell painting, resulting in 2,090 unique morphological features per extract. In conjunction with the TargetMol library of 4,400 compounds with known mechanisms of action, 198 unique hierarchical clusters were established after a feature selection strategy known as Fast Correlation-Based Filtering, which reduced the number of features to 429. An overall activity heuristic called CP score was calculated for each sample. After integrating CP score and spectrometric data, a molecular network containing higher CP scores was constructed and the compounds with high activity were targeted and being identified. The molecular network showed that American *Aconitum* is more active than non-American species in the cell painting assay.

Keywords: *Aconitum,* cell painting, mass spectrometry, molecular network, drug discovery

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Antibiotic discovery from a bacterial colony: isolation of new peptidic antibiotics using robotics, 3D printed bioassays, and MS-based bioinformatics

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Traditional front end antibiotic discovery methods such as microbial library generation, strain fermentation, and in vitro screening of natural product fractions have afforded most antibiotics currently used in the clinic (Newman et al, 2020). However, in the past few decades these methods have been far less likely to yield new therapeutic leads (Koehn et al, 2005). Further, as pharmaceutical companies continue to divest from antibiotic discovery it is becoming increasingly important for academic labs to fill this gap. In order to address this, our lab has developed several methods designed to increase the throughput and efficiency of academic antibiotic discovery programs. These methods focus on analysis *of single bacterial colonies* in the initial steps of a discovery program and include i) use of front-end, untargeted robotic colony picking; ii) development of novel, reusable, 3D printed high-throughput agar competition bioassays; and iii) integration of *i* and *ii* with our open-access MS-based bioinformatics platform as a means to prioritize hits and reduce sample redundancy. This pipeline led to the discovery of a rare class of tetronate polyketide antibiotics active against *Staphylococcus aureus* and *Borrelia burgdorferi* (Lee et al, 2023), in addition to novel peptides that exhibit activity against *S. aureus* (led by a middle school student in partnership with a community center in Chicago). The discovery of these new antibiotics from two strains will be presented in the context of introducing an innovative, efficient front end antibiotic discovery pipeline designed for academic labs.

Keywords: antibiotics, bacteria, MALDI-TOF, robotics, bioinformatics

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Bioactive triterpenic acid metabolism investigation through mass spectrometry approaches

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Despite the well-established therapeutic value of natural products, their valorization by the pharmaceutical industry in recent years has fallen behind. One example of this phenomenon is the natural resin of the endemic Mediterranean shrub *Pistacia lentiscus* var. *Chia,* a unique PDO product produced exclusively in the southern part of the Greek island of Chios. Chios mastic gum's (CMG) ethnopharmacological background dates back to antiquity, while modern research and a monograph published by the EMA further validate its traditional use. CMG's bioactivity has mainly been attributed to its unique triterpenoids, and most notably to its characteristic compounds, namely masticadienonic and isomasticadienonic acids; nevertheless, CMG has been largely limited to the supplement stage, with its isolated constituents having been poorly investigated (Pachi et al., 2020).

Taking the above into consideration, the current research aims to shed light on this unique phytotherapeutic product, with data obtained from *in vitro*, *in vivo* and human trials, with special focus on bioavailability and metabolism aspects. Through the exploitation of recent technological advances in the fields of mass spectrometry and metabolomics, we attempt to divulge the triterpenoids' fate within the gastrointestinal tract, as well as to propose structure – activity relationships that could be further applied to the versatile triterpenic skeleton in general. Overall, our results suggest that the presence of circulating triterpenic acid metabolites in plasma might hint to the idea that the true active forms of CMG's constituents are none other than biotransformation products, created by Phase I and II metabolic reactions within the host's organism.

Keywords: mastic, mass spectrometry, metabolism, metabolomics, triterpenic acids

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Applications of ChemGPS-NP in natural product research – exploring the Outer Rim

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The concept of chemography, *i.e.* navigating chemical space, has over the last decade been applied to a number of studies of natural products.

It was early demonstrated that proximity in the ChemGPS-NP eight-dimensional physico-chemical property space can be interpreted as a measure of molecular similarity (Rosén et al., 2009). In subsequent studies it was further concluded that this measure provided additional information to that of other commonly used methods *e.g.* structure fragment based molecular fingerprints (Buonfiglio et al., 2015).

One of the most appealing aspects of chemography is that patterns can be readily visualized. This can also be combined with methods to define volumes from asymmetric 'clouds' of compound representations. Many studies up to date have been focused on small, low molecular weight, druglike molecules. However, we are now exploring the outer rim of this chemical property space including sets of polypeptides, polyketides and other larger and highly aberrant natural products.

In this study some implications of such exploration are demonstrated and discussed.

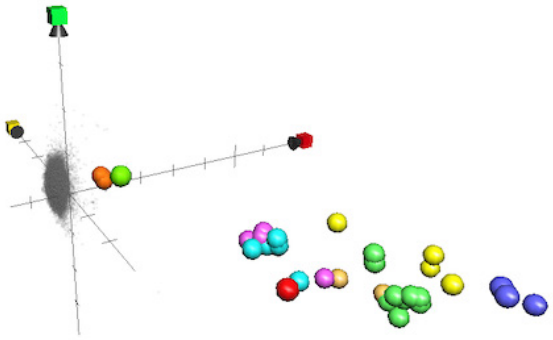

Fig. 1. ChemGPS-NP-based analysis for a series of larger natural product molecules, including representatives of groups of polypeptides, combined with low molecular weight compounds from the Maybridge screening set (gray). In the score plot of the three dimensions PC1 (red box) describes size and shape; PC2 (yellow box) the aromatic- and conjugation-related properties; and PC3 (green box) lipophilicity, polarity, and H-bond capacity.

Keywords: Chemography, ChemGPS-NP, polypeptides, polyketides, hyper-dimensionality

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Addressing the challenge of protein target identification of bioactive phytochemicals with a yeast-3-hybrid screening

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Although phytochemicals are nature's treasure box and are intensely studied for their biological effects in human cells, knowledge on direct protein interactions is scarce. A small molecule may have strong biological effects, but its protein target and molecular mechanism may remain unknown. These are, however,essential for developing the compound as a possible drug candidate (Bunnage *et al.*, 2015). Indeed, affected networks and downstream or upstream signaling pathways are often the missing link to completely understand the mechanisms leading to the activity of a small molecule and its cellular effects.

Un-biased target identification, or 'target fishing', is a challenging and often laborious task (Tabana *et al.*, 2023). Not only does the interaction partner need to be identified and selected out of a vast pool of potential hits obtained *via* diverse screening methods, the confirmation and validation of a putative target requires expertise in a variety of fields, depending on the target.

A potent yet complementary method to classical chemical proteomic approaches consists in the yeast three-hybrid (Y3H) system. The Y3H system was first described by Licitra and Liu in 1996 (Licitra *et al.*, 1996), and offers several benefits over affinity based methods for finding protein targets, one of the most important one is the fact that it is an *in vivo* method. In our lab, we have recently set up an improved Y3H system, and have demonstrated its power with the identification of a previously un-known target of the widely used contraceptive ethinylestradiol (Wang *et al.*, 2022).

We are now ready to apply the Y3H system as target identification platform for natural products, and are further planning to improve the versatility and usefulness of the system towards natural products.

Keywords: target identification, yeast-3-hybrid, phytochemicals

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Remediation of plant extracts using liquid-liquid chromatography: removal of pesticides and tetrahydrocannabinol from hemp extracts as a case study

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Liquid-liquid chromatography (LLC) is a preparative separation technique where the upper and lower phases of a biphasic solvent system are used as the mobile and stationary phases. Typically, LLC is employed for the purification of target compounds, such as bioactive natural products (Luca et al., 2020, 2021). In this work, we propose a model-based approach for the remediation of plant extracts with LLC. As a case study, we have selected the removal of intrinsic (i.e., tetrahydrocannabinol/THC) and extrinsic (i.e., pesticides) toxicants from hemp extracts. By taking advantage of a fully predictive thermodynamic model (COSMO-RS), a considerable number of biphasic solvent systems were initially screened. After validation in shake-flask experiments, three solvent systems were chosen: heptane/methanol/water (I), heptane/acetone/water (II), and heptane/acetonitrile/water (III). For pesticide remediation, a classification system ranking 59 pesticides in terms of their difficulty to be removed from hemp extracts was proposed. It was shown that the pesticide classification system can be used to select the appropriate solvent system for removing the majority of the contaminating pesticides. For THC remediation, various LLC operating modes were evaluated. Batch elution mode (conventional pulse injection) provided a THC-free hemp extract with a good productivity, whereas trapping multiple dual mode showed a lower productivity than batch. The sequential (continuous) mode afforded a THC-free extract with a similar composition to the batch mode and offered the advantage of continuous operation and superior productivity. The proposed approach is general and can be explored for the remediation of other contaminants from natural products.

Keywords: *Cannabis sativa* L., cannabidiol, tetrahydrocannabinol, pesticides,

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nmrXiv enhances the utility of NMR spectroscopic data of natural products

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Nuclear magnetic resonance (NMR) spectroscopy is a key tool in natural product (NP) analysis for the identification of unknowns, dereplication of knowns, metabolomic analysis, determination of relative and absolute configuration, and relative and absolute quantitation even of unweighable samples. Following the development of various first-generation databases for NMR data, recent efforts aim at broader scientific communities, seeking to enable direct data depositions connected to publications and projects. Our nmrXiv project, hosted at nmrXiv.org, addresses the particular needs of the NP and metabolomics communities by fostering FAIR principles of original data sharing, open licences, as well as structure and feature search capabilities.

A host of remarkable features makes nmrXiv stand out. At its core, nmrXiv serves as a versatile and long-term data archive, fostering collaboration and transparency in the scientific community. The advanced search and retrieval functionalities of nmrXiv empower researchers to access relevant NMR data and expedite their work. nmrXiv strongly emphasises open access and advocates for open science approaches that enhance rigor and reproducibility and drive innovation. Utilising state-of-the-art web tools, the nmrXiv platform fosters collaboration and knowledge sharing among researchers by creating an interconnected community rather than being repository-/storage-only. Stringent measures for data standardisation and quality control ensure the reliability and reproducibility of deposited data. Integration with popular NMR data formats and Electronic Lab Notebooks streamlines data processing and entry and promotes consistency. An interactive NMR spectra visualisation tool, NMRium [1], provides instrument-independent data processing capabilities and facilitates NMR data interpretation. Compliance with metadata standards (DataCite and BioSchemas) enhances the utility of NMR datasets.

nmrXiv is fully accessible to the global NP research community. It offers authors a streamlined submission tool for raw NMR data associated with publications, helping them to comply with contemporary FAIR requirements set forth by major publishers and funding agencies. Our contribution will explain the key tools available in nmrXiv and show how specifically NP researchers can benefit from engaging in both sharing of their own data as well as analyzing and mining data shared by fellow scientists.

Keywords: raw NMR data initiative, FAIR principles, data sharing

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Natural product-inspired chemical biology: development of highly sensitive labeling reagents for amino acids and scarce natural products

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Scarce natural products that possess unique biological activities have been ideal drug leads for decades. Among them medium-sized peptide molecules have received much attention as good motifs in drug design as they have intermediate properties between those of small molecule pharmaceuticals and antibodies. However, their identification and structural determinations are problematic owing to sample amount limitation.

Inspired by an extremely rare marine natural products yaku'amides, our highly sensitive labeling reagents L-FDVDA (1-fluoro-2,4-dinitrophenyl-5-L-valine-*N*,*N*-dimethylethylene-diamineamide) and L-FDLDA (1-fluoro-2,4-dinitrophenyl-5-L-leucine-*N*,*N*-dimethylethylene-diamineamide) that are powerful tools for detection of chiral amino acids were recently developed. By fusion of the Marfey's reagents with the key C-terminus structural motif of yaku'amides, the detection sensitivities of the reagents L-FDVDA and L-FDLDA for proteinogenic amino acids and non-proteinogenic amino acids such as all structural isomers and enantiomers of aminobutyric acid were drastically enhanced in LC-MS analysis, whose method named "the highly sensitive-advanced Marfey's method" (Kuranaga et al., 2020). Additionally, our method enabled to separate and identify the D/L-forms of short-chain peptides as well as the amyloid b fragment peptides with racemised and isomerised aspartic acid residues (Ozaki et al., 2023).

Our sensitivity-enhancement design concept was also applicable to the development of reagents for labeling saccharides and reactivity-guided isolation of electrophilic scarce natural products. we succeeded in detection of presaccharothriolide Z, the 10-membered microbial macrolide anticancer produced by a rare actinomycete *Saccharothrix* sp. A1506, using our original molecular probe A, and accomplished the total synthesis of presaccharothriolide Z (Kuranaga et al., 2021).

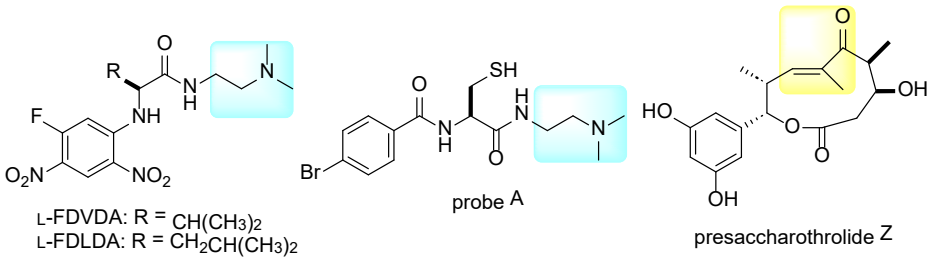

Fig. 1. Structures of L-FDVDA, L-FDLDA, probe A, and presaccharothriolide Z

Keywords: labeling reagents, LC-MS, amino acids, scarce natural products, chemical biology

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Role of Metabolomics in natural product discovery and chemical ecology

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Microbes whether commensal or pathogenic respond to the surroundings via small molecule natural products. Many of these small molecules are inspired by the biochemical modification of simple molecules that are an integral part of our cell biology, yet their discovery is challenging. This presentation will cover the development and application of mass spectrometry-based untargeted metabolomics methods for the discovery and dereplication of small molecule natural products mediating microbe-drug, microbe-microbe, and microbe-host interactions. By coupling untargeted metabolomics with specific microbial phenotypes such as response to antimicrobial compounds, chemoinformatic methods such as molecular networking, sub-structure and chemical class identification, isotope labeling in cell culture, we will describe discovery of a strain specific pathway involved in inactivation of the antibiotic trimethoprim and the production of homogentisic acid derived natural products from a human mucus-dwelling microbial pathogen. Second application of these methods will describe the discovery of a siderophore degrading pathway mediating an interaction between coral mucus-dwelling commensal and pathogenic microbes. Finally, challenges and opportunities of mass spectrometry-based metabolomics will be discussed in discovery of natural products and their role in chemical ecology across biological systems.

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Session IIA: Total synthesis and biomimetic synthesis of natural products

Topical Lecture

Olea europaea: a compelling source of health promoting biomolecules and an inspiration for the semi-synthetic design of bioactive analogues

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Olea europaea and its main products olive oil and olive drupes, have attracted scientific attention in recent years because of its biological activities and its attribution in many aspects of human health. Although olive oil primarily consists of oleic acid (up to 80 %) and other fatty acids, some minor phenolic compounds, comprising the 1-2 % of the total content, are generally considered to be responsible for the various health benefits of olive oil. The most characteristic compounds in this group are the simple phenylethyl alcohols such tyrosol, hydroxytyrosol, the seco-iridoids oleacein and oleocanthal, the decarboxylated aglycons of oleuropein and ligstroside respectively. These two compounds, and especially oleocanthal, have been identified as the agents responsible for the pungency of extra virgin oil. Oleocanthal's recent discovery as COX inhibitor, with similar effect to that of ibuprofen, has dramatically increased its interest both for the study of biological properties but also for the development of new non-steroidal anti-inflammatory drugs based on its structure. Additionally, according to many data, both compounds demonstrated promising anticancer and neuroprotective activities with no toxic effects. Prompted by the outstanding interest of these high-value natural compounds we have developed of a concise and scalable procedure for the synthesis of various analogues. The synthesis is performed by a convenient biomimetic and stereo-controlled approach, starting from oleuropein, isolated from olive leaves, an abundant biorenewable raw material and hydroxytyrosol the major compound of olive mill waste water.

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Synthetic homoisoflavonoids to treat wet-stage macular degeneration

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The occurrence of ocular angiogenesis resulting in choroidal neovascularisation (CNV) is the key process that leads to the wet-stage form of macular degeneration (wet AMD). This debilitating disease is the main cause of blindless in the aging population, but many patients are resistant to current therapies (Schwikkard et al., 2019). Existing treatments for wet AMD target the pro-angiogenic vascular endothelial growth factor (VEGF) pathway and consist of monoclonal antibodies and decoy receptors to VEGF. Due to the high molecular weights of these biologics, treatments are administered via intravitreal injection, a painful and costly process associated with undesirable side effects such as ocular haemorrhage (Tolentino, 2011). Thus, the discovery of a small molecule biologic to treat wet AMD could provide a less invasive route of administration, and potentially tackle the problems associated with current therapies. Homoisoflavonoids are a class of naturally occurring compounds often isolated from plants in the Hyacinthaceae family, whose *Eucomis* genus initially gained attention from researchers due to its use by African traditional healers (Mkhumbeni et al., 2022). Both naturally derived and fully synthetic homoisoflavonoids have previously been shown to exhibit anti- angiogenic effects against human retinal endothelial cells (HRECs) *in vitro* (Schwikkard et al., 2019). This provides an exciting opportunity for the potential to prevent CNV. Here, we describe the full syntheses of non-naturally derivatised homoisoflavonoids, many of which exhibited anti-angiogenic effects, with growth inhibitory concentrations (GI_{50}'s) as low as 1.48 μM against the proliferation of HRECs *in vitro*.

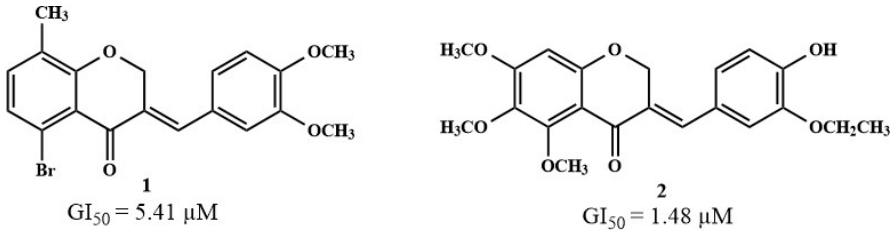

Fig 1. Synthetic homoisoflavonoids and their GI_{50}'s against the proliferation of HRECs.

Keywords: full chemical synthesis, homoisoflavonoid, macular degeneration, anti-angiogenic activity

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Minor Amaryllidaceae alkaloids as inspiration for development of highly selective butyrylcholinesterase inhibitors: design, synthesis, and biological evaluation

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Plants of the family Amaryllidaceae have a long history of usage as herbal remedies all over the world to cure different ailments and diseases. Since the isolation of the first Amaryllidaceae alkaloid (AA), lycorine, more than 600 AAs have been isolated and studied in terms of their biological activities. Within the last 10 years, more than 100 AAs of different structural types have been isolated in our research group from the genera *Zephyranthes*, *Narcissus*, *Nerine*, and others. Isolated compounds have been tested for biological activity connected with the potential treatment of neurodegenerative, oncological, and infectious diseases. Newly isolated dimer alkaloids from *Narcissus pseudonarcissus* cv. Carlton, named carltonine A and B, showed strong selective inhibition activity against butyrylcholinesterase (BuChE) in nanomolar range (IC_{50} = 31-910 nM) (Mamun et al., 2020). Unfortunately, these alkaloids are present in plant material only in trace amounts, and they cannot be isolated for either more detailed biological investigation or commercial use. Thus, we decided to preserve some of the crucial structural requirements from carltonine A/B that are plausibly responsible for high BuChE inhibition activity, i.e. the 4-[2-(benzylamino)ethyl]phenol moiety, and modify other molecular regions to elucidate SAR. Up to date, more than 120 compounds, structurally inspired from minor AAs carltonine A and B, have been designed, synthesized, and evaluated for their acetylcholinesterase (AChE) and BuChE inhibition properties. Compounds that revealed BChE inhibition below 100 nM were selected for detailed biological investigation. The CNS-targeted profile of the most active compounds was confirmed theoretically by calculating the BBB score algorithm, these data were corroborated by determining the permeability *in vitro* using PAMPA-assay for the most active derivatives. Moreover, a crystallographic study was performed to inspect the binding mode of compound **87**, revealing essential interactions between **87** and hBChE active site (Pidany et al., 2023).

Keywords: Amaryllidaceae, butyrylcholinesterase, PAMPA-assay, crystallographic study

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Naturally based prototypes for vector-control: synthesis and modification of carlina oxide scaffold

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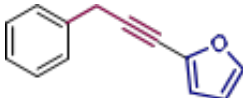
Vector-borne diseases cause public health concern worldwide and vector-control represents an everlasting challenge (Chala and Hamde, 2021). Thus, the research for novel effective tools is encouraged and compounds from botanical sources are gaining more interest as alternatives to conventional insecticides (Isman, 2020). Carlina oxide (**1**), isolated from the roots of *Carlina acaulis*

L. (Asteraceae), resulted effective against insect vectors, agricultural and stored-product pests (Spinozzi et al., 2023). Nevertheless, the plant species protection in several countries and the lack of a cultivation system are the main limiting factors for the exploitation of this molecule. This presentation will cover the development of a synthetic protocol to produce **1** (Fig. 1) through a one- step Sonogashira coupling and the synthesis of analogues featuring modifications to the chemical scaffold of the parental compound. These modifications were applied on the furan moiety and on the propynyl linker.



1

Fig. 1. Carlina oxide (**1**) scaffold.

The compounds were tested for acute toxicity on larvae of *Aedes albopictus* (Skuse) and *Anopheles stephensi* Liston and their safety profile was assessed on human keratinocytes (HaCaT) through MTT assay. Among the compounds tested, the analogue bearing the butynyl linker resulted more effective on the two insect species (LC_{50} of 5.8 and 3.1 µg/mL on *Ae. albopictus* and *An. stephensi*, respectively) than its natural counterpart also displaying lower cytotoxicity (IC_{50} > 100 µg/mL). The results demonstrated the possibility of synthesizing **1** and producing analogues with improved bioactivity and reduced cytotoxicity on keratinocytes.

Keywords: Vector-control, *Carlina acaulis* L., carlina oxide, synthesis, larvicidal

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Design and anti-inflammatory evaluation of ellagitannin-derived postbiotic metabolite conjugated with serotonin and dopamine

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Urolithin A (UA) is a postbiotic metabolite produced by gut microbiota following oral administration of ellagitannin-rich plants, and it is known for its significant anti-inflammatory, anti-cancer, and anti-aging biological activities (García-Villalba et al., 2022). However, UA conjugation with glucuronic acid *in vivo* leads to the production of metabolites with reduced biological activity (Bobowska et al., 2021). In response to UA's limitations, a series of UA derivatives (UADs) have been synthesized through conjugation with nonsteroidal anti-inflammatory drugs, with their identity, stability, anti- inflammatory efficacy, and intestinal bioavailability being thoroughly assessed (Korczak et al., 2022, 2023). Recently, the synthesis of novel UADs linked to neurotransmitters, specifically serotonin (UASer) and dopamine (UADop), was proposed. The purity and identity of obtained conjugates were verified through HPLC-MS/MS and NMR. The cytotoxicity and anti-inflammatory effects of these novel UADs were examined using immortalized bone marrow-derived macrophages. qPCR experiments showed that the mRNA levels of pro-inflammatory markers such as IL-1β, IL-6, iNOS, and TNF-α were significantly reduced by both UASer and UADop. Their influence on NF-kB signaling was also investigated using a NF-kB LUC – HEK reporter cell line. Additionally, preliminary *in vivo* studies on *Caenorhabditis elegans* were conducted to assess, among others, toxicity and potential life span extension by these newly synthesized compounds. In conclusion, the structural modification of natural products-derived postbiotic metabolites is presented as a promising approach, serving as a foundation for further investigations into their application in neuroinflammation.

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Keywords: ellagitannins, urolithin A, gut microbiota, neurotransmitters, inflammation

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Session IIB: Genetic engineering of food crops and medicinal plants

Topical Lecture

Unravelling the fitness programs that steer plant metabolism allows creating novel tools for the engineering of plant-based production of bioactive metabolites

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Nature has invented strict, yet not fully understood, regulatory networks that control plant metabolism. These networks safeguard plant fitness in a continuously changing environment. By investigating the reprogramming of plant metabolism by developmental and environmental cues, we aim to advance our fundamental understanding of the mechanisms that steer plant metabolism. We specifically focus on jasmonate (JA), the phytohormone that steers the delicate balance between growth and defense programs across the plant kingdom, including the production of bioactive specialized metabolites. In parallel, this enables us to unlock plant specialized metabolism for numerous human applications given that our findings serve simultaneously as a novel resource for engineering tools that facilitates the creation of (plant-based) synthetic biology platforms for the sustainable production of high-value plant metabolites. I will exemplify this by reporting on our recent work on the biosynthesis of bioactive metabolites in model, crop and medicinal plants.

Keywords: plant metabolism, biosynthesis of bioactive metabolites

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Towards the engineering of the plant endoplasmic reticulum for sustainable production of specialized metabolites

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Plants are among the most creative survivors on the planet. They have optimized their own defence mechanisms against attackers, typically by producing a species-specific arsenal of specialized metabolites. These metabolites not only play vital roles within the plants themselves but also offer a multitude of applications as pharmaceuticals, fragrances etc. However, industrial production of these valuable compounds faces many bottlenecks and often requires intensive adaptations. To facilitate sustainable large-scale production of plant metabolites, we aim to develop a universal tool to boost plant-based production of specialized metabolites. Our strategy is based on recent findings in yeast, in which engineering of the endoplasmic reticulum (ER), which harbours a main part of the enzymatic machinery for many metabolite biosynthesis pathways, results in an enlarged ER size and an increased metabolite accumulation (Arendt et al., 2017). By interfering with the phospholipid biosynthesis pathway, a higher production of building blocks for the ER-membrane and ultimately an enlarged ER were achieved. Our project aims to translate the findings from yeast to plants. Thus far, we have obtained an increased accumulation of phospholipid building blocks in tomato (*Solanum lycopersicum)* roots, by knocking out the *PHOSPHATIDIC ACID PHOSPHOHYDROLASE* (*PAH*) genes. Currently, we are investigating whether this correlates with an increased production of bioactive specialized metabolites. Additionally, we are also exploring the potential of ER engineering by overexpressing other key phospholipid biosynthetic enzymes. With our obtained *in planta* results, we believe that ER engineering holds great promise for a more efficient and sustainable production of plant specialized metabolites.

Keywords: *Solanum lycopersicum*, endoplasmic reticulum, phospholipids, engineering, roots

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Session IIC: Medicinal natural products: from bench to bedside

A new cytotoxic diphenazine from a deep-sea fungus *Cystobasidium laryngis*

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We report here the isolation, structure elucidation, and anticancer and anti-neuroinflammatory activities of phenazostatin J (**1**) from *Cystobasidium laryngis* isolated from a deep-sea sediment sample taken at the Indian Ocean Ridge along with semi-synthesis of **1** and *in vivo* test result against the human stomach cancer line (NUGC-3). The *Cystobasidium* genus has been reported as one of the uncommon fungi in deep-sea environments (Wei et al., 2018). In addition, no studies were conducted on natural products from *Cystobasidium* sp. until we first reported new metabolites from the strain in 2019 (Lee et al., 2019). As part of our ongoing search for bioactive compounds from an extract of culture broth of *C. laryngis*, we additionally isolated six new diphenazine derivatives, phenazostatins E–J, and evaluated their anti-neuroinflammatory and cytotoxic activities (Lee et al., 2022). Among the isolated new diphenazines, only phenazostatin J (**1**) exhibited strong anti-neuroinflammatory (IC_{50} = 0.30 μM) and cytotoxic (IC_{50} = 7.7 nM) activities against the NUGC-3 cell line.

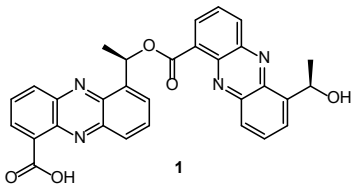

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Fig. 1. Structure of phenazostatin J.

To conduct an *in vivo* test against the NUGC-3 (stomach) cell line, we successfully semi-synthesized **1** using saphenic acid which is a monomer of **1**. Phenazostatin J (**1**) was administered intraperitoneally at different doses (1, 2, and 4 mg/kg) using a nude mouse subcutaneous model. An *in vivo* study demonstrated that **1** suppressed tumor growth in nude mice carrying NUGC-3 cells without significant secondary adverse effects. Tumor sizes and weights were reduced when treated with 4 mg/kg of **1**, with similar results to doxorubicin (2 mg/kg), a positive control. These results suggested that **1** has therapeutic potential as an anticancer candidate that deserves further investigation.

Keywords: *Cystobasidium laryngis*, phenazostatin J, semi-synthesis, cytotoxicity, *in vivo* test

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Safety and efficacy of topical Arnica tincture for the treatment of uncomplicated human cutaneous leishmaniasis

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Cutaneous leishmaniasis (CL) is classified as a neglected tropical disease for which new effective and safe therapeutic options are required. Arnica tincture (AT) is a herbal medicinal preparation with anti-inflammatory activity which is used traditionally for the topical treatment of blunt injuries among other uses. Its main bioactive constituents are sesquiterpene lactones (STLs) of the helenalin and 11α,13-dihydrohelenalin types. In our previous work, AT showed promising antileishmanial effects *in vitro* and *in vivo* (Robledo et al., 2022) and Glutathione conjugation plays a major role in the metabolism of these STLs (Jürgens et al., 2022a). Furthermore, *in vivo* dermal absorption, pharmacokinetic, and toxicity studies with STLs from AT were also reported (Jürgens et al., 2022b). As part of our continuing search for new alternatives to treat CL, an open-label, randomized, non-comparative phase Ib/II clinical trial was performed (NCT05094908). Adult volunteers with a parasitologically confirmed diagnosis of CL were randomly allocated to receive AT 3 times per day either for 4 or 6 weeks. Up to now (Feb. 2024), 7 out of 9 subjects screened have been included in the study. Cure has already been observed in all subjects in the 4- and 6-weeks group respectively (Fig. 1). Four patients so far have finished the 6-month follow-up period without showing relapses. The patients still under observation also demonstrate promising results. Furthermore, AT proved to be safe, and the adverse events reported are local, around the area of application of the tincture, and of mild intensity.

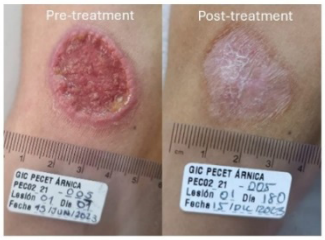

Fig. 1. Appearance of CL before and after treatment with AT

In conclusion, AT appears to be an efficacious, safe and well-tolerated intervention to treat CL.

Funding: This research was funded by the Wilhelm Doerenkamp-Foundation, grant specification: NATVANTAGE RESEARCH GRANT 2018.

Keywords: *Leishmania braziliensis,* clinical trial, sesquiterpene lactones, therapeutical response, adverse effects

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Mesembryanthemum tortuosum improves Parkinson's Disease symptoms in a zebrafish larvae model

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterised by the loss of dopaminergic neurons in the brain. The exact cause of PD is still not fully understood however, oxidative stress, inflammation, and genetic and environmental factors have been implicated (Olanow and Tatton, 1999). The pathological repertoire of PD can be induced in zebrafish larvae using reserpine (Puttonen et al., 2017). Currently, PD has no cure, however, medicinal plants such as *Mesembryanthemum tortuosum* with documented central nervous system properties may provide some benefit. The aim of the study was to evaluate the neuroprotective and neurorestorative effects of *M. tortuosum* extracts on reserpine-induced deficits, using a zebrafish larvae model. To achieve this, methanol and alkaloid-rich extracts were prepared from *M. tortuosum* raw material, while Zembrin® (commercial extract of *M. tortuosum*), the two alkaloids (mesembranol and Δ^7 mesembrenone) and L-dopa (positive control) were commercially sourced. Zebrafish larvae were treated with the extracts and the effect on locomotion, reactive oxygen species (ROS) and total glutathione (tGSH) was monitored (Fig. 1).

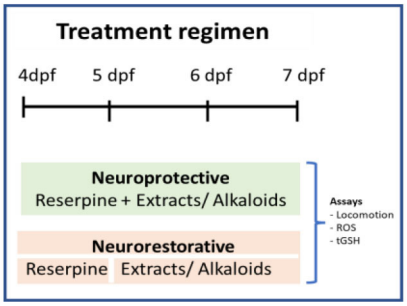

Fig. 1. The model for neuroprotective and neurorestorative treatment regimen

The results demonstrate that Zembrin® exhibited superior anti-PD activity by improving locomotion, reducing ROS and subsequently increasing tGSH in both the neurorestorative and neuroprotective assays. The alkaloid-rich extract did not improve locomotion but increased tGSH. The individual alkaloids enhanced movement, although not to the same extent as L-dopa and Zembrin®. *Mesembryanthemum. tortuosum* extracts and alkaloids improve reserpine-induced PD like effects in a zebrafish larvae model.

Keywords: *Mesembryanthemum tortuosum,* parkinson's disease, reserpine, zebrafish larvae, locomotion

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*Presenting author. E-mail address: LepuleKH@tut.ac.za (Keagile Lepule).

Tailoring plants as photosynthetic production chassis for small molecules

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Plant natural products are utilised in many industries including as dyes, scents, pharmaceuticals and agrochemicals. Chemical synthesis has provided easy and cheap access to some molecules but remains challenging or uneconomical for many molecules. In the past decade, aided by advances in genomics and synthetic biology, biosynthetic pathways have been elucidated and rebuilt in so-called 'chassis organisms'. To date, most work has been done in microbes, in which it has been shown that yields can be maximised by engineering precursor availability, balanced expression of pathway genes, and removing enzymes that compete for substrates or derivatise intermediates (Cravens et al., 2019). The model plant *Nicotiana benthamiana* is an increasingly attractive organism for the heterologous biosynthesis of high-value, biologically active molecules (Stephenson et al., 2019), however little has been done to optimise production. Previously, our lab observed that the relative positions of genes within synthetic constructs affects both transient and transgenic expression as well as product yields (Kallam et al., 2023). Further, genome editing techniques can be used to remove molecules and enzymes that interfere with production and purification techniques (Dudley et al., 2022; Vollheyde et al., 2023). In this study, we investigate how the expression of synthetic genes are affected by their neighbours (Fig.1A), and how knowledge of enzyme kinetics and rate limiting steps can be used to improve construct design. We also describe the production of genome-edited plants with multiplexed mutations (Fig.1B), tailored for triterpene production.

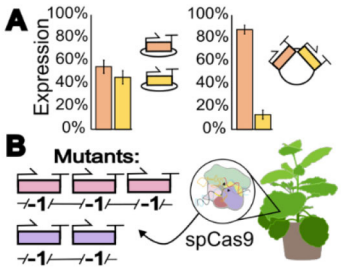

Fig. 1. Optimising natural product biosynthesis in plant chassis (A) The expression of synthetic genes is affected by transcription of upstream genes and can inform optimal construct designs (B) Genome editing of competing metabolic pathways.

Keywords: metabolic engineering, *Nicotiana benthamiana, gene expression, synthetic biology, triterpenes*

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Session III: Natural cosmetics, nutraceuticals, and food supplements

Topical Lecture

How to harness the potential of eutectic solvents for the formulation and enhancement of natural ingredients

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Natural deep eutectic solvents (NaDES) are being studied as an alternative to conventional solvents for preparing cosmetic ingredients from biomasses. These solvents are prepared by mixing plant cellular constituents, such as sugars, or amino acids. Their unique hydrogen bond network provides them high extraction power and the ability to stabilize sensitive active ingredients. To provide innovative natural cosmetic active ingredients, NaDES used must comply with international cosmetic regulations (Europe, China), which excludes NaDES based on choline chloride. Judiciously chosen NaDES-based extracts could be included directly in cosmetic products, making the solvent removal step in the extraction process unnecessary. Therefore, it is necessary to investigate the impact of various types of NaDES on the stability of active ingredients and the properties of cosmetic products. We will present the extraction and formulation of two examples of cosmetic active ingredients in a eutectic environment. First, we will examine an example of phycobiliproteins enriched extract, derived from microalgal biomasses. These extracts were prepared using an innovative solid/liquid/liquid extraction/purification approach (Hilali et al. 2024). The purified fractions were then incorporated into a gel formulation, demonstrating the successful stabilization of fragile phycobiliproteins within the gel. Additionally, sensory properties were enhanced. The following section presents an active ingredient from marigold (*Calendula officinalis*) that contains flavonoids and carotenoids. This ingredient is obtained through an innovative mechanical process that does not involve heating and is then incorporated into a cream formulation. (Boudesocque et al., 2024). These studies showcase the potential of eutectic solvents for extracting and formulating cosmetic ingredients.

Keywords: Eutectic solvent, natural cosmetic, natural active ingredient, sensory, stability

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Soukaina Hilali, Louise Van Gheluwe, Mervé Yagmur, Laura Wils, Myriam Phelippe, Barbara Clément-Larosière, Bénédicte Montigny, Johan Jacquemin, Emilie Thiery, Leslie Boudesocque-Delaye, NaDES-based biorefinery of Spirulina (Arthrospira platensis): A new path for sustainable high value-added metabolites, Separation and Purification Technology, 2024, 329, 125123.

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A multi-target approach to evaluate the efficacy of a capsiate rich fraction of *Capsicum annuum* fruit against typeII diabetes and obesity

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The fruits of a nonpungent cultivar of *Capsicum annuum* are rich in capsiate which is a nonpungent analog of capsaicin. This study was carried out to investigate the effects of a capsiate-rich fraction of *C. annuum* (germplasm 509-45-1) fruits (CR) on various nuclear receptors involved in multiple signaling pathways related to carbohydrate and lipid metabolism. The effects of CR on the muscular glucose uptake and adipogenesis were also determined to evaluate its anti-diabetic and anti-obesity properties.

At a concentration of 100 µg/mL, CR caused the activation of PPARα, PPARγ, LXR and NRF2 in hepatic cells (2 to 4-fold increase). CR was effective in decreasing adipogenesis and caused a 23% decrease in lipid accumulation in adipocytes. Moreover, the adipogenic effect induced by rosiglitazone was also antagonized by CR. Further studies in differentiated muscle cells indicated an increase of glucose uptake by 30%. Gene profiling studies showed a decrease in C/EBPα and an increase of KLF4 expression.

In conclusion, the study revealed a strong agonistic action of CR on multiple nuclear receptors which was associated with enhanced glucose uptake and decreased fat accumulation indicating a potential utility of the capsiate-rich fraction of *C. annuum* as a natural product that could ameliorate the symptoms of diabetes and obesity without the adverse effect of pungency. As a multiple agonist of PPARα, PPARγ and LXR, CR may also prevent the undesired adipogenic effects of antidiabetic drugs that are full PPARγ agonists. Further studies are warranted in animal models of TypeII diabetes and obesity.

Keywords: *Capsicum annuum*, capsiate, nuclear receptors, adipogenesis, glucose uptake

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Cimicifuga racemosa extract Ze 450 mediates resilience against oxidative stress, inflammatory responses and UV-induced aging through metabolic rewiring

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Mitochondria are the key organelles of energy metabolism and play critical roles in neurodegenerative diseases, in (neuro-)inflammation and also in processes of aging. Impaired mitochondrial metabolism featuring dysfunctional oxidative phosphorylation (OXPHOS) is regarded as a hallmark in paradigms of cellular dysfunction and death. Here, we investigated the effects of the *Cimicifuga racemosa* extract Ze 450 in settings of mitochondrial disorders with specific focus on metabolic alterations mediated by the extract.

Employing a systems approach with proteomics, metabolomics and metabolic flux characterization, we examined the effects of Ze 450 on the overall metabolic phenotype in different model systems of disease. Ze 450 demonstrated a robust ability to induce a metabolic switch in hippocampal HT22 cells, in RAW 264.7 macrophages and primary mouse embryonic fibroblasts. This shift was associated with a decrease in mitochondrial respiration and an increase in glycolytic activity to meet the cellular energy demands. Importantly, the metabolic rewiring was responsible for the resilience against oxidative stress, but also for the prevention of the LPS-induced inflammatory response in mouse macrophages. Moreover, the preserved mitochondrial integrity and reduced ROS levels following reduced mitochondrial respiration mediated by Ze 450, mitigated UV-induced cell death and senescene in MEF cells. Strikingly, we identified the glutamine-derived α-ketoglutarate anaplerotic flux to be critical for the beneficial effects of Ze 450 against ferroptosis and inflammation.

Our results highlight the importance of mitochondrial function and dynamics in regulating cell fate decisions and anti-inflammatory functions, but also the potential of *Cimicifuga racemosa* extract Ze 450 to interfere these processes.

Keywords: Ze 450, *Cimicifuga racemosa,* metabolic reprogramming, mitochondria, ferroptosis, inflammation, uv-induced cellular damage

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Phenolic-rich *Cratoxylum cochinchinense* leaves extract: a promising natural cosmeceutical

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Human skin is frequently subjected to oxidative stress due to reactive oxygen species (ROS) and the lack of intracellular antioxidants. UV overexposure is one of the primary causes of oxidative stress, which accelerates inflammatory responses and the skin aging processes. Our previous work revealed that *Cratoxylum cochinchinense* leaves extract (CCE) possessed high total phenolic content along with strong antioxidative activities (Tan et al., 2022). In addition, mangiferin, a phenolic compound, was identified as the main phytochemical in CCE (**Fig. 1**).

Fig. 1. The leaves extract of *Cratoxylum cochinchinense* contained mangiferin as the main phytocompound

Hence, the aim of this study was to assess the potential of CCE as an anti-aging and sun protective agent. The anti-aging ability of CCE was examined using *in vitro* anti-tyrosinase and anti-elastase activity, while sunscreen ability was determined using *in vitro* SPF method. The anti-inflammatory efficacy of CCE was evaluated via a protein denaturation assay using bovine serum albumin (BSA). The plant extract was able to reduce tyrosinase activity by 38.95 ± 0.86% at 60 µg/mL. Moreover, CCE at similar concentration demonstrated anti-elastase activity as effective as the positive control, ascorbic acid, by reducing elastase enzymatic activity by 62.11 ± 4.17%. Moreover, a dose-dependent increment of SPF values was observed for CCE indicating its efficiency as a sunscreen agent. Anti-inflammatory effect was also demonstrated by CCE via inhibition of BSA denaturation. In conclusion, *Cratoxylum cochinchinense* leaves extract is indeed a promising cosmeceutical agent that could be incorporated into anti-aging skin care products.

Keywords: *Cratoxylum cochinchinense*, anti-aging, sun protective, anti-inflammatory, cosmetics

Reference

Tan, S.-A., Yam, H. C., Cheong, S. L., Chow, Y. C., Bok, C. Y., Ho, J. M., Lee, P. Y., Gunasekaran, B. 2022. Inhibition of *Porphyromonas gingivalis* peptidyl arginine deiminase, a virulence factor, by antioxidant-rich *Cratoxylum cochinchinense*: *In vitro* and *in silico* evaluation. Saudi J. Biol. Sci., 29(4), 2573–2581.

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Dual ^{13}C NMR and LC-MS^2 analysis for chemical profiling of Polynesian plant extracts

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Five Polynesian plants among the most traditionally used in cosmetopeia and pharmacopeia were studied: *Calophyllum inophyllum*, *Gardenia taitensis*, *Curcuma longa*, *Cordia subcordata*, and *Ficus prolixa*. Their extracts were obtained by eco-extraction performed by ultrasound-assisted extraction (UAE) using ethanol:water (7:3, v/v) solvent. Liquid-liquid partition of crude extracts using stepwise gradients (heptane, EtOAc, n-butanol, and water) led to four extracts, and EtOAc extracts were fractionated by centrifugal partition chromatography (CPC).

The dereplicative chemical profiling method, combining ^{13}C NMR and LC-MS^2 analysis, was performed on CPC fractions. NMR analysis were carried out and hierarchical clustering analysis (HCA) was performed on ^{13}C signals with the Permut Matrix application. The resulting map included several clusters corresponding to molecular structures (Hubert *et al.*, 2014). Custom databases were created with VersaDB (Cordonnier *et al.*, 2024) and used to assign compound structures for each cluster. Right structures were then confirmed manually by 2D NMR analysis. LC-MS^2 data were processed via MZmine 3. Molecular networks were built using GNPS Feature-based molecular networking (Nothias *et al.*, 2020) and annotation was performed with SIRIUS.

The intelligent use of these two complementary methods allowed accelerated and efficient characterization of main compounds in complex mixtures. The known chemical composition of plant extracts such as *C. longa* and *C. inophyllum* allowed the validation of this process. Compounds identified and annotated for *F. prolixa* and *C. subcordata* were described for the first time in these two indigenous Polynesian tree extracts.

This powerful strategy led to the quick characterization of cosmetics with antioxidant and inflammatory properties.

Keywords: NMR; LC-MS^2; centrifugal partition chromatography; molecular networking; dereplication

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Chemical composition and nutritional value of the Sonora gum

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The Sonora gum is an exudate produced by female insect *Tachardiella fulgens* Cockerell (Kerriidae), a host of the tree *Coursetia glandulosa* A. Gray (Fabaceae). The hard exudate (lac) encases the insect bodies and eggs and is valued as a medicinal and flavoring agent in the Northern gastronomy of Mexico. Previous investigations revealed the presence of lacaic acids in the lac, an unusual type of anthraquinones (Bisulca et al., 2018)

From an aqueous extract of the gum, a few specialized metabolites were isolated and characterized by spectrometric and spectroscopic analyses, including emodin, lacaic acid B, along with two new nerolidol derivatives, namely (10*S*,2*E*,6*E*)-10-hydroxy-2,6,10- trimetildodeca-2,6,11-trienóic acid and (2*E*,6*E*,10*Z*)-12-hydroxy-2,6,10-trimetildodeca- 2,6,10-trienoic acid.

Headspace–solid-phase microextraction technique was employed to determine the volatile composition of the Sonora gum using four different coated fibers. The volatilome was characterized by a high content of vinyl-γ-valerolactone, lilac aldehyde D, α-curcumene, and nerolidol.

According to the Association of Official Analytical Chemists standard methods (AOAC, 2023), proximate analysis was carried out to determine the nutrient composition of the gum. The average protein, fat, carbohydrate, moisture, ash, and fiber contents were found to be 14,3, 27, 0.5, 0.9, and 53 %, respectively. The energy value was 200 kcal/100g. The gum was characterized by a high phosphorous content, a key element in the energetic metabolism; the other minerals were present in the amount required for a balanced diet. Niacin and thiamine were the most important vitamins. Thus, the Sonora gum is a good source of natural minerals and other constituents essential in the human diet.

Keywords: *Tachardiella fulgens*, *Coursetia glandulosa,* (10*S*,2*E*,6*E*)-10-hydroxy-2,6,10- trimetildodeca-2,6,11-trienóic acid and (2*E*,6*E*,10*Z*)-12- hydroxy-2,6,10-trimetildodeca- 2,6,10-trienoic acid

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Cannabinoids and olive oil secoiridoids: a fascinating case of synergy

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This study presents an investigation of the synergistic pharmacological effects of cannabinoids, from *Cannabis sativa*, and secoiridoids, found in olive oil. These two categories of natural compounds share common pharmacological targets. TRPA1 channels, are upregulated in numerous pathological conditions such as cancer and neurodegenerative diseases (Bosson et al., 2017; Takahashi et al., 2018), are a potential synergistic target for these compounds. The study initially explored the synergistic modulation of TRPA1 channels by cannabinoids and secoiridoids, revealing enhanced efficacy through different binding sites interactions. Specifically, a mixture of oleocanthal and cannabidiol in a ratio of 3:1 yielded the best results. *In vitro* MTT assay was utilized to investigate the synergistic cytotoxic effect of these compounds on various cancer cell lines, including breast, liver, and melanoma lines. The identified synergistic effect based on the coefficient of drug interactions (CDI< 0.7) led to the *in vivo* evaluation of the antitumor effect in MDA-MB-231 xenograft model of cancer (50 mg/kg CBD/ 5 mg/kg secoiridoids). Electrophysiological recordings in hippocampal brain slices of mice revealed a synergistic action in the decrease of seizure-like activity upon simultaneous perfusion of the two compounds. Additionally, employment of electrophysiological recordings uncovered an increase in long-term potentiation suggesting an enhancement in synaptic plasticity following their perfusion. *In vivo* behavioral experiments using an Alzheimer's disease mouse model (5xFAD), indicated improved performance in several cognitive tasks. The acute oral toxicity of a mixture of oleocanthal and cannabinoids was also evaluated in mice, with the combination being nontoxic in doses up to 300 mg/Kg.

Keywords: cannabinoids, secoiridoids, cannabidiol, oleocanthal, TRPA1

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Lignosus rhinocerus extract ameliorates glutamate-induced neuronal toxicity

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Lignosus rhinocerus (LR) extract has been widely used in traditional folk medicine. However, its potential in anti-neurodegeneration has not yet been fully elucidated. So, this study was aimed at investigating the protective effects of LR extract on glutamate-induced toxicity. Cell viability, immunofluorescence staining, flow cytometry and Western blot analysis were performed *in vitro* using mouse hippocampal neuronal HT-22 cells. Moreover, the ability of likely active ingredients from LC-MS binding to NMDA receptor was analysed using *in silico* docking. Both ethyl acetate (LR-EA) and ethanolic (LR-EE) extracts of LR effectively decreased glutamate-induced neuronal apoptosis and intracellular ROS accumulation. Both extracts could significantly attenuate mitochondrial dysfunction and mitophagy overactivation corresponding to the decreased mitochondrial depolarization, the increased ATP production, and the modulation of mitophagy related proteins (LC3 and p62/ SQSTM1) and LC3 colocalization. Moreover, both extracts possessed neuroprotective effect, demonstrated by significant suppression of glutamate-induced ER stress through the decrease of calcium influx, the ratio of p-ERK/ERK, and CHOP expression. In addition, phytochemicals of both extracts and their competence on inhibiting NMDAR were resolved. *In silico* analysis, 4 phytochemicals (canthin-6-one, magnoshinin, royleanone, and cernuine) from LR-EA and 2 phytochemicals (cernuine and levopimaric acid) from LR-EE revealed that their binding to NMDAR was more efficient than the binding of NMDAR antagonist, memantine. Likewise, cernuine could be the possible active constituent in both LR extracts with the strongest binding energy against NMDAR. In conclusion, LR is potentially classified as a neuroprotectant with cernuine as an energetic component for the prevention of glutamate-triggered neuronal toxicity.

Keywords: *Lignosus rhinocerus*, glutamate-triggered neuronal toxicity, HT-22 cells, Neuroprotectant, cernuine

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Novel health potential of isomers of 13²-hydroxypheophytin a from *Arthrospira platensis*

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Spirulina is widely commercialized for its fitness and health benefits, however the compounds responsible for said activity are unknown. This work covers a bioassay-guided isolation and structural elucidation of bioactive natural products from *Arthrospira platensis*. Zebrafish larvae were used for bioactivity screening throughout the isolation process. Structure elucidation of obtained most active isolates revealed chlorophyll a derivative isomers 13²-R- hydroxypheophytin a (**1**) and 13²-S-hydroxypheophytin a (**2**).

A DCM:MeOH (2:1) extract of *Arthrospira platensis* was submitted for subsequential fractionations using flash chromatography and HPLC with Hex:EtOAc mixtures. Each step was intercalated with lipid reducing assays until complete isolation of **1** and **2**. For structure elucidation, HRESIMS/MS and 2D NMR spectroscopy were employed using COSY, HSQC and ROESY for stereochemistry determination**.**

The bioactivity of both compounds was compared for lipid reduction and 2 food uptake assays in zebrafish larvae, and for the reversion of steatosis in fatty acid overloaded HepG2 cells. Both compounds reduced active feed uptake with similar IC_{50} however, only compound **1** presented activity in passive food uptake (IC_{50} = 22.5 µM), indicating putative hormonal and neuronal interactions. Isomer **2** presented instead 12x more lipid reducing potential with IC_{50} = 1.18 µM, and 2x steatosis reversion capacity (IC_{50} = 4.8 µM) than isomer **1**.

In summary, differences in bioactivity were revealed for both isomers of 13²- hydroxypheophytin a indicating the importance of conformational change in their mechanism of action. This knowledge will be important for future development of potential health applications.

Keywords: *Arthrospira platensis,* chlorophylls, obesity, appetite, steatosis

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Green extraction *via* NaDES of waste and by-products of the Aglianico cv. vinery supply chain

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In recent years, as part of sustainable development policies, the revaluation of end-of-life products has become more and more increased. Indeed, their effective reuse becomes pivotal as a starting point for the obtainment of high-added-value products (Caponio et al., 2022), especially in those supply chains (*e.g.*, vinery), in which high amounts of waste are produced during the whole year, both in the field and transformation processes. In this context, although anthocyanins from the grape pomace have been previously proposed as active ingredients in cosmetics, herein a sustainable approach was used to recover them from the leaves and grape pomace of *Vitis vinifera* L. cv. Aglianico. Thus, the waste/by-products were subjected to maceration with Natural Deep Eutectic Solvents (NaDES), made by citric acid and sucrose (Paiva et al., 2014). The polyphenolic fraction, obtained after the removal of NaDES, was chemically analyzed using UHPLC-HR-MS/MS tools, and the biological activities were evaluated in cell-free and cell-based systems. Radical scavenging capacity was highlighted through ABTS and DPPH tests, while the cytotoxic screening, carried out on HaCaT cell line by MTT test, evidenced the absence of toxicity. Thus, wound healing properties were evaluated, suggesting a valuable applicability in the cosmeceutical sector.

Keywords: Vitis vinifera L., waste valorization, UHPLC-HRMS, NaDES, cosmeceuticals.

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Bioassay-guided isolation of potential cancer chemopreventive agents from the fruits of Rhodomyrtus tomentosa

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Based on cancer morbidity and mortality rates, cancer is a major threat to human health.. Undoubtedly, cancer chemoprevention is one of the most important strategies for cancer control. *Rhodomyrtus tomentosa* (Aiton) Hassk. (Myrtaceae) is a Thai medicinal plant used traditionally for various diseases. Notably, phytochemicals from this species possess antioxidant, anti-inflammatory, anticancer and antimicrobial activities (Nwabor *et al*., 2022). This study aimed to identify phytochemicals with cancer chemopreventive effect from *R. tomentosa* fruits following a bioassay-guided isolation approach. Dried ground fruits were sequentially Soxhlet-extracted with *n*-hexane, DCM and MeOH. Chemopreventive potential of crude extracts, fractions and isolated compounds was evaluated for Nrf2 induction potential using a cell-based luciferase assay in the AREc32 cell line. The MeOH and DCM extracts were the most active extracts with a 7.1-fold and 6.7-fold induction of luciferase activity (relative to control), respectively. As a result, the active MeOH and DCM extracts were selected for fractionation using SPE and VLC, respectively. Compounds were isolated from the most active fractions using TLC and reversed-phase HPLC. Structural elucidation of isolated compounds was achieved by extensive 1D and 2D NMR spectroscopic analyses. 3-*O*-Methylellagic acid 3′-*O*-α-rhamnopyranoside, a polyphenolic glycoside (Fig. 1), was isolated from the active MeOH fraction 2. Studies on the chemopreventive potential of this compound are under way. Similarly, several other compounds have also been isolated and their structures are under investigation. All these results will be presented during the oral presentation.

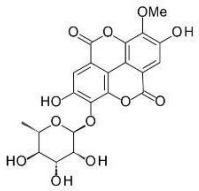

Fig. 1. Structure of 3-*O*-methylellagic acid 3′-*O*-α-rhamnopyranoside

Keywords: *Rhodomyrtus tomentosa,* myrtaceae, chemopreventive effect, bioassay-guided isolation

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Honeypot strategy for parasite weed control: An offer they cannot refuse

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Parasitic weeds pose a significant threat to agricultural productivity, necessitating the development of sustainable control methods. The use of traditional herbicides has led to the emergence of resistant weed varieties, highlighting the urgent need for eco-friendly and efficient alternatives. Allelopathy presents promising avenues for exploration, such as the honeypot strategy (Macías et al., 2020), which focused part of our continuing research on this topic. A primary approach involved the discovery of bioactive metabolites produced by host plants (biocommunicators), leading to the identification and study of promising compounds isolated from host species, such as strigolactones, sesquiterpene lactones, or α-tomatine. Implicit in this task was the development of a simple and reliable UHPLC-MS/MS method for the simultaneous analysis of strigolactones in exudates and extracts. This method was tested with limits of quantifications ranging from 0.05 to 0.96 µg/L. A second approach focused on synthesizing parasitic plant stimulants as strigolactone mimics and analogous. Encouraging results were achieved by adding a butenolide ring to phthalimides, isolated sesquiterpene lactones, and diterpenoids as starting materials, optimizing previously reported synthetic strategies (Zorrilla et al., 2022). The isolated and synthesized compounds demonstrated stimulating activity in bioassays with seeds from various *Orobanche* and *Phelipanche* species. Furthermore, a selection among the most promising compounds was encapsulated, yielding favorable results in bioassays. These findings introduce novel structures with the potential to serve as active ingredients in pre-emergence herbicides based on natural products, thereby stimulating further exploration and research into their efficacy and applicability.

Keywords: parasitic weeds, honeypot strategy, strigolactones, organic synthesis, encapsulations

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A (sub)cellular mapping of natural substances

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Phytohormones, preferentially auxins and cytokinins (CKs), control most aspects of plant growth, development and plasticity. Their distribution in plants has been described, but the importance of cell- and subcellular-type specific phytohormone homeostasis remains undefined. Recently, we revealed auxin and cytokinin distribution maps showing their different organelle-specific allocations within the Arabidopsis plant cells. More specifically, we revealed the intracellular distribution of auxin and CKs in nuclei, chloroplasts, mitochondria, endoplasmic reticulum (ER) and vacuoles. To achieve that, we introduced a breakthrough technique, so-called Fluorescence-Activated multi-Organelle Sorting (FAmOS), for the simultaneous fractionation of four organelle populations from a single sample. This time-efficient method is based on flow-cytometric principles, utilizes organelle-specific fluorophores and considers hormone metabolism stability. We combined our recently developed FAmOS tool with ultra-sensitive mass spectrometry-based methods. Apart from the four organelles isolated via FAmOS, vacuoles have been collected by density-gradient centrifugation to complete the subcellular hormone map. Altogether, this provides a unique approach for organelle-specific metabolic profiling of natural substances, auxin and CKs, combined with other "omics" applications such as proteomics and metabolomics. Shedding light on regulatory processes at the subcellular level will allow a deeper understanding of plant developmental processes and cellular homeostasis maintenance, as well as in intra- and intercellular communication.

Keywords: natural substances, phytohormones, auxin and cytokinin, (sub)cellular localization

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Utilizing natural products for weight management and longevity promotion

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Obesity presents a significant challenge to modern society, imposing a substantial burden on health systems and individuals alike. Nowadays, more than a billion people globally are considered obese, a condition that does not solely refer to weight management but rather to mitigating its far-reaching consequences on healthspan and lifespan (Savova et al., 2023). Individuals diagnosed with obesity are predisposed to comorbidities including type 2 diabetes, cardiovascular disease, and metabolic complications often referred to as metabolic syndrome. Most importantly, obesity is associated with a reduction in disease-free years, an excess risk of premature death, and accelerated aging (Savova et al., 2023; Todorova et al., 2024a). On the other hand, we live in a rapidly aging world, with an expectancy of more than a 2 billion people aged 65 years or older by 2050, alongside a rising proportion of age-related diseases. Thus, both obesity and ageing are, *per se*, different sides of the same coin and represent healthcare burden on our society (Savova et al., 2023; Todorova et al., 2024a). The development of strategies targeting both of these processes now becomes a challenge for science. Central to this pursuit is the recognition that metabolic health serves as a cornerstone for both healthy weight maintenance and prolonged lifespan (Todorova et al., 2024a). Additionally, key molecular pathways attributed to nutrient signalling that are implicated in obesity progression, intersect with those fundamental to longevity, suggesting potential shared targets for intervention (Todorova et al., 2024a). Utilizing the model organism *Caenorhabditis elegans*, along with molecular pharmacology approaches, our research focuses on the discovery of natural products that target these shared pathways (Savova et al., 2021; 2023; Todorova et al., 2024b). Through our approach, we aim not only to mitigate the adverse effects of obesity but also to uncover novel strategies for promoting healthy aging and longevity (Todorova et al., 2024a).

Keywords: obesity, aging, longevity, natural products

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Treatment strategies of pulmonary fibrosis using natural products

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Naturally occurring compounds have been shown to be non-toxic over wide dose ranges and are inexpensive and effective for treating various diseases. Idiopathic pulmonary fibrosis (IPF) is a chronic and irreversible lung disease characterized by limited treatment options, creating an urgent need for advancements in identifying new therapeutic targets. Currently, the Food and Drug Administration has only approved pirfenidone and nintedanib for IPF treatment. Although these drugs can delay disease progression and alleviate symptoms, they do not offer a cure for IPF or improve survival rates. Furthermore, they come with side effects like thrombocytopenia, gastrointestinal discomfort, and dermatological reactions. Consequently, there is a pressing medical need to develop new drugs targeting novel pathways to treat IPF. In this presentation, I will primarily focus on exploring the involvement of novel treatment targets in pulmonary fibrosis and showed the treatment strategy using natural products.

Keywords: pulmonary fibrosis, natural products, novel targets, non-toxic

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Coding metabolic profile of medicinal herbs using 2D-HPTLC

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The technology for classifying organisms through their genetic information is now universally utilized through gene coding (Zhu et al., 2022). However, classification technology using the metabolome of organisms is still in its very early stages. This is because it is challenging to find appropriate analytical techniques and methods that can effectively and universally represent and utilize metabolome profiles. In this presentation, we propose a systematic coding system that can universally code the metabolites of medicinal plants using 2-D HPTLC technology, and apply this system for the origin identification and quality control of medicinal plants. We determine the optimal 2-D development conditions that can comprehensively separate plant metabolites of various structures and obtain reproducible 2-D HPTLC chromatograms in the same analytical environment, including the development conditions. By visualizing the obtained chromatogram in various ways, we aim to encode the presence or absence of bands in each region of the TLC plate and assign a 2D-HPTLC code to each plant. In this presentation, we will introduce this system, discuss potential issues, and propose solutions to overcome them.

Keywords: species identification, metabolic coding, 2D-HPTLC, quality control

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Status and prospect of natural deep eutectic solvents in current natural products research

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Mixing salts and/or organic compounds can significantly reduce the melting point, converting them into liquids even at extremely low temperatures. The exploration of ionic liquids (ILs) began in the early 20th century with the investigation of the physical properties of ethylammonium nitrate. However, it is only in recent years that ILs and deep eutectic solvents (DES) have been reconsidered by chemical engineering for their potential as environmentally friendly mediums in chemical processing. Despite being hailed as promising candidates for green processing, ILs and DES are often composed of ingredients, predominantly synthesized chemically, that pose toxicity risks to both humans and the environment. Overcoming this challenge remains imperative for the widespread adoption of these solvents. Inspired by synthetic ILs, our research group has postulated the existence of a third type of liquid, distinct from water and lipids, hypothesizing that metabolites abundant in cells may give rise to such a medium (1, 2). Analysis of plant metabolomics data collected over several years revealed a striking similarity with synthetic ILs. This revelation challenges the long-standing notion that water is the sole medium in nature. This hypothesis could provide explanations for numerous scientific inquiries, including the low solubility of natural products, the mysteries surrounding biosynthesis, transportation, and storage of non-water-soluble metabolites, among others. Drawing from these insights and the mechanisms of ILs or DES, we propose the existence of a diverse array of natural deep eutectic solvents (NADES) in nature, comprising ILs or DES whose constituents are derived from natural sources. As an initial step to substantiate this concept, we experimented with various combinations of these potential candidates, resulting in the discovery of over 200 formulations yielding viscous liquids. Since the initial publication on NADES in 2011, a plethora of NADES have found applications across diverse fields of natural product research, including extraction for enhancing the concentration of bioactive molecules, serving as green reaction media for organic or enzymatic synthesis, stabilizing labile compounds, and formulating pharmaceuticals. In this presentation, I aim to showcase the diverse applications of natural products, focusing particularly on recent advancements such as their utilization as diffusion matrices for plant volatiles, while also addressing associated limitations.

Keywords: eutectic solvents, NADES, green technology, natural products

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Discovery and biosynthesis of specialized metabolites from human gut microbiome

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Gut microbes are considered as important contributors to human physiology and disease states, and they in the intestinal tract produce small molecule metabolites, which regulate diverse physiological responses (Donia and Fischbach, 2015). However, most of current research topics are focused on well-known gut bacteria, such as *Escherichia coli*, *Akkermansia muciniphila*, *Bacillus cereus*, *Clostridium* spp., *Enterococcus* spp., *Lactobacillus* spp., *Bacteroides* spp., and others. Other infrequent gut bacteria have relatively been neglected and therefore the majority of their metabolites remain structurally and functionally uncharacterized. *Aneurinibacillus aneurinilyticus* is a human gut bacterium, which might be involved in intestinal disease pathogenesis such as irritable bowel syndrome (Rajilić-Stojanović et al., 2011). This human bacterium has also been found from mouse lung tumors and showed probiotic effect in fish (Jin et al., 2018). Although they imply potential correlation between this bacterium and human health, the bacterial small molecule mediators remain largely unknown. In this presentation, we discover an angiogenesis inhibitory metabolite termed the "aneuristatin", which possesses unusual pyrrolo[1,2-a]pyrazine scaffold, from *A. aneurinilyticus* ATCC 12856 along with seven structurally related metabolites. We showed that aneuristatin is biosynthesized from three L-tyrosine molecules by stable isotope feeding experiment, *in vitro* reconstitution, and organic synthesis. Aneuristatin showed anti-angiogenesis activity in the mouse and zebra fish models. Our studies provide a molecular foundation for *A. aneurinilyticus*'s potential ability to prevent and treat hypervascularized diseases including cancer.

Keywords: human microbiome, gut bacteria, *Aneurinibacillus aneurinilyticus*, pyrrolo[1,2-a]pyrazine, anti-angiogenesis

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Posters

Marine organisms, fungi, microbes and plants as chemical factories

S1.P1 Anti-cancer effect of Willughbeia edulis Roxb. stem extract in hepatocellular carcinoma cells through triggering apoptosis and G2/M cell cycle arrest

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The medicinal plant *Willughbeia edulis* Roxb. (Apocynaceae), native to Vietnam, has a long history of medicinal uses by traditional doctors in the treatment of liver cancer (Chi, 2012). Our research aims to investigate the *in vitro* cytotoxic effect against liver cancer cells of *W. edulis* (WE) stem extract and apply Bioactive Molecular Networking (BMN) for guided isolation of bioactive components from WE stem extract. Both the crude extract and butanol layer (WEB) showed selective and significant cytotoxic effects on HepG2 cells, with IC50 values of 27.1 ± 2.7 and 26.06 ± 1.3 μg/mL, respectively. Furthermore, the cell cycle progression was analyzed by utilizing a flow cytometer following the treatment of cells with 16 and 32 μg/mL of crude extract and WEB for 24 or 48 hours. The results found that they induced apoptosis and G2/M cell cycle arrest in HepG2 cells. Then WEB was fractionated using Diaion HP-20 chromatography column, yielding five fractions. These fractions were subjected to LC-MS/MS analysis and screened cytotoxicity assays. The obtained mass spectrum and cytotoxicity data were applied to bioactive molecular networking workflow (Nothias et al., 2018), leading to the isolation of five known compounds from the butanol layer of the stem of *W. edulis*. These isolates, cinchonain Ia, cinchonain Ib, and cinchonain IIa, with high bioactive scores in the molecular network, exhibited potent cytotoxic effects in HepG2 cells with IC50 values of 39.4 ± 3.2, 38.5 ± 2.6, and 17.0 ± 1.5 μM, respectively.

Keywords: *Willughbeia edulis* Roxb., liver cancer, G2/M cell cycle arrest, bioactive molecular networking

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S1.P2 Chemical constituents isolated from leaves of seashore plant *Aglaia rimosa*

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Multiple pieces of evidence have shown that there are various types of compounds including amides, flavonoids, lignans, steroids, triterpenoids, rocaglamide, and dammarane in the *Aglaia* genus, and some of these compounds exhibit diverse biological activities (Harneti et al., 2021; Bueno Pérez et al., 2014). Eleven compounds, stigmasta-5,22E-diene-3β,7β-diol (**1**), stigmasta-5,22-diene-3β,7α- diol (**2**), stigmast-5-ene-3β,7α-diol (**3**), 7β-hydroxysitosterol (**4**), 5α,8α-epidioxy-24-methyl-cholesterol-6,22-dien-3β-ol (**5**), 4',5,7-tri-O-methyl-kaempferol (**6**), 5,6-desmethylenedioxy-5-meth-oxy-aglalactone (**7**), yangambin (**8**), eudesmin (**9**), 2,4-dimethexy-6-hydroxyl benzoic acid (**10**) and dehydroodorin (**11**), were isolated from the acetone extract of leaves of the seashore plant *A. rimosa*, a member of the Meliaceae family. Among these compounds, six compounds (**2** and **5**-**9**) were subjected to anticancer activity testing against oral cancer cells (SCC2095), breast cancer cells (MCF- 7), and gastric cancer cells (SC-M1). Compound **5** exhibited cytotoxic activity against MCF-7 cells (IC50 = 11.2 ± 1.3 μM), while **6** demonstrated cytotoxicity against SCC2095, MCF-7, and SC-M1 cell lines (IC50 = 16.3 ± 2.9, 24.8 ± 0.9, and 14.6 ± 0.7 μM, respectively) (Fig. 1). These findings highlight the potential of compounds from *A. rimosa* as promising candidates for further exploration in cancer therapy.

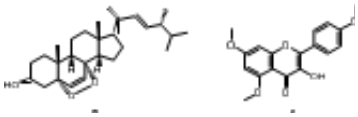

Fig. 1. Structures of compounds **5** and **6**.

Keywords: *Aglaia rimosa*, cytotoxicity, oral cancer cells, breast cancer cells, gastric cancer cells

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S1.P3 Fragment-guided genome mining leads to unprecedented structural complexity in natural products

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Here we reported the discovery and characterization of an unprecedented 6/12/6/6/6/6/6/6 fused octacyclic cyclophane-containing alkaloid, octacyclin A (1), by fragment-guided genome mining. The biosynthesis pathway of 1 involving 14 genes was completely unraveled by a combination of heterologous expression, feeding experiments and biochemical assays, showing that the complicated ring system was constructed by two single-module non-ribosomal peptide synthetases (NRPSs) and three cytochrome P450s. Briefly, NRPS OcyN with a noncanonical terminal condensation (CT) domain showed a novel function by modifying the piperazine cyclophane moiety to form a tetrahydroquinolin-4(1H)-one moiety. Subsequently, two P450s OcyM and OcyL mediated cascade cyclization with a cryptic protection process to forge the octacyclic system.

Keywords: oxidative cyclization, genome mining

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S1.P4 OSMAC approach, the tool to unlock the biosynthetic potential of deep-sea actinobacteria

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Research on natural products has played a major role in the discovery of new drugs. The class Actinomycete comprises a group of microorganisms highly prolific in the production of NP (natural products) with several relevant bioactivities (Murtaza et al., 2023). It is known that actinobacteria include in their genome multiple biosynthetic gene clusters (BGC), meaning that a single strain may be capable of producing several NP. In the recent years, the OSMAC (One Strain - Many Compounds) strategy has revealed very promising in activating the expression of dormant BGC by varying culture conditions (Le Loarer et al., 2023), and its application has allowed the discovery of novel bioactive compounds (Hug et al., 2018; Hussain et al., 2017). In this study, the OSMAC approach was applied to four actinobacterial strains isolated from deep-sea samples collected at Madeira and Azores islands, in order to find novel bioactive compounds with pharmaceutical relevance. Three different culture conditions were applied to each strain and the capacity of these conditions to induce different bioactivities was analyzed. The results showed that the conditions applied stimulated significant antimicrobial and anticancer activities in the analyzed strains. The bioactive actinobacterial extracts were subjected to a metabolomics analysis, included mass spectrometry-based dereplication and molecular networking analyses. These results revealed the presence of 5 possible new compounds. We are currently performing guided mass isolation of these potential new NP. This study shows that the OSMAC approach is a promising strategy for the discovery of new molecules with biotechnological potential.

Keywords: Actinomycete, OSMAC approach, Bioprospecting, secondary metabolites, pharmaceutical potencial

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S1.P5 Antiprotozoal activity of alkaloid fractions and isolation of four new steroidal alkaloids from *Buxus obtusifolia*

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As part of our continuing search for new antiprotozoal compounds derived from Buxaceae family (Szabó et al., 2021), we investigated the dichloromethane extract from the leaves of the East African *Buxus obtusifolia* (Mildbr.) Hutch. *B. obtusifolia* is an evergreen shrub utilized ethnomedicinally in many parts of E. Africa to treat chest-related ailments (Beentje et al., 1994). Fractionation of the alkaloid enriched fraction by centrifugal partition chromatography (CPC) yielded a total of 16 sub- fractions. The anti-protozoal activity of the crude extract, the alkaloid-enriched fraction, and the 16 CPC subfractions was assessed against *Trypanosoma brucei rhodesiense* (*Tbr*) and *Plasmodium falciparum* (*Pf*) using established standard protocols (Szabó et al., 2021). The alkaloid fraction displayed stronger activities against *Tbr* and *Pf* (IC50 = 8.07 and 0.69 µg/mL, respectively), compared to the crude extract (IC50 = 16.05 and 1.08, respectively). Sub-fractions 3 (IC50 = 0.76 µg/mL) and 4 (IC50 = 0.77 µg/mL) demonstrated the highest antitrypanosomal activity. Additionally, promising antiplasmodial effects with IC50 values ranging from 0.93 – 1.89 µg/mL were observed for fractions 3 to 16. Bioassay-guided separation of CPC sub-fractions 3 – 5 using prep-HPLC resulted in the isolation of four new steroidal alkaloids including cycloprotobuxoline-C (**1**), cycloprotobuxoline-C *N*-oxide (**2**), 3-aminocycloprotobuxoline-C (**3**), dehydrocyclovirobuxeine-B (**4**) along with the known cyclovirobuxeine-A (**5**) (Atta-ur-Rahman et al., 2001) (Fig. 1). *In vitro* antiprotozoal efficacy studies of the isolated compounds are underway.

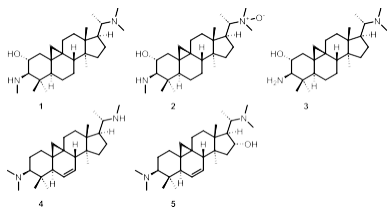

Fig. 2: Structures of compounds **1 – 5** isolated from *Buxus obtusifolia* (Mildbr.) Hutch.

Keywords: *Buxus obtusifolia,* Buxaceae, steroidal alkaloids, *Trypanosoma brucei rhodesiense, Plasmodium falciparum*

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S1.P6 Genome mining-based discovery of new glycosylated terpenoids from *Micromonospora* sp. isolated from *Leontopodium nivale* subsp. *alpinum* rhizosphere

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Representatives of the bacterial genus *Micromonospora* have been isolated from rhizosphere soil of the rare medicinal plant *Leontopodium nivale* subsp. *alpinum* (Oberhofer et al., 2019). Genome sequencing and analysis of one of the *Micromonospora* isolates led to the identification of a gene cluster presumably specifying biosynthesis of a glycosylated terpenoid. Transformation-associated recombination-assisted assembly of this cluster in yeast and its heterologous expression in an engineered *Streptomyces* host resulted in the production of a novel brasilicardin-related terpenoid which, however, was not glycosylated. At the same time, targeted screening of extracts from the native *Micromonospora* host strain by means of LC-MS allowed the identification of glycosylated congeners of this terpenoid. The latter suggested that the heterologous *Streptomyces* host is deficient in either biosynthesis of particular sugars or in expression of the cluster-specific glycosyltransferases. To alleviate this problem, several plasmids containing different cassettes for the biosynthesis of activated deoxysugars were introduced into the recombinant *Streptomyces* host harboring the terpenoid cluster. Analysis of the extracts from these recombinant strains revealed the production of several novel glycosylated terpenoid analogs. Currently, different approaches are being explored aimed at producing various glycosylated terpenoids via combinatorial biosynthetic engineering of this gene cluster.

Keywords: genome mining, glycosylated terpenoids, *Micromonospora*, combinatorial biosynthetic engineering, heterologous expression

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S1.P7 Changing paradigms in natural product discovery: A molecule to microbe approach

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Traditional approaches to microbial natural product discovery take a microbe first approach in which individual strains are cultured in the lab and bioassays used to guide compound isolation. While once productive, the limitations to this approach are now well documented and include the recognition that only a small percentage of the bacteria present in the environment are readily obtained in culture. We have developed a new approach to microbial natural product discovery in which compounds are isolated directly from the environments in which they are produced thus bypassing the initial need for laboratory cultivation. This culture independent approach, which we call the Small Molecule In Situ Resin Capture (SMIRC) technique, is agnostic to the biological source of the compounds and requires no up-front knowledge of cultivation requirements or the cues needed to induce biosynthesis. SMIRC deployments in marine habitats yielded extensive chemical diversity including compounds previously reported from marine plants, invertebrates, and bacteria. Mining compounds that could not be identified has yielded unprecedented carbon skeletons and demonstrated that sufficient yields can be obtained for NMR-based structure elucidation. These results, along with "omic" efforts to identify the producing organisms, suggest a path forward to access chemical space natural product diversity without the need for cultivation.

Keywords: natural product discovery

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S1.P8 Bioactive scalarane sesterterpenoids and polybrominated diphenyl ethers from the two sponges *Hippospongia* sp. and *Dysidea* sp.

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One new scalarane sesterterpenoid (**1**), along with four known sesterterpenoids (**2**–**5**), were isolated from the sponge *Hippospongia* sp. Five known polybrominated diphenyl ethers natural compounds (**6**–**10**) were isolated from the sponge *Dysidea* sp. The structure of marine natural products **1**–**10** was established by NMR spectral data analysis. Moreover, the structure of brominated diphenyl ethers **6**, **9** and **10** was further confirmed by X-ray single-crystal diffraction analysis (Fig. 1).

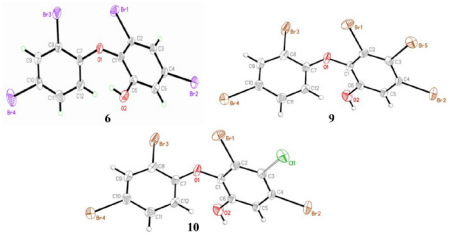

Fig. 1. Molecular structures of **9** and **10** based on X-ray analysis.

The study assessed the cytotoxicity of compounds **1**–**10** against human cholangiocellular carcinoma (HuCCT1) and human colonic adenocarcinoma (SW620). The findings indicated that sesterterpenoids **2** and **5** demonstrated substantial cytotoxic effects on these cancer cell lines. They significantly reduced cell viability and promoted apoptosis in a dose-dependent manner. Furthermore, the application of these compounds was associated with an increased expression of apoptosis-related proteins, particularly caspase-3, caspase-7, and PARP, highlighting their potential as therapeutic agents.

Keywords: sesterterpenes, polybrominated diphenyl ethers, sponge, cytotoxicity

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S1.P9 New triterpenoid saponins from the aerial parts of *Glinus oppositifolius* and their anti-adipogenic activity.

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Glinus oppositifolius L., Molluginaceae family, has a long-standing history of utilization as both a vegetable and a medicinal agent across numerous countries (Burkill, 1985). As a part of our ongoing study to find natural anti-adipogenic agents from Vietnamese plants, an alcoholic extract from the aerial parts of *G. oppositifolius* was found to exhibit an inhibitory effect on adipogenic cells. This presentation will cover the isolation of six new triterpenoid saponins (**1**, **2**, **6**-**9**) along with three known compounds (**3**-**5**) (Fig. 1). The structure elucidation was performed by a combination of extensive NMR analysis, HRFABMS, and DFT calculations.

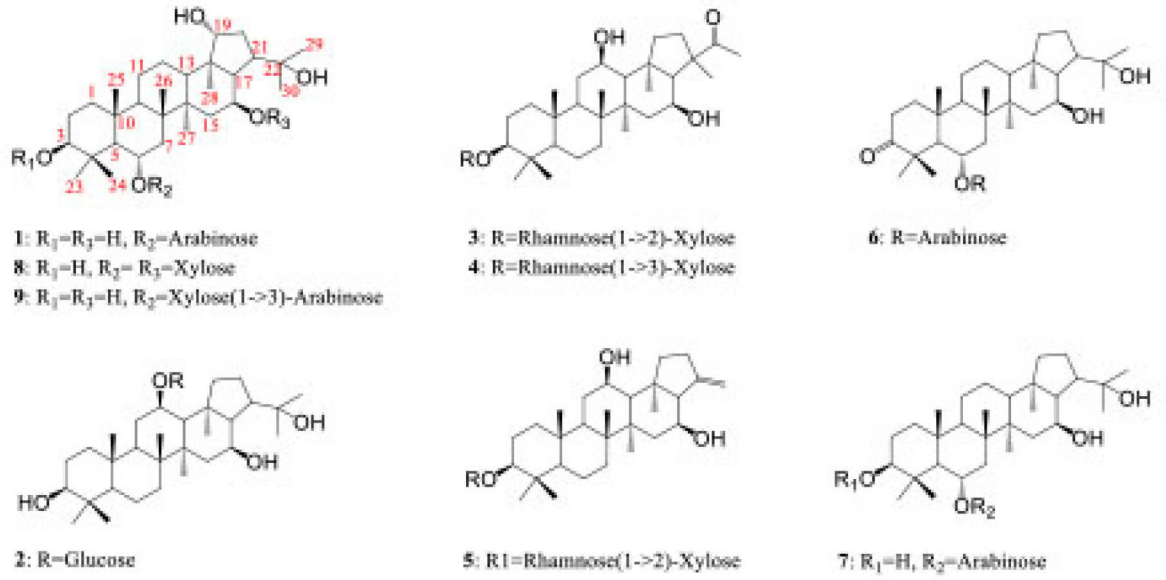

Fig. 1. Structures of isolated compounds (**1**-**9**) from *G. oppositifolius*.

Through the adipogenesis assay, compounds **5** and **7** significantly suppressed preadipocyte differentiation of 3T3-L1 cells with IC_{50} values at 35.4, 32.7, and 40.9 μM, respectively, compared to that of epigallocatechin-3-gallate (EGCG) previously reported as a positive control (Lee et al., 2013).

Keywords: *Glinus oppositifolius*, hopane-type saponin, anti-adipogenic activity, 3T3-L1, obesity

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S1.P10 Chlorine-containing polyacetoxybriaranes from the octocoral *Junceella fragilis*

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Chemical screening of the octocoral *Junceella fragilis* has led to the isolation of five chlorinated briarane-type diterpenoids, including three known metabolites, gemmacolide X (**1**), frajunolide I (**2**), and fragilide F (**3**), along with two new analogs, 12α-acetoxyfragilide F (**4**) and 12α-acetoxyjunceellin (**5**) (Fig. 1).

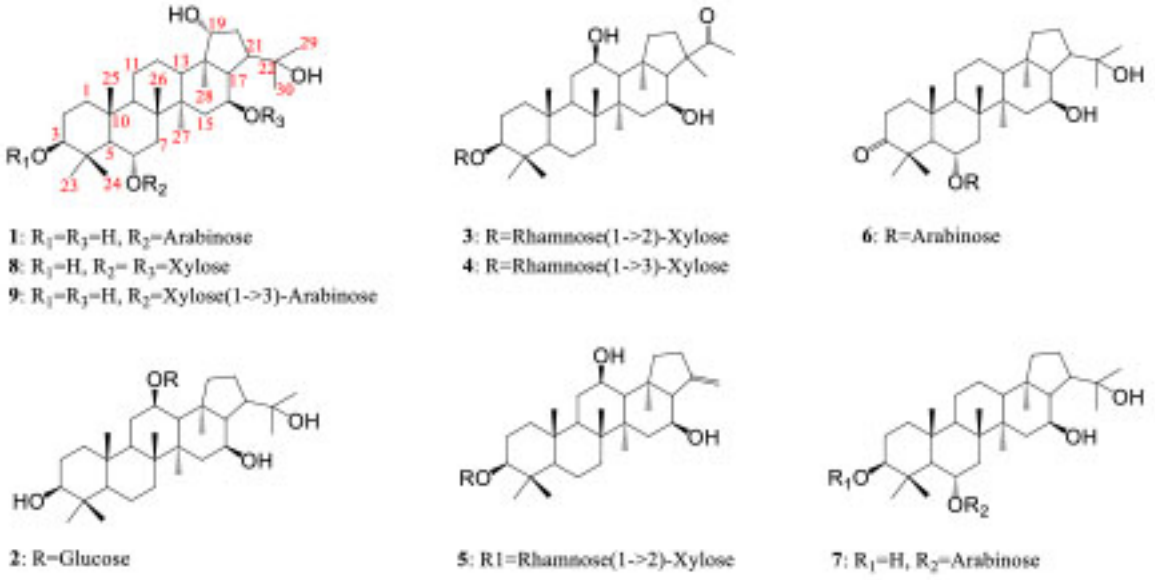

Fig. 1. Structures of gemmacolide X (**1**), frajunolide I (**2**), fragilide F (**3**), 12α-acetoxyfragilide F (**4**), 12α-acetoxy- and junceellin (**5**); ORTEP revealing the structures of **1** and **2**.

Single-crystal X-ray diffraction analysis established the complete absolute stereochemistry of natural products **1** and **2** as depicted, while the structures of new compounds **4** and **5** were ascertained with 2D NMR experiments. An evaluation of the conformation of frajunolide I (**2**) was carried out. Briaranes **4** and **5** exhibit cytotoxicity toward MG63 human osteosarcoma cells at concentration of 10 μM.

Keywords: *Junceella fragilis,* Ellisellidae, octocoral, cytotoxicity, osteosarcoma

References

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S1.P11 Apoptotic agents from endolichenic fungi against chemoresistant cancers

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In 2020, cancer emerged as a prominent global cause of death, with breast, lung and colon cancers being the most prevalent. Overcoming chemoresistance represents a major challenge in cancer treatment. A potential breakthrough lies in endolichenic fungi, non-pathogenic fungi found in lichens, which offer new bioactive compounds with anticancer potential. Endolichenic fungi (ELF) were previously isolated from lichens collected in the Nouvelle Aquitaine region (France) and a screening of 20 endolichenic extracts against the human colorectal cancer cell line (HT-29) highlighted their antiproliferative potential, with IC50 values ranging from 2 to 60 µg/mL (MTT assay). EtOAc extracts from strains PA08 and XC04 were the most active, with IC50 values below 6 µg/mL against HT-29. However, only the extract PA08S was active on triple-negative human breast cancer (MDA-MB-231), with an IC50 of 4.1 µg/mL. Solid-state fermentation of PA08 and XC04 on appropriate medium yielded extracts of 5 g EtOAc each. Evaluation of the antiproliferative activity of their sub-extracts highlighted the potency of PA08S-H and PA08S-M on HT-29 with IC50 values of 16 and 3 µg/mL respectively. For XC04P, the two precipitates and the intermediate fractions from the liquid-liquid partition displayed IC50 values on HT-29 of 4.9, 4.3 and 1.1 µg/mL respectively. The main compound isolated from XC04P-I was active with IC50 of 1.5 and 3.7 µM on HT-29 and MDA-MB-231 cells respectively. Purification of bioactive fractions is underway to isolate further bioactive compounds, and their bioactivity will be investigated through apoptosis studies on MDA-MB231 and HT-29 cells.

Keywords: lichens, endolichenic fungi, antiproliferative activity, colorectal cancer, triple negative breast cancer

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S1.P12 *Aetokthonos hydrillicola* – an epiphytic cyanobacterium with surprising toxic potential

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Cyanobacteria are infamous producers of toxins. While the toxic potential of planktonic cyanobacterial blooms is well documented, the effects of toxigenic benthic and epiphytic cyanobacteria are an understudied threat. In a previous study, we showed that the freshwater epiphytic cyanobacterium *Aetokthonos hydrillicola* produces a neurotoxin, aetokthonotoxin (AETX, **1**), causing the fatal disease Vacuolar Myelinopathy that affects a wide array of wildlife in the southeastern United States (Breinlinger et al., 2021).

In an assay for cytotoxicity, however, we found the crude extract of the cyanobacterium to be much more potent than pure AETX, prompting further investigation. This led to the isolation and structure elucidation of the aetokthonostatins (AESTs, **2**), linear peptides belonging to the dolastatin compound family, featuring a unique modification of the C-terminal phenylalanine-derived moiety. We confirmed that AEST potently impacts microtubule dynamics and can bind to tubulin like dolastatin 10, and we showed that AEST inhibits reproduction of the nematode *C. elegans*. Bioinformatic analysis and biochemical studies revealed the AEST biosynthetic gene cluster encoding a nonribosomal peptide synthetase / polyketide synthase accompanied by a unique tailoring machinery (Schwark et al., 2023).

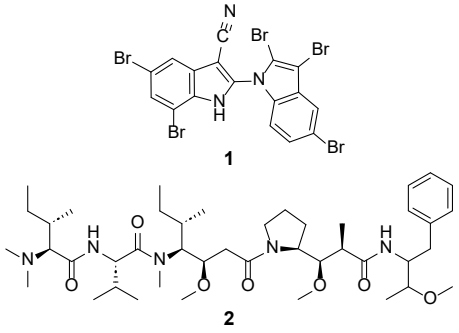

Fig. 1. Structures of aetokthonotoxin **1** and aetokthonostatin **2**

Keywords: *Aetokthonos hydrillicola,* cyanotoxin, dolastatin, biosynthesis

References

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S1.P13 Cellulamides, a new family of marine-sourced peptides from a seaweed-associated *Cellulosimicrobium*

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Mining the secondary metabolism of underexplored Actinomycetota taxa from unique marine sources represents a valuable route to uncover novel chemistry. These bacteria often live in symbiosis with other organisms, as seaweeds, prompting a prolific – yet poorly explored – hotspot for the synthesis of new molecules (Girão *et al*., 2019). In this work we report the isolation, structure elucidation and bioactivity screening of cellulamides A-C (**1**-**3**), a family of novel linear peptides isolated from cultures of the seaweed-associated strain *Cellulosimicrobium funkei* CT-R177. The host macroalgae *Codium tomentosum* was collected in the northern Portuguese coast and, as part of a bioprospection program to explore its associated actinobacterial community, CT-R177 was isolated, taxonomically identified and screened for the production of antimicrobial and anticancer compounds. Dereplication of CT-R177 crude extract using LC/HRMS unveiled a putative novel natural product, cellulamide A (**1**), that was isolated following mass spectrometry-guided fractionation. Throughout the chromatographic process two other analogs, cellulamides B and C (**2, 3**), were also obtained and chemically characterized. Using a combination of 1D/2D NMR spectroscopy and HRMS, the structure of the novel peptides was elucidated. Cellulamide A was subjected to a set of bioactivity screenings, with no positive results. More assays are being conducted to uncover its potential biotechnological application. Cellulamides represent the first family of natural products reported from the Actinomycetota genus *Cellulosimicrobium* – a bacterial group known for their industrial-value in cellulose degradation with unreported secondary metabolite production – showcasing not only the potential of less-explored taxa but also host-associated marine strains for novel chemistry discovery.

Keywords: Actinomycetota, *Cellulosimicrobium*, linear peptides, natural products, seaweed- associated;

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S1.P14 A high-performance thin layer chromatography (HPTLC) method to identify lichen compounds and highlight their antibacterial activity

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Rennes University, ISCR, CNRS, UMR 6226, CorInt team, Rennes, France

Lichens are symbiotic organisms that produce unique specialized metabolites. Phenols and terpenoids are the major ones and are often biomarkers used for lichen recognition (Lohezic-Le Dévéhat, 2012). Moreover, some of them are described for their bioactivities (Kello et al., 2023). This explains the challenge of the present work, which consists on the development and optimization of a new efficient analytical method to identify lichen compounds and verify the presence of biological activities. One analytical technique that meets both these objectives is HPTLC. Indeed, it allows the user to quickly visualize the major compounds in a lichen extract. Moreover, it is an interesting way to analyze UV absorbing compounds (phenols) and also those that are not visible under ultraviolet wavelengths (terpenoids), thanks to a derivatization step that reveals the compounds as colorful spots (Gerlach et al., 2018). Two different HPTLC methods (terpenoids/phenols) were developed in different modes (gradient/isocratic). Hundreds of standards were analyzed, enabling the creation of databases in order to gather all information (R*f*, color of the spots before and after derivatization step, UV spectra data) and facilitate the compounds identification in a lichen extract. Once the compounds were well separated and recognized, bioautographies were performed on new plates. Culture medium and bacteria (*Bacillus subtilis*) were poured onto the plates after the migration step. After incubation and derivatization (methyl thiazolyl tetrazolium bromide), the zone of inhibition was measured to highlight the antibacterial activity of the identified lichen compounds. Results will be discussed in the presentation.

Keywords: lichens, HPTLC, identification, bioautography, antibacterial

References

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S1.P15 Potent antiproliferative dimeric biaryl-cyclohexapeptides from *Lentzea flaviverrucosa*

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Rare actinomycetes represent an underexploited source of new bioactive compounds. Using a targeted metabologenomic approach we identified piperazyl compounds in the rare actinomycete *Lentzea flaviverrucosa* DSM 44664. These efforts to identify molecules that incorporate piperazate building blocks resulted in the discovery and structural elucidation of two dimeric biaryl-cyclohexapeptides, petrichorins A (**1**) and B. Petrichorin B (**2**) is a symmetric homodimer similar to the known compound chloptosin, but petrichorin A (**1**, Fig. 1) is unique among known piperazyl cyclopeptides because it is an asymmetric heterodimer. Due to the structural complexity of petrichorin A, solving its structure required a combination of several standard chemical methods plus in silico modeling, strain mutagenesis, and solving the structure of its biosynthetic intermediate petrichorin C (**3**) for confident assignment. Furthermore, we found that the piperazyl cyclopeptides comprising each half of the petrichorin A heterodimer are made via two distinct nonribosomal peptide synthetase (NRPS) assembly lines, and the responsible NRPS enzymes are encoded within a contiguous biosynthetic supercluster on the *L. flaviverrucosa* chromosome. Requiring promiscuous cytochrome p450 crosslinking events for asymmetric and symmetric biaryl production, petrichorins A and B exhibited potent in vitro activity against A2780 human ovarian cancer, HT1080 fibrosarcoma, PC3 human prostate cancer, and Jurkat human T lymphocyte cell lines with IC50 values at low nM levels.

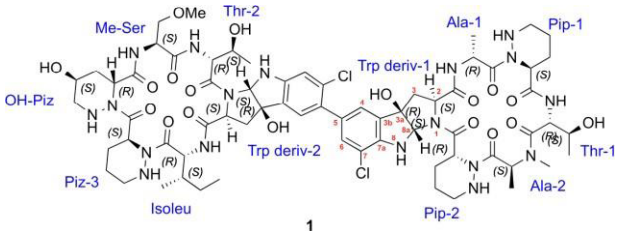

Fig. 1. Structure of petrichorin A **1**.

Keywords: *Lentzea flaviverrucosa,* dimeric biaryl-cyclohexapeptides, nonribosomal peptide synthetase, p450, NMR, antiproliferative activity

References

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S1.P16 A validated RP-HPLC-DAD method for analyzing flavonoids in Caatinga Brazilian green propolis from *Mimosa tenuiflora* (Willd.) Poir. produced by *Apis mellifera* L.

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There are several types of propolis in Brazil produced by *Apis mellifera* (Santos et al., 2021), and the Caatinga biome green propolis stands out for its high content of flavonoids (Son et al., 2022). Hence, the presentation will cover the flavonoids isolated from Brazilian green propolis of Caatinga *Mimosa tenuiflora*, and the development of a reliable RP-HPLC quantitative method using a Shim-pack VP-ODS column (250 × 4.6 mm i.d., 5 µm) with nonlinear gradient elution and UV visualization at 280 nm. Additionally, a sample preparation method for extracting flavonoids using 96% ethanol and caffeic acid as the internal standard was developed.

The developed method allowed an excellent detection response, in which the LOD (limit of detection) and LOQ (limit of quantification) reached ranges of 0.65-2.08 µg/mL and 1.97-6.31 µg/mL, respectively. The maximum RSD (relative standard deviation) was 4.61%. Thirteen flavonoids, comprising santin (**1**), ermanin (**2**), sakuranetin (**3**), quercetin 3-methyl ether (**4**), viscosine (**5**), eriodictyol 5-O-methyl ether (**6**), isokaempferide (**7**), kaempferide (**8**), penduletin (**9**), quercetagetin 3,6,7-trimethyl ether (**10**), cirsimaritin (**11**), 3,3'-*O*-dimethylquercetin (**12**), and luteolin (**13**), were quantified (Fig. 1).

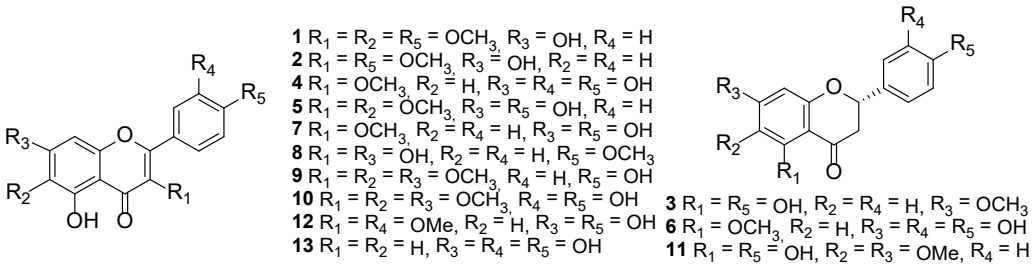

Fig. 1. Chemical structures of compounds **1**-**13**.

The developed method met all the parameters set by the international guidelines for developing analytical methods. The current analytical method is reliable for the quality control of *M. tenuiflora* green propolis and its related products.

Keywords: *Apis mellifera*, *Mimosa tenuiflora*, Caatinga, Fabaceae, flavonoids, RP-HPLC-DAD

References

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S1.P17 Functional oligosaccharides derived from the degradation of natural *Dendrobium* polysaccharides by human gut microbes regulate physiological factors associated with type-2 diabetes

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Dendrobium, a noble traditional Chinese herb medicine, has been found to be rich in glucomannan and possesses significant bioactivities in regulating blood sugar and blood pressure (Pan et al., 2014). Herein, we selected a unique hybrid species, *Dendrobium Taiseed Tosnobile* (DTT), which is derived from the crossbreeding of *D. tosaense* and *D. nobile*, as our research material. The acetylated glucomannan, isolated from DTT, consists of mannose to glucose at a ratio of 5.5:1 and has an average molecular weight of 194 kDa. After administering isolated DTT polysaccharide (DTTPS) to diabetic model mice for 7 weeks, notable improvements in body weight, appetite, and postprandial blood glucose levels of the mice were observed. Furthermore, the abundance of probiotics such as *Akkermansia*, *Lactobacillus*, and *Bifidobacterium* significantly increased in mice fed with DTTPS compared to those in the control group. To further investigate the mechanism of action of DTTPS, *Bacteroides ovatus* was chosen to ferment DTTPS under carbon source-depleted conditions. In this study, small molecular weight acetylated glucomannan, ranging from 1-2 kDa, were successfully isolated. These oligosaccharides demonstrated potent activities toward promoting GLP-1 secretion and inhibiting DPP-4 degradation, along with moderate inhibitory activity against α-glucosidase, revealing a comprehensive function in regulating physiological factors associated with type-2 diabetes.

Keywords: *Dendrobium* spp., glucomannan, gut microbes, GLP-1, DPP-4

References

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S1.P18 Discovering original antiplasmodial compounds through mass-guided exploration of the alkaloid diversity in the *Strychnos* genus

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Several monoterpene indole alkaloids from various plants of the *Strychnos* genus have shown promising antiplasmodial activity. In front of the parasites' growing resistance to existing treatments, the alkaloid content from 44 crude extracts of 28 *Strychnos* species are explored using the molecular networking technique with the aim of identifying new antimalarial compounds. Thanks to public MS/MS spectral databases and the antiplasmodial activities of studied crude extracts, clusters containing mainly unknown metabolites potentially active against malaria were highlighted and targeted (Fig. 1) (Bonnet *et al.*, 2022a).

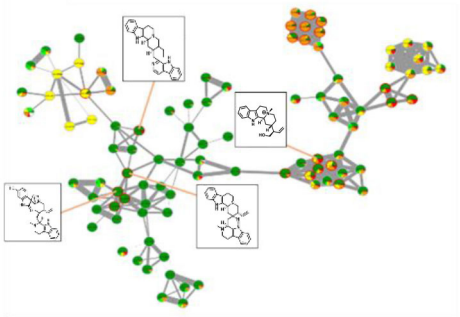

Fig. 1. One cluster from GNPS-annotated molecular network of 44 crude extracts. (Colors of antiplasmodial activities: Dark green= < 5 µg/mL; Light green= 5-15 µg/mL; Yellow= 15-30 µg/mL; Orange: 30-50 µg/mL; Red: > 50 µg/mL).

Exploring the annotated molecular network led to reveal unexpected presence of strychnine in seven species (Bonnet *et al.*, 2022b), and to select *Strychnos usambarensis* leaves and *Strychnos longicaudata* trunk barks for in-depth studies. Using bio- and mass-guided fractionation, several unknown metabolites were isolated, and are currently being identified using NMR and MS/MS data.

Keywords: *Strychnos*, alkaloids, molecular networking, mass spectrometry, malaria

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S1.P19 Characterization and antimicrobial activity of extracts from microalgae *Chlorococcum* sp. with green organic solvents

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The processes involved in extracting and characterizing natural compounds are crucial for enhancing the environmental sustainability of industrially relevant products, particularly when aimed at discovering novel chemical structures for drug development. Various extraction methods utilizing environmentally friendly organic solvents (Byrne et al., 2016) are employed to obtain bioactive compounds from natural sources. Microalgae, rich in bioactive compounds such as carotenoids, polyphenols, phytosterols, and tocopherols (Sarkar et al., 2020), are gaining attention from numerous agro-food and pharmaceutical industries as a source of natural products. This study focuses on extracting the lipophilic fraction from the microalgae *Chlorococcum* sp. and identifying compounds with antimicrobial properties against *Escherichia coli*, *Bacillus megaterium*, and *Bacillus subtilis* through principal component analysis (PCA) of GC-MS spectra of the whole extracts. The extraction process relies on green organic solvents, aiming to demonstrate their efficacy as replacements for conventional organic solvents like chloroform (Patras et al., 2011). The proposed approach seeks to offer alternative methods to the laborious extraction and material-consuming steps typically involved in isolating and characterizing individual compounds. Antimicrobial tests were conducted using the agar well diffusion method (Valgas et al., 2007) and the broth dilution method (Rubin, 2013), revealing significant antimicrobial activity in the extracts. Overall, the study underscores the potential of microalgae as a valuable source of bioactive compounds and investigates the processes involved in their extraction, characterization, and assessment of antimicrobial activity.

Financial support from the European Union – Next Generation EU in the frame of the "National Biodiversity Future Center"(CUPn°B83C22002930006) is gratefully acknowledged.

Keywords: microalgae, green organic solvent, extractions, antimicrobial activity, extract characterization, PCA

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S1.P20 Exploration of new biological activity of *Mikania micrantha*, a widespread weed in Asia

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Mikania micrantha Kunth (family Asteraceae), a fast-growing perennial herbaceous vine, has been regarded as a widespread weed in Asia. However, originates from the tropical South and Central America, *M. micrantha* (MM) has been used as medicinal plant. Pharmacological activities such as anti-inflammatory, antioxidant, antiviral and cytotoxicity towards various cancer cells have been previously reported (But et al., 2009; Dong et al., 2017; Rios et al. 2014). This study aimed to identify the active fraction of MM which may account for the cytotoxic activity in human cancer cells, using bioassay-guided fractionation. The aerial parts of MM were extracted with 80 % ethanol under reflux, followed by sequential partition with petroleum ether, ethyl acetate and *n*-butanol. The active fraction was then subjected to column chromatography to yield subfractions for further evaluation on the cell viability of different human cancer cells and normal fibroblasts Hs27 using MTT assay. Results showed that the ethyl acetate fraction significantly reduced cell viability of MDA-MB-231 (breast), HT-29 (colon) and EC-109 (esophageal) cancer cells with IC50 of 12.9, 21.5 and 46.9 mg/mL, respectively, but not Hs27 (IC50 > 200 mg/mL) cells, suggesting its selective cytotoxicity. Moreover, in MDA-MB-231 and HT-29 cells, the ethyl acetate fraction induced G2 phase arrest of cell cycle in a concentration dependent manner. The subfraction E2 maintained selective cytotoxic activity in MDA-MB-231 cells (IC50 at 46.8 mg/ml). Our findings demonstrated the potential of this subfraction in suppressing breast tumor growth, which warrant further *in vivo* investigations to verify the new bioactivity of this weed.

Keywords: *Mikania micrantha*, Asteraceae, cytotoxicity, breast cancer, colon cancer

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S1.P21 Preclinical study on the anti-proliferative effects and underlying mechanisms of broussoflavonol F in colon cancer

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Broussoflavonol F (BFF), a natural prenylated flavonol isolated from *Macaranga indica* Wight, has been reported to possess cytotoxicities against various cancer cells, including MCF7, A549, HepG2 and Hela (Segun et al., 2019; Vu et al., 2021). However, there is no report on the anti-proliferative activity of BFF in colon cancer. This study investigated on the anti-tumor effect of BFF and its underlying mechanisms in colon cancer. The anti-proliferative effects of BFF on human colon cancer HCT116 and LoVo cells were evaluated using MTT, BrdU, and colony formation assays, cell cycle and cell apoptosis analysis. Besides, network pharmacology analysis was used to predict the effects of BFF on the expression of key proteins in intracellular signaling pathways, with validation using western blot. The anti-tumor effect of BFF was also examined in HCT116 tumor-bearing nude mice. Our results showed that BFF significantly exhibited anti-proliferative activities in both HCT116 (2.5-7.5 μM) and LoVo (5-10 μM) cells via inducing apoptosis and cell cycle arrest at the G0/G1 phase. Further investigation revealed that BFF (1.25-5 μM) inhibited cell proliferation by downregulating the expression of HER2, RAS, p-BRAF, p-MEK and p-Erk. In addition, intraperitoneal administration of BFF (10 mg/kg) suppressed tumor growth and expression of Ki-67 and CD31 in the tumor tissues of HCT116 tumor-bearing mice. This is the first report on the *in vitro* and *in vivo* anti-proliferative effects of BFF in colon cancer, through regulation of HER2-RAS-MEK-Erk pathway. Our findings support further research development of BFF as an anti-cancer agent for colon cancer.

Keywords: broussoflavonol F, *Macaranga indica*, colon cancer, anti-proliferative, anti-tumor

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S1.P22 Isolates of *Pavonia multiflora* as alternative control of the phytopathogen *Fusarium solani piperis*

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Fusarium species, responsible for a significant proportion of crop fungal diseases, act a substantial threat to global agriculture, resulting in considerable economic losses. Environmental and health concerns associated with synthetic fungicides have prompted the exploration of natural products as alternative control measures. Antifungal compounds derived from the endemic Brazilian species *Pavonia multiflora* (Malvaceae) emerge as ecologically friendly alternatives for *Fusarium* control. In this study, *in vitro* assays were conducted to screen eight *P. multiflora* isolates (Lopes et al., 2016) and identify compound(s) with fungicidal efficacy and safe cytotoxic profile. Four isolates: vanillic acid (MFC = 200 μg.mL^{-1}), loliolide (MFC=400 μg.mL^{-1}), vomifoliol (MFC=200 μg.mL^{-1}), and 4,5- dihydroblumenol A (MFC = 400 μg.mL^{-1}) exhibited fungicidal capacity, remaining biocompatible up to 50 μg.mL^{-1} with no significant metabolic changes in basal cells (Raw 267.4). However, distinct cytotoxic profiles were observed after exposure to a metabolizing system (Fraction S9) (Gonçalves et al., 2016) under HepG2 cells. Vanillic acid showed decrease of cytotoxicity (IC50 54.31 to 226.3 μg.mL^{-1}), while other isolates displayed increase of cytotoxicity. Given the use of only Phase I metabolism cofactors, a more precise evaluation, including Phase II cofactors and mutagenicity assays, is required in future works. However, this study showed these compounds hold promise as fungicides, particularly vanillic acid and it marks the first reporting of antifungal and cytotoxic activities of isolate 4,5 - dihydroblumenol A. Considering the long-term consequences of synthetic pesticides (carbendazim and tebuconazole), this work paves the way for safe and effective alternatives of *Fusarium* species control.

Keywords: phytopathogen, fungicides, natural products, cytotoxicity, vanillic acid

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S1.P23 Co-culturing *Streptomyces albidoflavus*, an epiphyte isolated from *Lavandula angustifolia* Mill., increased the anti-*Porphyromonas gingivalis* effect in exo-metabolome extracts

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Background: *Porphyromonas gingivalis* (*Pg*) is a keystone pathogen in periodontitis, which affects ~20% of the global population and represents a societal burden of USD$298 billion/year (Listl et al., 2015). Common treatments are tissue debridement and antibiotics administration. However, between 13% to 76% of the clinical isolates display high antibacterial resistance (Ardila et al., 2020). **Aim:** Our aim is to identify novel anti-*Pg* agents by bioprospecting the phytomicrobiota of medicinal plants, an underexplored niche of microbial and chemical diversity (Newman and Cragg, 2020). **Methods**: Bacterial endophytes and epiphytes from *L. angustifolia* were isolated from 2021 to 2023. A screening panel of 20 isolates was characterized and confronted against *Pg* ATCC 33277 using our modified version of the "agar-overlay antibacterial assay" tested in different culture media. Then, ethyl-acetate extracts obtained from co-culturing the bioactive strains together with *Pg* were tested anaerobically in the microdilution assay to determine the Minimum Inhibitory Concentration (MIC). **Results**: An epiphytic *Streptomyces albidoflavus* isolate (*Sa*_LaEp14) grown in supplemented Potato Dextrose media presented the highest bioactivity against *Pg* (inhibition halo:1.2 cm) (Fig. 1). Extracts from co-cultures with *Pg* showed a stronger anti-*Pg* effect (MIC: 0.25 µg/mL) than extracts from pure *Sa*_LaEp14 cultures (MIC: 2 µg/mL).

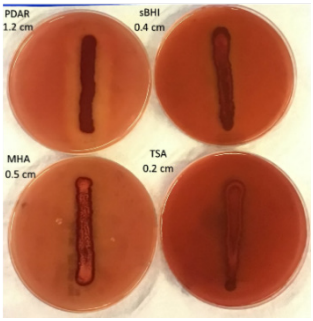

Fig. 1. Modified "agar overlay assay" showing *Sa*_LaEp14 (seeded in the middle) forming an inhibition halo against *Pg*.

Conclusions: This is the first report demonstrating that co-culturing *Pg* and the epiphyte *Sa*_LaEp14 increases the production of anti-*Pg* secondary metabolites by *Sa*_LaEp14. Currently, the identity of the bioactive secondary metabolites is under analysis.

Keywords: phytomicrobiota, lavender, co-culture, *P. gingivalis*, antibacterial

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S1.P24 Tyrosinase and hyaluronidase inhibitors in *Eleutherococcus divaricatus*

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For centuries, plants have been a major source of medicines for the treatment of many diseases. The development of modern research methods has made it possible to isolate the active components of plants and develop much more effective and safer drugs (Katiyar et al. 2012). Currently available compounds, which inhibit tyrosinase or hyaluronidase, are characterized by a few unfavorable properties such as low persistence, poor absorption, or carcinogenicity (Shakya, 2016). The aim of the present study was to determine the usefulness of Eleutherococcus divaricatus in the treatment of tyrosinase- and hyaluronidase-related diseases. These enzymes represent potential therapeutic targets in the inflammatory-related diseases. Based on the results, *E. divaricatus* inhibited tyrosinase and hyaluronidase activity. The ethyl acetate fraction showed the strongest inhibition (bovine hyaluronidase - IC50=27.5 µg/ml, fungal tyrosinase - IC50=65.5 µg/ml, respectively). The extract was also active against human hyaluronidase from plasma (55.83 µg/ml). In both cases, the extract inhibited the enzyme stronger than aescin, used as the positive control. The fraction was rich in rosmarinic acid (26.62 mg/g) and caffeic acid (24.02 mg/g). The results support the traditional use of the roots in enzymes-related diseases. The use of ethyl acetate fraction demonstrates its inhibiting effect especially towards the hyaluronidase activity, which may additionally indicate its anti- inflammatory property. Thus, the results mean that the fraction may be used in inflammatory-related diseases.

Keywords: *Eleutherococcus divaricatus,* Araliaceae, hyaluronidase, tyrosinase, human serum

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S1.P25 Four sesquiterpene-acylated flavonol glycosides from the sprouts of *Astragalus membranaceus*

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Sprouting as a cultivation technique has shown promise in enhancing the metabolite profile of medicinal plants, leading to potential new applications in functional foods (Benincasa et al., 2019). The present research investigated *Astragalus membranaceus* Bunge, a medicinal plant widely utilized for its roots in Oriental medicine, focusing on its sprouts which have not been extensively studied for their secondary metabolites (Hou et al., 2023). Initially, feature-based molecular networking workflow was employed to distinguish the metabolites in *A. membranaceus*' sprouts from those found in its roots. Next, compounds were annotated by matching the MS/MS spectrum with those in public databases, leading to the discovery of compounds not previously reported. Then, the targeted isolation and structure elucidation of these compounds from *A. membranaceus*' sprouts were carried out. As a result, four unreported sesquiterpene-acylated flavonol glycosides (**1**–**4**) were identified as compounds specific to *A. membranaceus* sprouts (Fig.1.). The chemical structures of compounds **1**–**4** were determined through a comprehensive analysis of their spectroscopic data (UV, HR-MS, 1D- and 2D-NMR, and CD), along with hydrolysis.

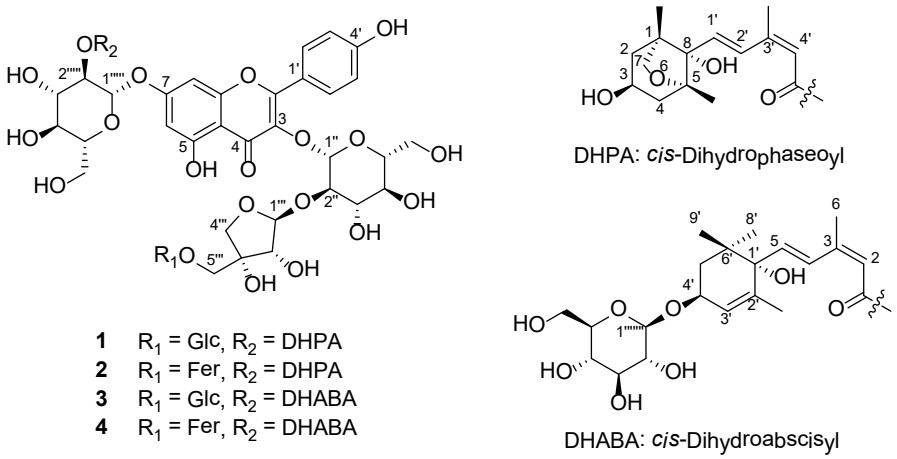

Fig. 1. Sesquiterpene-acylated flavonol glycosides from the sprouts of *A. membranaceus*

Keywords: *Astragalus membranaceus* Bunge, sprouts, flavonol glycosides

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S1.P26 Effects of Astraflavonoids A and D, flavonol glycosides from the aerial parts of *Astragalus membranaceus*, on TNF-α-induced human dermal fibroblasts

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Astragalus membranaceus, a commonly used medicinal herb, is primarily valued for its root, while the aerial parts are typically discarded (Guo et al., 2022). To utilize this agricultural waste, the present study focused on the anti-skin aging properties of the aerial parts of *A. membranaceus* and their major constituents. First, a LC-MS analysis identified flavonoid glycosides as the major constituents in the aerial parts of *A. membranaceus* extract. Following this, two significant acylated flavonol glycosides—astraflavonoids A and D (**1** and **2**; Fig. 1.)— were isolated, with astraflavonoid D (**2**) being a previously unreported compound. The chemical structures of the isolates were elucidated through HR-MS, UV, IR, 1D- and 2D-NMR analysis, acid hydrolysis, and comparison with existing literature (Hao et al., 2016). Subsequent experiments revealed that both astraflavonoids A and D (**1** and **2**) have antioxidative properties, reducing ROS and MMP-1 level stimulated by TNF-α in NHDF. Specifically, astraflavonoid A (**1**) also inhibited the expression of pro-inflammatory cytokines (IL-6 and IL-8). Additionally, the expression of inflammatory mediators (NF-κB and COX-2) was suppressed by astraflavonoid A (**1**). These findings indicate that astraflavonoids A and D (**1** and **2**) from the aerial parts of *A. membranaceus* are active principles to possess anti-skin aging properties, particularly astraflavonoid A (**1**), as a potential inhibitor for inflammatory skin diseases.

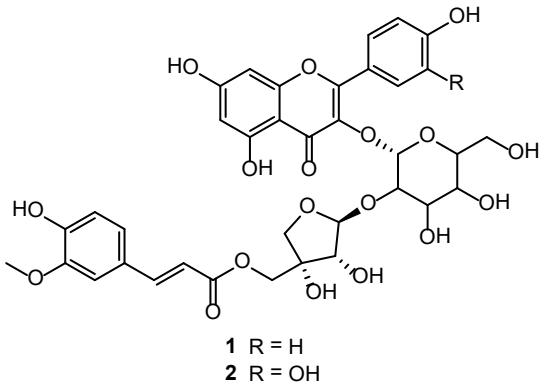

Fig. 1. Structures of compounds **1** and **2**

Keywords: *Astragalus membranaceus* Bunge, reactive oxygen species, MMP-1, COX-2

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S1.P27 Harnessing the microbial richness of *Ips perturbatus* for novel antimycotic agents

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This presentation will provide an exploration of the potential of the bacterial symbionts of northern spruce engraver beetle (*Ips perturbatus*) as novel antimicrobial sources. Previously complex interplay between this beetle and its associated microorganisms has demonstrated biological novelty. For example, a new species of fungus, *Leptographium fruticetum* sp. nov., was identified from *Ips perturbatus* collected in northern British Columbia and Yukon Territory, with a possibly specific symbiotic relationship between the beetle and this fungus highlighted (Alamouti et al., 2006a). Additional studies have revealed the presence of a diverse range of ophiostomatoid fungi associated with *Ips perturbatus* (Alamouti et al., 2006b), along with a significant chemical component to the holobiont's interaction with its environment (Holsten et al., 2001). This variety of ecological interaction involving insects, plants and microorganisms has previously been shown to be associated with rich bacterial chemistry (Beemelmanns et al., 2016). Nevertheless, the capacity of the bacteria within this holobiont to produce unique chemistry has not been explored. This presentation describes our work as we begin to unravel the potential chemistry of this microbiome. As part of our continuing search for new antimycotics active against both plant and human pathogens, the analysis of the bacterial community of *Ips perturbatus* collected in the Yukon Territory demonstrated a community rich in Actinobacteria. Examination of the resulting isolates with bioassays, spectroscopic and genomic data, continues to drive our interest in the bacteria of this underexplored microbiome.

Keywords: microbiome, antimicrobial, bioactive compounds, insect-associated bacteria

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S1.P28 Insights into the mechanism of antibacterial action for prenylated (iso)flavonoids from Fabaceae: a systematic study on inhibition and permeabilization of Methicillin-resistant *Staphylococcus aureus*

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Prenylated (iso)flavonoids are plant-derived compounds with potent antimicrobial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA). They primarily act on the bacterial membrane and disrupt membrane integrity by fast permeabilization (Araya-Cloutier et al., 2018; Sun et al., 2022). Very potent antimicrobial diprenylated (iso)flavonoids, however, did not show membrane permeabilization in *Listeria monocytogenes* (Araya-Cloutier et al., 2018). So far, the permeabilization capacity of prenylated (iso)flavonoids is only assessed at singular concentrations, making it impossible to establish dose-response relationships and accurately compare permeabilization potencies. In this study antimicrobial activity and permeabilization capacity of 36 different prenylated (iso)flavonoids were assessed against MRSA. Antimicrobial activity was determined by the broth microdilution assay and membrane permeabilization by measuring the uptake of propidium iodide using fluorescence microscopy and spectroscopy. Dose-response curves were established and used to extract effective concentrations (EC10). No relationship between antimicrobial activity and permeabilizing capacity was found for compounds having good antimicrobial activity (minimum inhibitory concentration (MIC) ≤ 50 µg/mL). Luteone (**1**) (EC10: 23±7 µg/mL) and neobavaisoflavone (**2**) (EC10: 28±8 µg/mL) showed most potent permeabilization but were not the most potent antimicrobials (MIC of 25 and 38 µg/mL, respectively). Molecular descriptors analyses revealed that permeabilization (EC10) could not be explained by relative hydrophobic surface area, as found for *Listeria monocytogenes*. Conversely, polar surface area was positively correlated to pEC10, suggesting that membrane permeabilization occurs through interactions with polar phospholipid headgroups. These results highlight the complexity of prenylated (iso)flavonoids' antimicrobial action, suggesting other (membrane) effects besides permeabilization.

Keywords: prenylated (iso)flavonoids, methicillin-resistant *Staphylococcus aureus*, antimicrobial activity, permeabilization, propidium iodide uptake

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Sun, L., Tang, Z., Wang, M., Shi, J., Lin, Y., Sun, T., Zou, Z., Weng, Z. (2022). Exploration of Antimicrobial Ingredients in *Psoralea corylifolia* L. Seed and Related Mechanism against Methicillin-Resistant *Staphylococcus aureus.* Molecules, 27(20).

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S1.P29 *Porphyridium cruentum* as a chemical factory: valorization of high-value phycobiliproteins and lipids using an innovative solid-liquid-liquid method based entirely on eutectic solvents

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Microalgae are being studied as sustainable factories for sourcing pharmaceutical, cosmetics, and nutraceutical molecules (Sonani et al., 2014). As an example, *Porphyridium cruentum*, a red microalgae, is a valuable source of metabolites with high added value, such as free fatty acids and pigments like phycobiliproteins. Natural Deep Eutectic Solvents (NaDES) are a new environmentally friendly substitute for petrochemical solvents. NaDES are green, non-toxic, economical, and bio-based solvents formed from plant metabolites such as sugars (Dai et al, 2013). We recently developed an innovative solid/liquid/liquid (SLL) strategy for one-step extraction and purification of metabolites using a combination of polar and nonpolar NaDESs (Fig. 1) (Hilali et al., 2024).

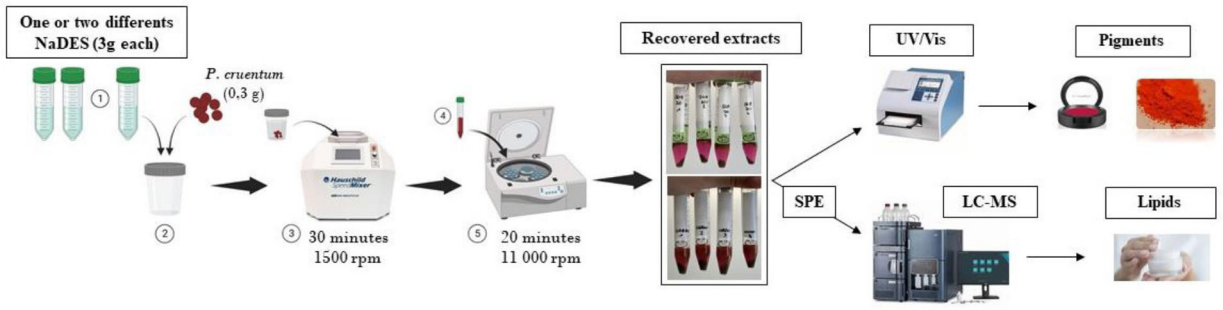

Figure 1 : Workflow for extracting *P. cruentum* using two different processes.

A screening process was conducted on a large library of NaDES to identify the most suitable polar-nonpolar NaDES partners. Compared to the classical solid/liquid approach, the SLL process significantly increases the extractive performance of non-polar NaDES. Among all NaDES combinations screened, we highlighted good combinations to improve phycobiliprotein stability over time or to maximize lipid and phycoerythrin recovery.

Keywords: *Porphyridium cruentum,* solid-liquid-liquid extraction, phycobiliproteins, free fatty acids, NaDES

Reference

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S1.P30 Formicine ant venoms put to the acid test - novel antimicrobial peptide families revealed by proteotranscriptomics

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Research on venoms of ants (Hymenoptera: Formicidae) dates back to Samuel Fisher´s first report on the isolation of formic acid from wood ants (*Formica* sp.) in 1670. Since, studies have revealed a plethora of natural products from ant venoms, including alkaloids, terpenoids, and peptides with neurotoxic, insecticidal, or antimicrobial activities (Touchard et al., 2016). Among ants, venom sprays containing concentrated formic acid are exclusively found in the Formicinae subfamily. Formicine ants use their multipurpose venoms in predation and defense, for disinfection in brood- and self-grooming, and, when swallowed, gut microbial control. (Pull et al., 2018; Tragust et al., 2020) Despite their biological importance, little is known about the composition of formicine ant venoms, and the occasionally presumed presence of uncharacterized peptide natural products has never been substantiated (Flury, 1919; Osman and Brander, 1961). Pursuing this enigma, we analyzed the venom of the carpenter ant *Camponotus nicobarensis* combining mass spectrometry and RNA sequencing. Next to various lipids, we identified three novel peptides in the venom. These peptides, the formicitoxins, are encoded by two distinct gene families exclusive to formicine ants. Intriguingly, they share the tendency to adopt amphipathic alpha-helical structures – a common feature of many antimicrobial peptides. Indeed, we found two formicitoxins to inhibit growth of the entomopathogenic fungi *Metarhizium robertsii* and *Beauveria bassiana*. In conclusion, we for the first time characterize peptides from a formicine ant venom. Crucially, these may function as antimicrobials in external immune defense and thus represent an adaptation to the microbial challenges threatening colony health.

Keywords: *Camponotus nicobarensis,* Formicidae, venom, peptides, antifungal

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Tragust, S.; Herrmann, C.; Häfner, J.; Braasch, R.; Tilgen, C.; Hoock, M.; Milidakis, M.A.; Gross, R.; Feldhaar, H., 2020. Formicine ants swallow their highly acidic poison for gut microbial selection and control. eLife 9, e60287.

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S1.P31 Antibacterial activity of a novel callus culture line from *Cistus creticus*

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Callus cultures are currently the most used biotechnological platform used to provide year-round and low cost production of natural compounds. This method can also greatly contribute to the study of specific biosynthetic pathway, speeding up the process. In the wide-ranging plant kingdom, *Cistus creticus* (Cistaceae) is a source of specialized metabolites mainly represented by catechins, monoterpenes and sesquiterpenes (Zalegh et al., 2021). Accordingly, this species has long been reported to be a medicinal plant and to be marketed as herbal infusions and dietary supplements (Viapiana et al., 2017). This is specifically due to the antimicrobial properties of *C. creticus* extract tested against several viruses (Ebola virus, HSV-1, PI-3, and dengue virus) and bacteria (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*) causing severe human diseases (Zalegh et al., 2021). Our work aimed to develop a novel callus culture line from *C. creticus* hypocotyl and explore the potential of its extract against foodborne pathogens (Fig. 1. (B)).

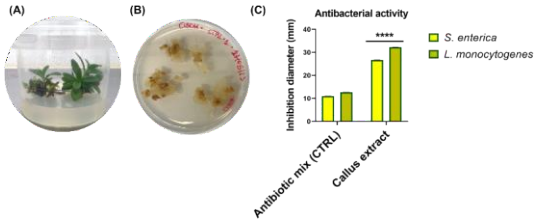

Fig. 1. (A) *Cistus creticus in vitro* plant; (B) Hypocotyl-derived calli; (C) Antibacterial activity of hypocotyl-derived callus extract compared to an antibiotic mixture.

As shown in Fig. 1. (C), the antibacterial activity of the methanolic extract was tested against *Salmonella enterica* (Gram negative) and *Listeria monocytogenes* (Gram positive) by the disk diffusion method. The results demonstrated that the hypocotyl-derived callus extract was more than twice as effective as the antibiotic mixture (combination of ampicillin and clavulanic acid) in reducing bacterial growth. This confirms the outstanding potential of cell cultures in the production of natural phytochemicals. Further investigations need to be performed to better elucidate the specific molecules that might be involved in this function.

Keywords: Cistaceae, cell cultures, specialized metabolites, antimicrobial potential

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S1.P32 Secondary metabolites produced by *Nigrospora sphaerica* associated with the invasive weed *Cenchrus ciliaris*

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Due to the urgent need for new strategies in managing the agricultural pests, the production of phytotoxic compounds from fungal pathogens could represent a new frontier for weed management. Phytopathogenic fungi can be employed as biocontrol agents representing good alternatives for weed management that allow reducing hazardous effects of synthetic pesticides (Masi et al. 2017A and B; Siciliano et al., 2023). *Nigrospora sphaerica* is known to be a plant pathogen, causing diseases in various plants including fruits and vegetables. In this respect, the pathogenic association of this fungus with invasive weeds suggests a possible production of phytotoxic compounds which could help in the development of new weed control strategies. A screening of secondary metabolites produced in mycelial and culture filtrate extracts of a foliar strain of *N. sphaerica* isolated from buffelgrass (*Cenchrus ciliaris*) was conducted after observing an intense activity of the organic extract on buffelgrass seed germination and subsequent seedling growth. The present communication reports the structural and stereostructural elucidation of a new triol, nigrosphaeritriol (**1**) and a new lactol, nigrosphaerilactol (**2**), together with the identification of several known compounds. Some of them are reported in Fig. 1.

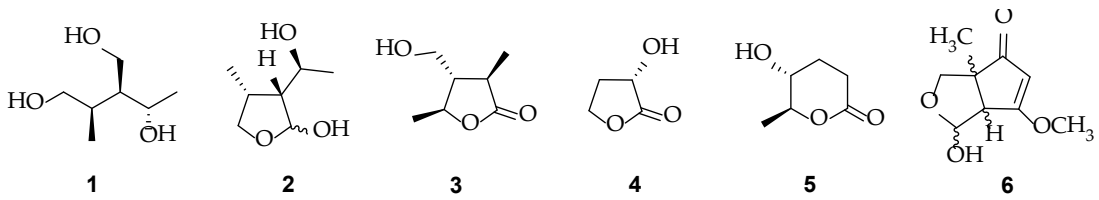

Fig 1. Structures of nigrosphaeritriol, nigrosphaerilactol, nigrosphaerilactone, (*S*)-hydroxybutyrolactone, lupinlactone, nigrosporione A (**1**-**6**).

Keywords: weed, bioherbicides, specialized metabolites, Integrate Pest Management, buffelgrass

References

Masi, M., Meyer, S., Clement, S., Pescitelli, G., Cimmino, A., Cristofaro, M., Evidente, A., 2017A. Chloromonilinic acids C and D, phytotoxic tetrasubstituted 3-chromanonacrylic acids isolated from *Cochliobolus australiensis* with potential herbicidal activity against buffelgrass (*Cenchrus ciliaris*). J. Nat. Prod. 80, 2771-2777.

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S1.P33 Anti-inflammatory activity of a methanol extract of *Adiantum capillus-veneris L.*

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Four highly programmed phases are involved in wound healing, namely, haemostasis, inflammation, proliferation, and remodelling. A normal inflammation process is necessary for removing contaminated factors. However, prolonged inflammation occurs in certain circumstances, such as incomplete clearance of micro-organisms. This often leads to the transition from acute wounds to chronic wounds, resulting in a severe impact on patient's quality of life (Guo et al., 2010).

The fern *Adiantum capillus-veneris* L. (Pteriaceae) has been used traditionally as an analgesic for relieving headache, preventing hair loss, and treating asthma (Dehdari et al., 2018). In our previous research, the methanol extracts of *A. capillus-veneris* promoted proliferation and migration of human HaCaT keratinocytes at low concentrations. As part of our continuing research, we explored its anti-inflammatory activity on LPS-stimulated RAW 264.7 mouse macrophages. Using ELISA, we found that the methanol extract significantly inhibited LPS-induced release of three inflammation-related cytokines (TNF-a, IL-6, IL-1β), and three chemokines (CXCL2, CXCL10, CCL2) in a concentration-dependant manner (0.1, 1, 10, 50 µg/mL). None of the tested concentrations showed cytotoxic effects in RAW 264.7 cells. In addition, Western blot analyses showed that LPS-activated mitogen-activated protein kinases (MAPK) p38 MAPK and ERK1/2 were also inhibited by the methanol extracts of *A. capillus-veneris* in RAW 264.7 cells. Here, we provide first evidence that the anti-inflammatory activity of *A. capillus-veneris* observed in vivo may be mediated by its inhibitory effect on release of CXCL10, CCL2, CXCL2 and IL-1β, and inhibition of activated p38 MAPK and ERK1/2 kinases under inflammatory conditions.

Keywords: medicinal plants, *Adiantum capillus-veneris L.,* anti-inflammatory activity, wound healing

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Acknowledgment

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S1.P34 Fatty acid profile of lipids from microalgae grown on media with hydrolysed agro-food biowaste

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The growing demand for natural products for the nutraceutical, pharmaceutical and cosmetic industries has led to investigate micro and macroalgae as a source of bioactive compounds of interest, including carotenoids, polyphenols and fatty acids (Levasseur et al., 2020). Moreover, as microalgae often grow in mixotrophic conditions, enriching the culture media with a carbon source coming from biowaste, is an interesting perspective to develop sustainable processes. This presentation will concern the study of the fatty acid profiles obtained from the microalgae *Chlamydomonas reinhardtii* (CC-503 and CC-5163) and *Chlorococcum* sp. The microalgae were grown in mixotrophic conditions, employing TAP or BG-11 media containing variable percentages (5% and 15%) of hydrothermally hydrolysed biowaste coming from the agri-food industry or the environment such as orange peels, hemp or the algae *Cladophora glomerata*. In a typical procedure, the microalgae biomass was harvested after 10 days from inoculum by centrifugation and washed with water to eliminate media residues and finally freeze-dried. The fatty acids fractions were obtained by rupture and extraction of the microalgae biomasses (2-3 mgs) in two-steps carried out with 0.5 mL 0.035 M KOH in methanol (keeping the sample in these conditions overnight) and sonication for 5 min (step 1), then 0.1 mL of 2% H2SO4 in methanol is added (Step 2). Next, the sample was extracted with 0.6 mL of petroleum ether and analysed by gas chromatography (GC-FID)(Cooney et al., 2009). The microalgae growth and the fatty acids profiles were influenced by the addition of the biowaste hydrolysate, which significantly increased the percentage of unsaturated fatty acids, C16:2, C16:3, C18:2 and C18:3.

Financial support from the European Union – Next Generation EU in the frame of the "National Biodiversity Future Center"(CUPn°B83C22002930006) is gratefully acknowledged.

Keywords: microalgae, lipid, mixotrophy growth, *Chlamydomonas reinhardtii, Chlorococcum sp*

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S1.P35 Cytotoxic cyclopeptides from the fungus *Gymnopus fusipes* (Omphalotaceae)

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In our ongoing research focused on identifying fungal natural products with anticancer potential, we have discovered novel cyclic octadecapeptides from the mushroom *Gymnopus fusipes* (Bull.: Fr.) Gray. Commonly referred to as spindleshank, *G. fusipes* is a parasitic species (Camy et al., 2003) within the Omphalotaceae family, native to woodlands of Europe and Asia. The mushroom material was freeze-dried followed by extraction using methanol. The resultant crude extract was subjected to normal-phase flash chromatography. The ultimate purification was accomplished through reversed- phase HPLC, leading to the novel gymnopeptides C and D, besides the previously known gymnopeptides A and B (Ványolós et al., 2016). The structure elucidation of the isolated metabolites was performed by extensive NMR and MS analysis. The absolute configurations of the amino acid residues were examined using HPLC–MS. Initially, the cyclopeptides underwent acidic hydrolysis, followed by derivatization of the resultant amino acid mixture with Marfrey's reagent. Subsequently, HPLC–MS analysis was conducted, wherein the retention times corresponding to the accurate mass values of the derivatized amino acids in the extract ion chromatograms were compared with those observed for the derivatized reference L,D-amino acid pairs. The antiproliferative properties of the isolated peptides were determined by means of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on human cancer cell lines. Gymnopeptides A-D demonstrated very strong cell growth inhibitory activity with nanomolar IC50 values.

Acknowledgements: The authors are grateful for the support of the National Research, Development and Innovation Office, Hungary (grant: OTKA NKFIH FK-146487).

Keywords: *Gymnopus fusipes,* Omphalotaceae, cyclic peptides, cytotoxicity, fungus

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S1.P36 Cytotoxic hederagenin saponins from the seeds of *Oxybasis rubra* (L.) S. Fuentes, Uotila & Borsch (Amaranthaceae)

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This work reports isolation, structure elucidation and cytotoxic activity evaluation of two triterpene saponins (**1,2**) from *Oxybasis rubra* (L.)S.Fuentes, Uotila & Borsh (Amaranthaceae). The species (syn. *Chenopodium rubrum* L.) is an annual plant, widely distributed in temperate zone, with characteristic shiny leaves. It has been mainly used as a dye, and as a food ingredient, but is also a folk medicinal anticancer remedy (Kokanova-Nedialkova et al., 2009).

Our on-going studies on triterpene saponins from various plant sources are focused on the evaluation of their cytotoxic potential. As Amaranthaceae is a known source of saponin-plants we decided to analyze *O. rubra*, which is a phytochemically under-explored species. MeOH extracts from different plant parts were initially tested for cytotoxic activity (Mynarski et al. 2018). Chromatographic separation of fruit extract (MPLC, CC) led to the isolation of two compounds, which, following extensive NMR spectroscopy and HRESIMS, have been identified as 3-O-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl] hederagenin **1** (a new structure) and 3-O-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl] hederagenin 28-O-β-D-glucopyranosyl ester **2** (Fig. 1).

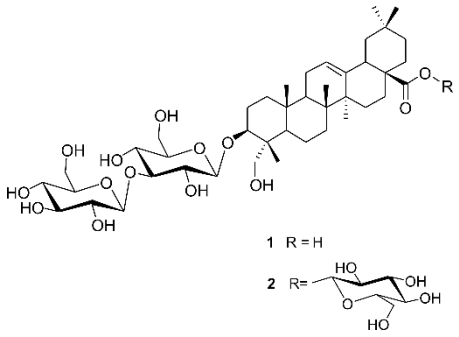

Fig. 1. Structures of hederagenin saponins **1** and **2** from *Oxybasis rubra*

Both saponins were tested on a wide panel of 11 human cancer cell lines using colorimetric LDH assay. Normal human cells were included to assess selectivity of action. In general, the bidesmosidic saponin was less active compared to the monodesmoside. Both saponins showed moderate activity and were most active against lung cancer A549 (IC_{50} 8.24 and 11.8μg/mL) while best selectivity was observed for **2** (IC_{50} 21.09 μg/mL and >100μg/mL, against cancer PC3 and normal prostate PNT2 cells, respectively).

Keywords: *Oxybasis rubra,* Amaranthaceae, hederagenin saponins, cytotoxicity

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S1.P37 Untargeted and targeted LC-MS metabolomic analyses of putative chemical defenses in *Taenaris* butterflies

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The potential chemical defense mechanisms of *Taenaris* butterflies, a group of 25 species found in New Guinea and nearby islands, was investigated. While the chemical defense of herbivorous insects in temperate regions are reasonably well-studied (Agrawal, 2017), tropical taxa, including *Taenaris*, have received limited attention. *Taenaris* butterflies exhibit characteristics of being chemically defended (aposematic), such as mimicry by other species (Lohman et al., 2020). We examined the hypothesis (Parsons, 1984, 1998) that adult *Taenaris* butterflies are defended by cycad-derived compounds, including cycasin, macrozamin, and methylazoxymethanol acetate, acquired through larval or adult feeding. Contrary to previous findings, our comprehensive metabolomic analyses fail to detect these compounds in *Taenaris* samples. This suggests that cycad-derived compounds are not present in *Taenaris* butterflies, though compound degradation, instrument limitations, or sample handling may have prevented detection. A multivariate analysis highlighted metabolomic differences between *Taenaris* and their close relative *Faunis eumeus*, which prompted further exploration into taxon-specific defensive chemical features. Field observations on Yapen Island suggest that *Taenaris* adults may acquire defensive compounds via pharmacophagy from sources other than cycads, challenging published observations suggesting that species rely on cycad-derived toxins. These findings contribute to our understanding of the ecological and evolutionary dynamics of butterfly communities in tropical regions.

Keywords: chemical defense, Cycadaceae, cycasin, LC-MS metabolomics, mimicry

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S1.P38 Multimodal analysis of oak wood metabolites for a comprehensive understanding of the ageing process in French typical spirits barrels

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Oak wood is vital in the barrel ageing process of spirits, impacting their flavor and quality (Canas, 2017). To optimize this process, understanding the dynamics of wood metabolites is crucial. In our study, we employed a comprehensive and multimodal analytical approach to investigate the spatial distribution of central and specialized metabolites in oak wood. To achieve this, we conducted a detailed exploration using various analytical techniques such as Mass Spectrometry in tandem, Laser Desorption/Ionization, and Time-of-Flight Secondary Ion Mass Spectrometry (Vanbellingen et al., 2016), to study the distribution of metabolites in oak wood according different parameters. We analyzed metabolite profiles of samples from freshly cut or toasted oak compared to a 150-year-old oak stave. By mapping the distribution of lignins, polysaccharides, lipids, and phenolic compounds at the subcellular level and across the stave thickness, we gained a comprehensive understanding of the metabolite dynamics within the wood. One notable finding was the significant decline in polysaccharide fragment ions observed in the 150-year-old oak stave. This decrease suggests potential degradation of polysaccharides over time, which could have implications for the ageing process of spirits in oak barrels. In addition, investigation of the spatial distribution of inorganic compounds within the oak wood revealed unique patterns that could influence oak growth and durability. Overall, our study provides a fresh perspective on the shifts in metabolites within oak wood over time. The insights gained from this research can contribute to the development of refined barrel aging techniques, ultimately leading to better spirit quality.

Keywords: oak wood, TOF-SIMS, LC-MS, LDI-MS, molecular network

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S1.P39 Exometabolites from the Mediterranean sponge *Agelas oroides*: where is oroidin?

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Sponges are filter-feeding sessile marine animals that are keystone species of various benthic ecosystems. They are known to produce a plethora of structurally diverse metabolites. Some of them are part of their toolbox for defense and communication strategies. When released, these (exo)metabolites may contribute to distant species interactions (Hay 2009; Santonja et al. 2018). In the course of our studies on the chemical diversity of exometabolites from sponges, we wanted to know whether *Agelas oroides* (Schmidt, 1864), a rather dominant species of Mediterranean marine ecosystems, releases its characteristic bromo-pyrrole alkaloids. A series of captures and enrichment experiments were performed in aquaria and in situ (Mauduit et al. 2023). MS-based metabolomics revealed the paucity of bromo-pyrrole alkaloids as exometabolites. Oroidin was not detected in seawater despite being the most abundant compound in *A. oroides* extract (Fig. 1), and as opposed to published results with *Agelas conifera* (Richelle-Maurer et al. 2003).

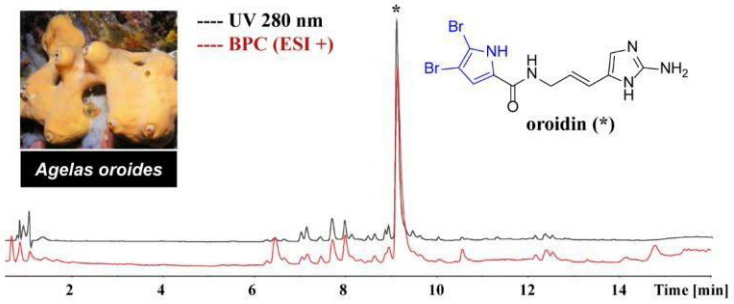

Fig. 1. Stacked chromatograms of *A. oroides* extract containing 615% w/w of oroidin.

Such findings prompted us to explore the fate of oroidin from the sponge biomass to the seawater. The presentation will delve into these experimentations, and illustrate how examining organisms in their ecosystems can lead to new discoveries even for extensively studied species.

Keywords: sponges, exometabolites, oroidin, biotransformation, MS-based metabolomics

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S1.P40 Anti-platelet aggregation and vasorelaxant activity of rapanone from *Ardisia crenata* Sims

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The results of the study that will be presented concern the isolation and biological evaluation of naturally occurring benzoquinone, rapanone, from leaves of *Ardisia crenata* Sims (Primulaceae). This shrub can achieve 1-2 m of height, has characteristic dark green leaves with tightly waved edges and originally red berries. *A. crenata* occurs naturally in Southern and Eastern Asia and in Europe it is cultivated as a greenhouse plant due to its ornamental value (Larson et al., 2023; Lim et al., 2012). As rapanone (Fig.1) was recognized as an anti-inflammatory and antioxidant agent, we decided to check its potential value in non-communicable diseases which are characterized by chronic inflammation, such as e.g. cardiovascular disorders (Geto et al., 2020). Thus, its anti-platelet and vasorelaxant activity was evaluated. Rapanone has been isolated form the air-dried leaves of *A. crenata* using extraction protocol developed by us previously (Wróbel-Biedrawa et al., 2022). Ethyl acetate UAE extract was separated by chromatographic methods (CC, pTLC) to obtain rapanone. The purity and identity of the isolate was checked by LC-MS/MS and ^{13}C NMR.

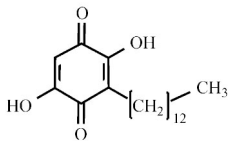

Fig.1. Structure of rapanone.

Rapanone was tested in an *in vitro* aggregation test and was shown to inhibit collagen induced platelet aggregation with IC50=40.2±18.6 μM. Furthermore, in an *ex vivo* assessment of the effect in the rat aorta precontracted with phenylephrine, the compound exhibited concentration-dependent (in the range of 0.3 – 100 μM) relaxation in endothelium intact aortic rings, reaching maximal effect at the level of almost 50%.

Keywords: *Ardisia crenata,* Primulaceae, anti-platelet, vasorelaxation, cardiovascular diseases

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S1.P41 Unlocking bioactivity of endophytic fungi from Mokrzański Forest in Wroclaw, Poland

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This presentation will cover the isolation of fungal endophytes from some plant species of a local forest in Wroclaw, Poland, and the testing of several biological activities of their extracts. Currently, the search for new bioactive compounds with innovative modes of action and chemistry is desperately needed to discover new drug leads (Miethke et al. 2021). Indeed, the increased emergence of drug- resistant microbes is a dramatic example of such pressing needs. Generally, forests are unique habitats where the growing plants face extreme environmental conditions, such as nutrient deficiency, high oxidative stress, etc (Terhonen et al. 2019). Therefore, the associated endophytic fungi of these plants are more metabolically active than their counterparts, and as a result, may biosynthesize novel bioactive chemical scaffolds (El-Sayed et al. 2021). With this view, the presented research was conducted for the isolation of fungal endophytes of twelve different plant species viz., *Larix decidua*, *Pinus sylvestris*, *Corylus avellana*, *Alnus glutinosa*, *Quercus robur*, *Fagus sylvatica*, *Picea abies*, *Populus tremula*, *Sorbus aucuparia*, *Acer platanoides*, *Betula pendula*, and *Robinia pseudoacacia*. A total of 46 endophytic fungi were isolated and tested for bioactive metabolites with antifungal, antibacterial, antioxidant, and anti-enzymatic (monoamine oxidase A, acetyl, and butyl cholinesterase inhibitors) properties in their extracts. Upon screening, several fungal isolates showed promising activities. Extracts with activities were fractionated by TLC and then re-tested. Current work is to chemically identify compounds responsible for these activities. The presented research also explains the high value of fungal endophytes from forest plants as untapped sources of bioactive metabolites.

Acknowledgments: The presented research is part of the BioExplor project No. 2021/43/P/NZ9/02241 co-funded by the National Science Centre and the European Union Framework Programme for Research and Innovation Horizon 2020 under the Marie Skłodowska-Curie grant agreement no. 945339.

Keywords: endophytic fungi, bioactivities, cytotoxicity, antimicrobial, anti-enzymatic

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S1.P42 HME-DDS-applied astaxanthin nanocapsule suppresses DNCB-induced atopic dermatitis-like inflammation in BALB/c mice

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Astaxanthin is a carotenoid as a natural product be found in various bacteria or microalgae. Among the different resources, microalga *Haematococcus pluvialis* is known for the most potential source for the biological production. Astaxanthin has broad latent abilities on skin health; anti-aging, anti-oxidant, and anti-inflammatory. Since the skin plays a crucial role as the outermost barrier against external surroundings, keeping the skin health is one of the important ways to keep the immunological balance. Though the high capacity on the skin biology and related application of astaxanthin, its poorly low solubility and difficult processes lower the possibility of utilization. The authors applied hot melt extrusion (HME)-drug delivery system (DDS) on the natural product (HDA) leading high contents and usages of astaxanthin. The authors investigated the effects of HDA on 2,4-dinitrochlorbenzene (DNCB)-induced atopic dermatitis (AD)-like skin inflammation in an animal model for 4 weeks. Comparison between HDA and non-process raw astaxanthin material (NRA) was conducted. Repeated toplication of DNCB caused the pathological symptoms on the dorsal skin including edema, redness, and dryness. Mice were orally administered with HDA showed relatively low itching scratching behavior frequency and less scaly areas than NRA-treated ones. Also, high skin barrier index was shown in HDA treated group than in the group with NRA; index was measured by transepidermal water loss and severity of symptoms. With these results, the authors assumed the HDA showed ameliorative effects on DNCB-induced AD mouse model comparing to the NRA.

Acknowledgments: This research supported by Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (grant # RS-2023-00254674).

Keywords: *Astaxanthin Nanocapsule, HME-DDS, DNCB, atopic dermatitis, skin barrier*

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S1.P43 Spermidine alkaloids of the native Australian plant *Acacia auriculiformis*

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Australian Aboriginal culture is one of the oldest enduring cultures on Earth. This ethnomedicinal knowledge offers a curated library of traditionally used and medicinally active plants (Mani et al. 2021). Our group is exploring the phytochemical profile of a range of native Australian plants used in traditional Aboriginal medicine. *Acacia auriculiformis* from the family *Fabaceae/Leguminosae* is a small tree native to northern regions of Australia and parts of Indonesia and Papua New Guinea (Boland et al., 1990). More commonly known as the 'ear-pod wattle', the pods and leaves have been used traditionally by Aboriginal communities to prepare an antiseptic skin wash and to relieve itching skin conditions. Crushed plant material may also be thrown in to watering holes to kill fish for later collection (Smith et al., 199q; Marrfurra, 1995). Our preliminary investigations indicated that the leaves and seed pods were rich in alkaloids that had not previously been explored. We now report the isolation and structure elucidation of a family of alkaloids using 1D and 2D ^{1}H, ^{13}C and ^{15}N NMR techniques and high-resolution mass spectrometry. These compounds contain an unusual substituted perhydro-1,5-diazocine-2-one structure (Fig. 1) and are previously unreported.

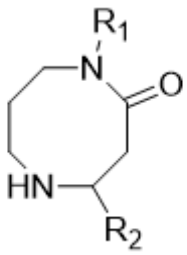

Fig. 1. General structure of isolated perhydro-1,5-diazocine-2-one compound family.

Keywords: *Acacia auriculiformis*, ethnopharmacology, alkaloids, diazocine

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S1.P44 New bioactive peptaibols from a soil fungus, *Trichoderma strigosum* (Hypocreaceae)

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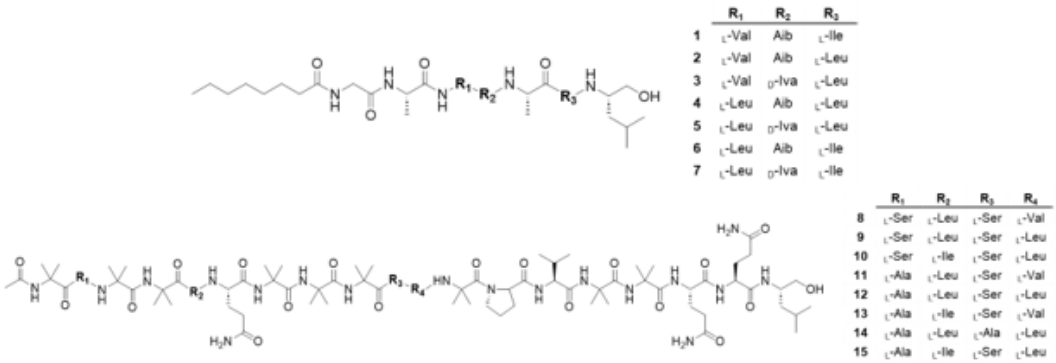
The genus *Trichoderma,* commonly found in soil, is known to produce cytotoxic peptides with unique amino acid sequences, known as peptaibols. In an effort to discover bioactive secondary metabolites from fungi, *Trichoderma strigosum* was isolated from soil in north part of Seoul. Large scale culture for chemical investigation followed by LC-MS-guided isolation led to isolation five new lipopeptaibols (**1**-**5**) and eight new 19-residue peptaibols (**8**-**15**) along with two known lipopeptaibols, lipovelutibols C (**6**) and D (**7**). The planar structures of the newly discovered peptaibols (**1**-**5**, **8**-**15**) were elucidated through a comprehensive analysis of 1D, 2D NMR spectral data and UPLC-MS/MS data. Additionally, the determination of absolute configuration for new peptaibols (**1**-**5**, **8**-**15**) was achieved using the advanced Marfey's method and GITC (2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate) derivatization. Co-cultivation with a plant pathogen, *Fusarium* sp. increased the production of peptaibols, supporting that *Trichoderma* in soil might help to protect plants from external pathogens. In addition, *T. strigosum* extracts exhibited a remarkable 72.4% inhibition against the triple-negative breast cancer cell line, MDA-MB-231 in a preliminary cytotoxicity test. Detailed discussion on biological activities of the isolated peptaibols (**1–15**) will be presented.



	R_1	R_2	R_3
1	L-Val	Aib	L-Ile
2	L-Val	Aib	L-Leu
3	L-Val	D-Iva	L-Leu
4	L-Leu	Aib	L-Leu
5	L-Leu	D-Iva	L-Leu
6	L-Leu	Aib	L-Ile
7	L-Leu	D-Iva	L-Ile

	R_1	R_2	R_3	R_4
8	L-Ser	L-Leu	L-Ser	L-Val
9	L-Ser	L-Leu	L-Ser	L-Leu
10	L-Ser	L-Ile	L-Ser	L-Leu
11	L-Ala	L-Leu	L-Ser	L-Val
12	L-Ala	L-Leu	L-Ser	L-Leu
13	L-Ala	L-Ile	L-Ser	L-Val
14	L-Ala	L-Leu	L-Ala	L-Leu
15	L-Ala	L-Ile	L-Ser	L-Leu

Keywords: *Trichoderma strigosum,* peptaibols, UPLC-MS/MS, advanced Marfey's method, cytotoxicity

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S1.P45 Evaluation of bioisosteric 6,5-fused heterocyclic salvinorin A analogues

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Salvinorin A (**1**) is a neoclerodane diterpenoid isolated from the leaves of *Salvia divinorum*. It represents a pharmacologically unique natural product in that it is a subtype-selective opioid receptor agonist with high binding affinity towards the kappa opioid receptor (Roth et al., 2002). Additionally, it exhibits a comparatively safe physiological profile, being well tolerated in clinical trials, and is supported by a growing body of literature identifying potentially useful clinical applications, including: antinociceptive, antiaddictive, and neuroprotective effects (Moreira et al., 2023). These potential applications support the continued characterization of the structure-activity-relationship of salvinorin A through the development of analogues as essential molecular probes.

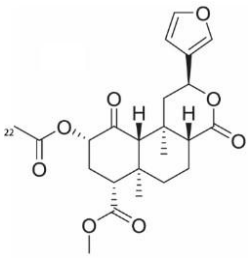

Fig. 1. Structure of salvinorin A with the C(22)-substituted position indicated.

In this study, we evaluated a series of C(22)-6,5-fused heterocyclic salvinorin A analogues by exploring the effect of bioisosteric replacement through incorporation of divalent substitutions into the fused five-member ring. *In vitro* evaluation consisted of competitive radioligand binding affinity at delta, kappa, and mu opioid receptors, followed by functional [^{35}S]GTP[γS] binding activity assays (Leon et al., 2013). This resulted in analogues exhibiting mix-opioid receptor binding affinity and functional activity (EC50 < 200 nM). However, a declining trend in both binding affinity and functional efficacy was observed in order of benzofuran > indole > benzothiophene in C(22)- substitution when compared to salvinorin A. These results support further pharmacological characterization, especially *in vivo* assessment, to ascertain the potential physiological consequences of these exhibited trends.

Keywords: *Salvia divinorum,* salvinorin A, opioid, SAR

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S1.P46 Two novel saturated alicyclic carboxylic acids in *Ginkgo biloba* (Ginkgoaceae) leaf extract EGb 761

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We report about the two saturated alicyclic carboxylic acids (1*S*,3*R*,4*S*)-3,4-dihydroxycyclo-hexane-1-carboxylic acid (**1**) and (1*R*,3*R*,4*R*,5*R*)-3,4,5-trihydroxycyclohexane-1-carboxylic acid ((1*R*)- dihydro-shikimic acid)) (**2**) (Fig. 1). The two compounds were purified from *Gingko biloba* leaf extract EGb 761® by preparative HPLC. Molecular formulae were determined by LC-HRMS and structural constitutions and relative stereochemical configurations were elucidated by NMR spectroscopy. Absolute stereochemical configurations were determined by stereoselective synthesis and hydrogenation of shikimic acid to afford **1** and **2**, respectively, followed by CD spectroscopy. The relative concentration of the two compounds in 5 batches of proanthocyanidin reduced EGb 761® were determined by HPLC coupled to a charged aerosol detector to be 2.6 % and 2.9 % for **1** and **2**, respectively. So far, these two substances have been described in literature as metabolites of quinic acid formed by microorganisms (Whiting et al., 1971) or rodents (Brewster et al., 1978). Our study is the first report of the two substances being present in EGb 761® and plant extracts in general to the best of our knowledge.

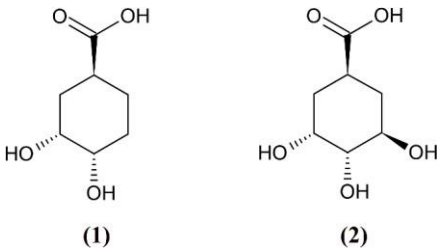

Fig. 1. Structures of the two saturated alicyclic carboxylic acids in EGb 761®.

Keywords: *Gingko biloba* leaf extract, Ginkgoaceae, structure elucidation, stereochemistry, alicyclic organic acids

Conflicts of interest: All authors are employees or were interns (JL) of Dr. Willmar Schwabe GmbH & Co. KG, Germany, manufacturer of *Ginkgo* leaf extract EGb 761®.

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S1.P47 Microalgae: versatile microbial factories for natural product synthesis

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Recent advancements in microbial biotechnology have underscored the potential of photosynthetic microorganisms, such as microalgae, for industrial substance production. This study focused on optimizing the growth conditions of the microalga *Chlorella sorokiniana*, under both autotrophic and mixotrophic conditions (Perez-Garcia et al., 2015), employing orange peel extract and spent mushroom substrate to stimulate biomass growth. The utilization of orange peel extract led to a 17- fold increase in biomass compared to autotrophic conditions. Furthermore, efforts were made to valorize the biomass by extracting the lipophilic fraction using green organic solvents like dimethyl carbonate and identifying compounds with antimicrobial properties through principal component analysis (PCA) of GC-MS spectra of raw extracts. Remarkable antimicrobial activities were observed primarily against Escherichia coli and Bacillus megaterium. Emphasis was placed on elucidating the impact of various growth conditions on the quantity and composition of lipids, particularly polyunsaturated fatty acids, with significant implications for healthcare and biotechnological applications (Lakshimi et al., 2023). However, extracts obtained from biomass grown under mixotrophic conditions exhibited a reduction in fatty acid content compared to those grown under autotrophic conditions. In conclusion, this study highlights the potential of microalgae to produce bioactive molecules, with particular attention to optimizing growth conditions and valorizing biomass for antimicrobial and nutritional applications.

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Keywords: microalgae, cyanobacteria, omega-3, extractions, extract characterization

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S1.P48 Chemical and biological characterization of species of the genus *Weinmannia* (Cunoniaceae) endemic to Reunion Island for valorization purposes

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This study focuses on the chemical and biological characterization of species from the *Weinmannia* genus endemic to Reunion Island. These plants belong to the Cunoniaceae family and two species are present on this territory: *W. tinctoria* or "tan rouge" and *W. mauritiana* or "petit bois de tan" ("Weinmannia L. | Plants of the World Online" n.d.). The "tan rouge" is an emblematic plant of Reunion Island and was used traditionally in tannery and for dyeing natural fabrics for the red color of its bark (De Lanessan, 1886). The lack of knowledge on the chemical and biological characterization of endemic Reunionese species of the *Weinmannia* genus makes them plants with a high potential for innovation in the search for original active substances. As part of the chemical and biological characterization of *Weinmannia* species, a library of extracts has been created from different plant organs collected. Then, the purification of the natural substances present in the fractions of interest derived from the plant was carried out. Moreover, the chemical profiling of plant extracts and fractions following a dereplication workflow based on both NMR and LC-HRMS2 data (Hubert et al., 2017) has been achieved, and additionally, biological assays has been carried out in parallel. Finally, to characterize the tinting strength, the coordinates in CIEL*a*b international color system from the plant extracts has been measured. By improving the knowledge of the specialized metabolites produced by these plants, this project will contribute to developing and conserving the natural plant resources of the southwestern Indian Ocean.

Keywords: *Weinmannia* spp, Cunoniaceae, characterization, chemical profiling, bioactivities

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S1.P49 Enhancing caffeoylquinic acids production in *Limbarda crithmoides* (L.) Dumort. through yeast extract elicitation in liquid root cultures

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In this research, we developed a liquid culture of adventitious roots of *Limbarda crithmoides* (L.) Dumort., aiming at generating extracts overproducing hepatoprotective caffeoylquinic acids through yeast extract (YE) elicitation. The initiation of adventitious roots occurred in leaves grown *in vitro* on MS medium enhanced with 1 mg/L indole-3-butyric acid (IBA). The growth curve and synthesis of secondary metabolites (phenolics, flavonoids, and hydroxycinnamic acids) were monitored weekly to ascertain the ideal time for harvesting. Then, the roots were subjected to elicitation using different concentrations of YE – 50, 100, and 200 mg/L for four weeks. Ethanol extracts were assessed for their hepatoprotective capabilities against ethanol-induced damage and chemically analyzed via high- performance liquid chromatography coupled to electrospray ionization mass spectrometry (HPLC- ESI-MS/MS). Roots from greenhouse-grown plants served as a comparison. The addition of IBA led to the successful development of adventitious roots, peaking in biomass and phenolic content during the fourth week. Application of YE enhanced the concentration of hydroxycinnamic acids, especially chlorogenic acid and di-O-caffeoylquinic acid isomers. The extracts demonstrated marked liver- protective effects with 50 mg/L YE elicitation. Therefore, the use of yeast extract to stimulate liquid root cultures of *L. crithmoides* proves to be an effective method for enhancing the production of caffeoylquinic acids, providing scientific validation for the traditional medicinal claims of liver protection attributed to this plant species.

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Keywords: golden samphire, Asteraceae, plant tissue culture, yeast extract

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S1.P50 Daphnane diterpenoids from the fruits of *Daphne pseudomezereum*

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Daphnane diterpenoid possess a 5/7/6 (A/B/C) *trans*-fused tricyclic structure, which is exclusively distributed in plants of the Thymelaeaceae and Euphorbiaceae families (Otsuki et al. 2023). Daphnane diterpenoids have been demonstrated to exhibit a wide range of biological activities, such as antineoplastic, antiviral, analgesic, and pesticidal effects. *Daphne pseudomezereum* A. Gray (Thymelaeaceae) is a deciduous shrub mainly distributed in Japan, China, and Korea. The fruits of this plant are known to be poisonous and may cause diarrhea and vomiting if accidentally eaten. Previous chemical investigations of *D. pseudomezereum* have been conducted on the leaves and reported the isolation of coumarins, flavonoids, lignans and their glycosides. As part of our research for searching bioactive diterpenoids from the plants of Thymelaeaceae family, herein, we report the isolation and structural elucidation of daphnane diterpenoids from the fruits of *D. pseudomezereum*. A methanolic extract of the fruits of *D. pseudomezereum* was partitioned between EtOAc and H2O, respectively. The EtOAc fraction was fractionated by Diaion HP-20 column chromatography and preparative HPLC, to afforded ten daphnane diterpenoids (**1**–**10**), including four new compounds (**1**– **4**). Their structures were determined by extensive spectroscopic analyses. The new daphnanes were structurally characterized by 1,2-dihydro structures for the A-ring or 4,6- and 4,7-ether structures for the B-ring. This study reveals the presence of daphnane diterpenoids in *D. pseudomezereum* for the first time, contributing to further understanding of bioactive diterpenoids in plants of the Thymelaeaceae family.

Keywords: *Daphne pseudomezereum,* Thymelaeaceae, diterpenoid, daphnane, phytochemical investigation

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S1.P51 Nephroprotective *Ganoderma sp.* extracts towards cisplatin *in vitro*: chemical composition, metabolomics study and potential impact on cancer cells

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This work aims to study the chemical composition, identify the bioactive compounds through metabolomics analyses and investigate the potential impact of nephroprotective extracts on lung cancer cells. For this, methanolic extracts of 5 mushrooms species from the *Ganoderma* P. Karst genus were first studied *in vitro* on Human Kidney (HK-2) cells as a 1 h pretreatment prior to cisplatin (20 µM) exposure (Sinaeve et al., 2022). The chemical content was then analyzed by a phytochemical screening, the determination of total triterpenes and the phenolic compounds content, TLC-MS dereplication, metabolomics study (LC-MS), identification of potential bioactive compounds (LC- MS/MS) and estimation of their nephroprotective effect *in vitro*. Finally, the potential impact of cisplatin on lung cancer cells (A549) growth was established in the presence of the nephroprotective extracts and the bioactive compounds. Results have shown that methanolic extracts of *G. parvigibbosum* Welti & Courtecuisse and *G. tuberculosum* Murill. (10 µg/mL), and their combination (5 + 5 µg/mL) were significantly nephroprotective on HK-2 cells, mainly through an inhibition of the apoptosis and its signaling pathway. The phytochemical screening and chemical content study demonstrated a high content of triterpenes. The metabolomics study resulted in the detection of 9 discriminant compounds. Among them, 6-hydroxycoumarin was identified for the first time in these species, and exhibited, at 10 µg/mL, a significant protective effect on HK-2 cells exposed to cisplatin. Finally, the nephroprotective extracts were tested on A549 cells and were found to potentiate cisplatin anti-cancer activity. The evaluation of 6-hydroxycoumarin on A549 cells is undergoing.

Keywords: cisplatin, metabolomic, nephroprotective, cancer, *Ganoderma*

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S1.P52 Phytochemical differentiation of wild thyme from thyme

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The *Thymus* L. is a large genus with about 350 species that are used as food and medicine. The most popular ones are Thyme (*Thymus vulgaris* L./*Thymus zygis* L.) and Wild Thyme (*Thymus serpyllum* L.) that are traditionally used against productive coughs mainly. In addition, recent studies with Wild Thyme extract have shown intestinal anti-inflammatory effects, metabolic improvements and modulation of the gut microbiota in rodents (Algieri et al., 2014; Ruiz-Malagón et al., 2022a, 2022b). A clinical study has confirmed the gastrointestinal benefits and the modulation of the Firmicutes/Bacteroidetes ratio in healthy volunteers (Knaub et al., 2022). To make sure that the intended species is used, distinguishing characteristics are of particular importance. In addition to pharmacopoeial morphological examination, our current study aims to differentiate between Wild Thyme and Thyme by phytochemical methods. The results identified characteristic HPTLC- fingerprints on flavonoids and on polar constituents of aqueous and methanolic extracts (Meier, 2011). The LC-HRMS identified more than 40 common substances, but one flavonoid (*m/z* 447.0933, C21H20O11) was present only in Thyme, while another (*m/z* 463.0882, C21H20O12) only in Wild Thyme. Salvianolic acid K was detected in both species but as different isomers. In addition, a flavonoid (*m/z* 299.0; C16H12O6) was present only in the Wild Thyme aqueous extract. The different Thymol:Carvacrol ratio (16:1 for Thyme and 1:1.2 for Wild Thyme) also distinguishes the two species. These results corroborate that the evaluated *Thymus* species differ not only morphologically but chemically, and support the differentiation in laboratory routines.

Keywords: *Thymus serpyllum* L, *Thymus vulgaris* L., *Thymus zygis* L., phytochemical profile

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S1.P53 Chemical ecology in marine fungi: diversity and dynamics of pyran-2-ones in a mussel- derived *Penicillium restrictum*

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Very little is known about chemical interactions between fungi and their marine mollusk hosts. Here, we investigated the metabolome of a *Penicillium restrictum* strain isolated from the blue mussel *Mytilus edulis* collected on the Loire estuary, France. Following the OSMAC approach, effects of salinity and of a mussel-derived medium on the metabolic expression of this strain were analyzed. An MS-based untargeted metabolomics study highlighted the high chemical diversity of pyran-2- ones that was found related to the presence of mussel lyophilizate in the culture medium. MS- and UV-guided purification allowed to isolate thirteen pyran-2-ones including five new compounds (Fig. 1), whose complete structure was elucidated using NMR, ECD and DP4 calculations (Le et al., 2021). A both untargeted and targeted time-scale metabolomics study has then been performed on the strain, which showed the dynamics of specialized metabolism with a variety of compounds produced at an early, intermediate or late stage of growth. One compound appearing at the very late stage was successfully isolated and characterized as (2*E*)-5-acetoxy-3-methoxy-2-pentenoic acid (Fig. 1), an unreported precursor of pyran-2-ones. Biosynthesis was further explored using a dynamic molecular networking approach, which highlighted the sequential biosynthetic steps leading to pyran-2-ones and the specific induction of some analogs when colonies reached confluence. These results illustrate the utility in using host-derived media for the discovery of new marine natural products and that combining metabolomics and dynamic molecular networking is a new approach to explore microbial biosynthesis, chemo-diversity and chemical induction.

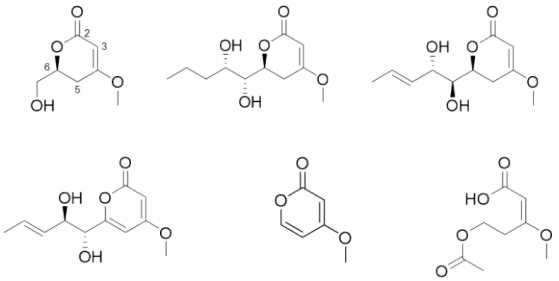

Fig. 1. Structures of new fungal pyran-2-ones.

Keywords: *Penicillium restrictum,* pyran-2-ones, metabolomics, dynamic molecular networking, ecological cultures

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S1.P54 Development and optimization of different eco-extraction and analytical methods on *Cannabis sativa* L.

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The cannabinoids, predominantly found in the glandular trichomes of *Cannabis sativa* L., have garnered significant scientific interest due to their diverse pharmacological properties (Pacher et al., 2006). These include analgesic, anti-inflammatory and psychoactive effects (Russo et al., 2011). The access to these compounds depends on the extraction methods used, which can vary in their yield and metabolite profile for the same plant. This study was focused on the development and optimization of green extraction methods in the laboratory scale and their transferability to industry. A variety of green extraction techniques were employed (Tiago et al. 2022) and results were compared according to their mass yields, total contents (polyphenols, flavonoids, chlorophylls) and quantifications. Ethanol was used as the reference solvent. The use of ultrasounds, pressure, microwaves methods significantly improved mass yield and desired molecules. The HRLC-MS/MS allowed for the monitoring of cannabinoid concentrations, facilitating the selection of the most appropriate technique for the industrial scale. In addition to these results, a statistical approach was applied and made it easier to sort the data, to compare and obtain the best compromises for metabolites of pharmaceutical interest. The notion of desirability was added and highlighted the possibility to choose the condition which minimazing undesirable molecules (e.g. degradation products) while maximizing interesting molecules and enhance entourage effect (e.g. polyphenols, terpenes). Among the various options tested, ASE at 75°C emerged as the most effective process (65% of global desirability) providing a balance between mass yields, total contents and concentration of cannabinoids.

Keywords: *Cannabis sativa* L., eco-extractions, total contents, quantification, statistic description

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S1.P55 Impact of biomass hydration on the valorization of non-polar metabolites from Spirulina using eutectic solvents

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Arthrospira platensis (Spirulina), is a cyanobacteria that contains non-polar value-added metabolites for nutraceuticals and cosmetics, such as carotenoids or free fatty acids (Wils et al., 2021). This work focuses on the use of custom-made Natural Deep Eutectic Solvents (NaDES), which are alternative biobased solvents, to target non-polar metabolites of Spirulina (Wils et al., 2021). Biomass pre- treatment is crucial to ensure data reproducibility and stability before scaling up any process. We then investigated different biomass preparations: fresh or flaked biomass provided by our industrial partner. Initial extraction tests were performed using fresh biomass. Even though promising levels of target compounds were obtained, the extraction recovery was not reproducible in all experiments. This lack of consistency is linked to the instability of fresh biomass over time, as revealed by further analysis (Fig. 1).

Fig. 1. Spirulina preparations after cultivation and biomass stability.

To tackle this challenge, extraction experiments were conducted using rehydrated Spirulina flakes with 20% dry matter instead of fresh biomass. This change prevented technical issues related to structural changes in the fresh biomass over time, ensuring the collection of reproducible data (Fig. 1). To investigate the effects of rehydration on extraction performance, experiments were conducted to determine metabolite recovery as a function of water rates ranging from 0 to 80%. These analyses show that it is possible to modulate the profile of extracted compounds depending on the rehydration rate used. This opens up various options for optimization prior to industrial scaling up.

Keywords: Microalgae, Spirulina (*Arthrospira platensis*), green chemistry, NaDES, extraction

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S1.P56 Chemical investigation of the beetle *Agrilus cyanescens* (Buprestidae)

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There are over 400,000 described species of beetles with many still unknown, making them the most diverse group of macroorganisms on earth. Many beetles have been shown to biosynthesize potently active molecules with the potential to act as new drug leads. *Agrilus cyanescens* is metallic wood- boring beetle (Buprestidae) native to Europe and Asia that is an invasive species in North America. To investigate the chemistry of adult *A. cyanescens*, we analyzed their chemical extracts via NMR spectroscopy and UPLC-HRMS. It was determined that *A. cyanescens* contains a mixture of buprestins (Fig. 1), defensive compounds known from other metallic wood-boring beetles. Interestingly, *A. cyanescens* contained all the known buprestins except for buprestin H, for which no evidence could be found. Studies of the defensive chemistry of invasive species are important to help understand the ecology of these organisms.

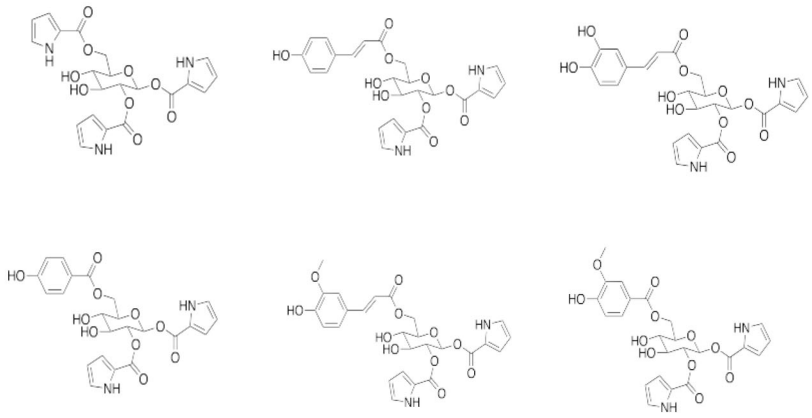

Fig. 1. Structures of buprestins A, B, and D-G.

Keywords: *Agrilus,* insect, defensive chemistry, buprestins, invasive species

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S1.P57 New lipopeptides from the marine bacteria *Pseudovibrio denitrificans*

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Microorganisms inhabiting distinctive and underexplored environments represent a rich source of bioactive compounds characterized by unique structures. Similarly, marine bacteria have garnered considerable interest due to their potential to yield unprecedented bioactive compounds. In this study, we isolated eight lipopeptides (**1–8**) from the seawater-derived bacterium *Pseudovibrio denitrificans* KCTC 62704. The chemical structures of the new compounds (**3–8**) were characterized by spectroscopic and spectrometric data interpretation, including NMR (^{1}H and ^{13}C NMR, ^{1}H–^{1}H COSY, HSQC, HMBC, and NOESY) and HRESIMS analysis. Experimental ECD data analysis was conducted to assign the absolute configurations of the new compounds. We propose that these lipopeptides are synthesized from hexanoic acid, L-phenylalanine, and additional building blocks by stable-isotope feeding experiments. AntiSMASH analysis of the genome of *P. denitrificans* KCTC 62704 revealed the potential biosynthetic gene cluster for the isolated lipopeptides.

Acknowledgements: This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korean government (MSIT) (Nos. 2021R1C1C1011045, 2022R1A6A1A03054419), by the BK21 FOUR Project.

Keywords: marine bacteria, *Pseudovibrio denitrificans*, lipopeptide, biosynthesis

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S1.P58 The Arabian Peninsula: A largely untapped resource for biotechnology

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The Arabian Peninsula is a desert environment surrounded by three seas, namely the Arabian Gulf, Arabian Sea and Red Sea. The Arabian Gulf is a semi-enclosed shallow sea with mean depth of about 35 m. Because of the arid climate there, the Gulf's evaporation greatly exceeds freshwater input from precipitation and river runoff causing Gulf waters to be hypersaline. With vast expanses of desert dominating the landscape, temperatures can soar to extreme levels, often surpassing 50°C degrees Celsius during the peak of summer increase the sea temperatures from 22 to 35°C. The relentless sun beats down on the arid terrain, resulting in high levels of UV radiation. The combination of blistering heat, high salinity and relentless UV rays makes the Arabian Peninsula's climate one of the most unforgiving on the planet, shaping the lifestyle and survival strategies of many organisms. Extreme climates have long been recognized as catalysts for genetic mutations, driving adaptation and evolutionary change in organisms inhabiting such environments. The extremities of temperature, pressure, salinity, and radiation encountered in extreme climates can induce mutations in the genetic material of living organisms, allowing them to better survive and thrive in these hostile conditions. These mutations may confer novel traits that enhance an organism's ability to withstand the challenges posed by extreme environments, such as heat-resistant enzymes or mechanisms for preserving cellular integrity in high-salinity environments. Extremophiles, organisms that thrive in extreme conditions, provide compelling examples of the genetic adaptations that arise from exposure to extreme climates. Studying extremophiles and their genetic makeup holds promise for diverse applications, including biotechnology, medicine, and environmental remediation, as these organisms offer valuable insights into the mechanisms of adaptation and resilience in the face of extreme challenges. Herein, we discuss our strategy for collecting/isolating macro and microorganisms from these extreme marine and terrestrial environments. Additionally, we highlight our methodology to activate silent biosynthetic gene clusters in order to upregulate/activate the production of secondary metabolites as well as evaluate these compounds for their medical or agricultural potential. Compounds showing biotechnological potential are purified and elucidated through HRMS and 1D/2D NMR spectroscopy.

Keywords: extremophiles, Arabian Gulf, biotechnology, polyextremophiles

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S1.P59 Valorisation of north atlantic macroalgae: antimicrobial activity of phlorotannins

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Multidrug-resistant bacteria are a severe threat to society. Discovery of novel antimicrobials with distinctive mechanisms of action is pertinent. Marine environments, specifically, seaweeds (macroalgae) are excellent sources to look for new bioactive compounds (Silva et al., 2020). Due to the harsh environment that seaweeds grow in, they produce a variety of bioactives with unique chemical scaffolds (Pérez et al., 2016). In this work, fourteen species of seaweed harvested in the North Atlantic (North Sea) waters were screened for their antimicrobial potential. Crude extracts were obtained by ultrasound-assisted extraction using sequential solvents of varying polarity. Their antimicrobial activity was assessed in a particularly sensitive agar-well diffusion assay against bacteria *Bacillus cereus, B. pumilus*, *B. subtilis, Micrococcus luteus*, and *Yersinia ruckeri* (NAT- screen, Pikkemaat et al., 2008). At a concentration of 1 mg/mL, 57 out of the 85 extracts showed antimicrobial activity, with the largest zones of inhibition (~7 mm) detected in highly apolar (hexane) and polar (methanol) solvent extracts. The most potent antimicrobial activity was observed for brown seaweed *Ascophyllum nodosum, Fucus vesiculosus, Sargassum pallidum,* and *Undaria pinnatifida.* As a highlight, methanol extracts (*A. nodosum* and *F. vesiculosus*) were further investigated by activity-guided fractionation through purification with FLASH chromatography and analysis by RP- UHPLC-PDA-ESI-MS. Bioactive fractions were found to be enriched in phlorotannins (polymers of phloroglucinol units **1**, such as fucophlorethol A **2**, Fig. 1.) (Vissers et al., 2017). We developed a highly sensitive pipeline for non-targeted screening and characterization of antimicrobial compounds in seaweeds, for the valorisation of this abundant marine resource.

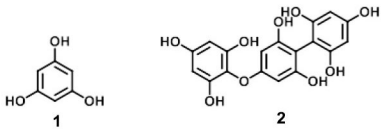

Fig. 1. Structures of phloroglucinol **1**, and fucophlorethol A **2**.

Keywords: antimicrobials, macroalgae (Seaweeds), NAT-screening, bioactive polymers, phlorotannins

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S1.P60 Assessment of the antioxidant potential of leaf extracts from twelve *Sambucus nigra* varieties and their phytochemical characteristic

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Elderberry flowers and fruits are a medicinal raw material known in phytotherapy (Stępień et al., 2023); however, the leaves have not yet been used in medicine. The study aimed to compare the antioxidant activity and phytochemical screening of 70% methanolic extracts obtained from the leaves of twelve elderberry varieties collected from the experimental station of the Central Center for Research on Cultivated Plant Varieties in Słupia Wielka (Poland). In this study, the ability of the extracts to scavenge free radicals (DPPH, ABTS), their properties to reduce transition metal ions (CUPRAC, FRAP), and the ability to chelate Fe^{2+} ions were tested. The total content of polyphenols and flavonoids (Studzińska-Sroka et al., 2022, 2021) and the content of rutin, isoquercetin and neochlorogenic acid (Paczkowska-Walendowska et al., 2021) were examined. To statistically evaluate the relationship between properties, principal component analysis was performed. The obtained results showed that the extract from the Black Beauty variety had the strongest antioxidant potential, which was correlated with the highest content of total polyphenols and a high content of total flavonoids. The analysis of the main components showed that the antioxidant potential of all tested extracts is strongly correlated with the total polyphenol content, moderately dependent on the content of isoquercetin and neochlorogenic acid, and weakly dependent on the content of rutin. Moreover, the biplot graph shows that among the analysed varieties, Samyl, Obelisk, and Sabbo are characterized by the lowest antioxidant activity and low content of the tested active compounds.

Keywords: black elderberry leaves, PCA, phytochemical screening, biological activity *in vitro*

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S1.P61 Unlocking the potential of *Geissospermum* trees: exploring bioactive alkaloids for neurodegenerative diseases

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The genus *Geissospermum*, belonging to the Apocynaceae family, consists of Amazonian trees native to Brazil, primarily found in the eastern region. Indigenous tribes utilize aqueous and ethanolic extracts from the bark of these trees for various ailments such as malaria, cancer, and bacterial infections. *Geissospermum vellosii*, a species within this genus, is particularly notable for its abundance in indole and β-carboline alkaloids. Despite limited phytochemical research on this genus, isolation of alkaloids remains largely unexplored. The biological activity of extracts obtained from *G. vellosii* closely mirrors traditional decoction uses. A preliminary screening study assessing cholinesterase inhibition of the bark extract revealed significant activity against huBuChE (with an IC50 value of 0.37 ± 0.05 μg/mL). Furthermore, analysis via gas chromatography-mass spectrometry (GC/MS) and thin-layer chromatography (TLC) identified at least 12 alkaloids. The primary ethanolic extract was obtained from 40 kg of dried, crushed bark, and subsequent preparation of the alkaloidal extract involved different solvents (diethyl ether and chloroform) based on polarity. The purified diethyl ether extract (53 g) underwent separation via column chromatography, resulting in 16 fractions. Following purification and crystallization processes, five compounds have been isolated thus far. The inhibitory activity against recombinant human AChE and BuChE, as well as GSK-3β, of these isolated alkaloids, was evaluated along with their ability to penetrate the blood-brain barrier.

Keywords: *Geissospermum vellosii,* Apocynceae, cholinesterase activity, alkaloids

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S1.P62 Bioguided-isolation of antimicrobial compounds against phytopathogens and human pathogens from lichen-associated bacteria

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The World Health Organization places Antimicrobial Resistance (AMR) to be one of the greatest threats to global health (Murray *et al.*, 2022). The ermergence of AMR in humans, animals and plants highlights the need for a "One Health" strategy (Despotovic *et al.*, 2023). This project goal is the investigation of the antimicrobial potential of a collection of 500 strains from the microflora of several lichen samples of *Rhizocarpon geographicum* against two phytopathogens: *Erwinia amylovora* (Piqué *et al.*, 2015), *Venturia inaequalis* (González-Domínguez, Armengol and Rossi, 2017) and against three human bacterial pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*). Lichen samples were collected from coastal and terrestrial areas, allowing them to undergo various biotic and abiotic stresses. Moreover, lichens, as holobionts, constitute micro- ecosystems that shelter unidentified microbial communities which could take part in the holobiont's defense against pathogens (Miral *et al.*, 2022). The first step of our study involved culturing 286 lichen-associated bacteria in a suitable medium, collecting the supernatant and pellet at an appropriate phase for the production of specialized metabolites and evaluating their activities against the targeted pathogens. Initial results performed on 150 freeze-dried supernatants highlighted the inhibitory activities of 10 supernatants against *E. amylovora*, 2 against *V. inaequalis*, 4 against *P. aeruginosa* and 1 against *E. coli*. Four of the most active strains were selected for production optimisation and large-scale cultivation leading to bioguided fractionation. The results of this fractionation to isolate bioactive compounds will be discussed.

Keywords: antimicrobial resistance, biocontrol, lichen microflora, bioguided fractionation, Rhizocarpon geographicum

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S1.P63 Cardiometabolic effects on obese mice of the Mediterranean seagrass *Cymodocea nodosa* hydroalcoholic extract

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Cymodocea nodosa (Ucria) Asch. (Cymodoceaceae) is a dioecious medium-size, fast-growing seagrass colonizing coastal waters of the Mediterranean and eastern Atlantic Ocean (Garrido et al., 2013). Due to its high plasticity, it can colonize different substrates and environments, tolerating salinity fluctuations and climate changes (Chefaoui et al., 2015). Flavonoids, steroids, polyamines, diarylheptanoids, and sulfated polysaccharides with interesting potential biological activity (Ben Abdallah Kolsi et al., 2017) have been previously extracted from its plant tissue (Smadi et al., 2017). This study aims to investigate the phytochemical profile of a leaf hydroalcoholic extract of *C. nodosa* collected in the Ligurian Sea (Italy) and its activity in a murine model of cardiometabolic disorder. UHPLC coupled to an Orbitrap-based HR-MS led to the characterization of phenolic bioactive substances including flavonoid glycosides (quercetin and isorhamnetin derivatives) and chicoric acid as the most represented compound (1.58 ± 0.3 mg/g fresh weight). Mice fed with an obesogenic diet were supplemented with the extract for 9 weeks and at the end of the experimental protocol a containment of body weight gain (increase of 32±2 vs 49.6 ± 8.7%) and an improvement of glycaemic profile, compared to animals supplemented with placebo, were observed. Glucose levels were 115±4 vs 138 ± 10 mg/dl and HbA1c levels were 32±0.4 vs 29.8±0.8 mmol/mol. These results suggest that the extract obtained has beneficial effects in the prevention of metabolic disorders associated with obesogenic diet. Further experiments will be carried out to explore the mechanisms involved in this beneficial profile.

Keywords: *Cymodocea nodosa,* phenols, chicoric acid, LC-HR-ESI/MS, cardiometabolic disorder

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S1.P64 Obtaining natural and sustainable blue dyes from the fungus *Terana coerulea* (Lam.) for textile application – The TEXTBLUESTYLE project

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Amongst the several categories of synthetic dyes, the azo is one of high concern as once under specific conditions, these dyes decompose generating aromatic amines classified as carcinogenic. This toxicity has led to legal impositions at European level, resulting in application restrictions in several industrial sectors (Keshava et al., 2023). Among the possible natural dyes' sources available, higher fungi stand out due to their ability to synthesize bioactive molecules and pigments, like the cobalt blue pigment generated by the fungus *T. coerulea*. Hence, the TextBlueStyle project aims at obtaining blue, stable, and natural dyes by applying green extraction techniques and using water as solvent, following the work performed within our research group (Maisterra et al., 2017). The obtained extracts are being characterized regarding antioxidant (DPPH, FRAP and reducing power methods), antimicrobial activity and toxicity. Previous results obtained pointed out that cytotoxicity of *T. coerulea* aqueous extract against non-tumor porcine liver cells primary culture, occurred at concentrations higher than 400 µg/ml, the maximum concentration tested (Maisterra et al., 2017), suggesting the low toxic character of the obtained extract. This work is still ongoing, being expected to achieve extracts with different shades of blue as well as antioxidant activity suitable for textile functionalization. This would allow to consider further development with the production of technical textile products, in a sustainable and eco-innovation context.

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Keywords: *Terana coerulea,* antioxidant extract, natural blue dye, textile application

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S1.P65 Using low levels of antimicrobials and antiseptics as elicitors for environmental isolates to activate or re-activate antimicrobial producing biosynthetic gene clusters

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This presentation will describe the results of screening >2000 environmental isolates against six antimicrobials at low levels to determine elicitation of antimicrobial activity. The overall aim of this study being the recovery of antimicrobial activity that has been lost from environmental bacteria following an initial agar plate based antimicrobial production assay. We compare the antimicrobial activity of >2000 environmental isolates against *Escherichia coli*, *Micrococcus luteus*, MRSA and *Candida albicans* with and without one of six elicitors: tetracycline (0.1 µg/ml), ampicillin (0.1 µg/ml), clindamycin (0.5 µg/ml), ciprofloxacin (0.01 µg/ml), CTAB (0.08 µg/ml) and Triclosan (0.003 µg/ml). These experiments showed a 25% success rate in eliciting antimicrobial production from the environmental isolates. As part of our wider antimicrobial discovery activity, we have

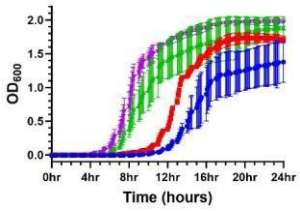

Fig. 1. The effect cell free supernatant from isolate X when elicited by triclosan (blue) and when not elicited (red) on the growth of susceptible *E. coli*. The effect of the triclosan concentration on *E. coli* growth is shown (green) and normal *E. coli* growth (purple).

assembled a large library of environmental microbes (currently >70,000), which we are screening for antimicrobial activity against the WHO priority pathogens *E. coli*, *C. albicans* and *Candida auris* (WHO 2017, WHO 2022). We found that approximately 40% of active isolates lose activity on secondary agar plate-based assays so aimed to re-activate antimicrobial production using the elicitors described above. We found a 23% elicitation on agar assays using tetracycline, CTAB and triclosan; however, this reduced to only 0.7% (1 isolate) elicitation in broth (Fig. 1). Sequencing and subsequent bioinformatics on the whole genome of this isolate showed likely biosynthetic gene clusters and the potential antimicrobials being produced.

Keywords: elicitation, antimicrobial production, environmental isolates, microbial

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S1.P66 Biotransformation of the secondary metabolites from *Picralima nitida* and their antibacterial activities

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This presentation will focus on the antibacterial activities of secondary metabolites from *Picralima nitida* (Apocynaceae) and their biotransformed derivatives. *Picralima nitida* Durand and Hook is a medicinal plant from the genus *Picralima* and family Apocynaceae. It is found in tropical African countries such as Ivory Coast, Nigeria, Uganda, and Gabon (Erharuyi et al., 2014; Olajide et al., 2014). This plant is used in traditional medicine for the treatment and management of malaria, abscesses, hepatitis, pneumonia, diabetes, and hypertension (Erharuyi et al., 2014; Teugwa et al., 2013). Several studies have previously shown that various extracts of this plant are good sources of phytochemicals such as glycosides, alkaloids, triterpenes, flavonoids, polyphenols, saponins, and tannins (Akinwunmi et al., 2019; Creedet al., 2020; Erharuyi et al., 2014). As such, in our continuous effort to discover new bioactive compounds, the fruits of *Picralima* nitida were collected in Cameroon. The air-dried powder was extracted with MeOH. The separation and isolation of compounds from the obtained extract were performed using classical chromatography methods (column chromatography, flash-chromatography) while the structures of the isolates were elucidated by interpretation of their spectroscopic data and by comparison with literature. Biotransformation was performed on the major compounds using some bacteria belonging to the *Bacillus*, *Nocardia* and *Streptomyces* genera. The antibacterial activity of natural compounds and their biotransformed derivatives was carried out using the broth microdilution method against human bacterial pathogens.

Keywords: *Picralima nitida,* Apocynaceae, biotransformation, antibacterial activities

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S1.P67 Natural products isolation from subterranean fungi of Wind Cave National Park (US)

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Since 2019 undergraduate students and faculty from the University of Northern Iowa have been active in a NASA sponsored research program to explore Wind Cave National Park (South Dakota, USA) as a terrestrial analog for a possible exobiological environment off earth. The subsurface lakes within Wind Cave contain fewer microbial cells (5 x 10^3 cells/mL) than nearly all other bodies of water on earth. It's thought that these isolated, nutrient-limited lakes may be an analog for such oceans on the moons of Saturn. One of the goals of this multi disciplined research project is to develop a genetic map of the Wind Cave microbial system. To accomplish this goal, microbes have been collected from the air, aqueous, and terrestrial environments from many areas of the cave not accessible to the general public. Though not part of the original scope of this project, our lab viewed these subterranean microbes as possible sources of novel, biologically active natural products. To date, we have purified and identified over 90 fungal isolates from the cave. This presentation will summarize the species diversity that has been isolated from the cave to date. Additionally, the antimicrobial activity of isolated fungal cultures and the structural characterization of compounds will be presented.

Keywords: antimicrobial activity, fungal isolate, cave microbes

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S1.P68 Chemical and biological study of *Glutamicibacter* sp. NCA-315 isolated from the sinkhole Tza- Itzá in the Yucatan Peninsula

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The Yucatan Peninsula in Mexico is an enormous reservoir of unique microbes for bioprospecting purposes (Suárez-Moo et al., 2022). In this work, the chemical and biological potential of a bacterium identified as *Glutamicibacter* sp. NCA-315, isolated from sediment of the sinkhole Tza-Itzá in the Anillo de Cenotes (Ring of Sinkholes) Reserve in the Yucatan Peninsula, was explored. The EtOAc extract of the supernatant of the strain cultivated in DSC-ASW media showed ≥50% inhibition against *E. faecalis*, MSSA, and MRSA. The chemical study of the extract yielded the bile acids cholic acid (**1**), 7-oxo-3,12-dihydroxycholic acid (**2**), glycocholic acid (**3**), and glycodeoxycholic acid (**4**) (Fig. 1). Compounds **3** and **4** inhibited 40% and 60% of the biofilm formation of MRSA, respectively, at 64 µg/mL (Martínez-Rodríguez et al., 2023). Finally, WGS was completed for the bacterium and BGC analysis was performed.

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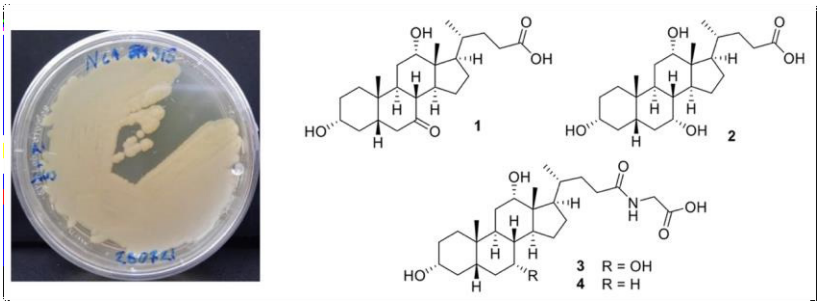

Fig. 1. *Glutamicibacter* sp. NCA-315 and isolated bile acids.

Keywords: *Glutamicibacter* sp., Tza-Itzá sinkhole, bile acids, MRSA biofilm inhibition

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S1.P69 Comparative analysis of chemical variations across plant parts, seasons, and species in three *Osmanthus* plants

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The genus *Osmanthus* is distributed mainly in East Asia, and is famous for *Osmanthus fragrans* (OF) having a strong scent. Due to its value as an ingredient of perfume, phytochemical and pharmacological studies on this genus have been focused on OF. Due to the lack of other species in this genus, we performed a comparative study on the chemical composition of three plants, OF, *Osmanthus fragrans var. aurantiacus* (OFA), and *Osmanthus* × *fortunei* (OxF). To this end, LC- MS/MS analysis of samples by parts (twig, leaf, and flower) and months (May, June, July, September, and October) of the three species was conducted. The differences between the parts were evident. The flavonoid class predominated in the leaves, while the lignan and phenylethanoid classes were dominant in the twig samples. Furthermore, the chemical dissimilarity within the same parts was observed. In the leaves, there was a tendency for OxF to exhibit a higher prevalence of flavonoid glycosides with two sugars rather than aglycones. For the twigs, OFA showed a different trend from the other species; lignans were notably shown in leaves of OFA, whereas these compounds were mainly detected in twigs of the other two species. Moreover, the phenolic compounds were prominently in OFA compared to the others. We assessed the ABTS and DPPH radical scavenging activity to know the relationship between chemical composition and physiological activity. Antioxidant capacity decreased until July and then increased after September. Consistent with this trend, the phenylethanoid class compounds mainly detected in the twigs of OFA exhibited high bioactivity scores. Therefore, it is considered most effective to collect OFA twigs after September to maximize the antioxidant activity of *Osmanthus* spp.

Keywords: *Osmanthus* spp., LC-MS/MS, metabolomics, antioxidant activity

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S1.P70 Siderophores-mediated antagonism of specialized metabolites in fungal-bacterial interactions

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Siderophores are specialized microbial metabolites that chelate iron and play multiple roles in the survival strategies of microorganisms. In a coculture model of 86 *Penicillium* species and *Bacillus subtilis*, we find that siderophores have an additional function of releasing toxic metabolites from partner organisms during competitive interactions. In expectation of the cocultivation-induced new metabolites to have antibacterial activity, the LC–MS/MS were analyzed to observe the changes in metabolomes. However, surprisingly, various secondary metabolites, including verruculogen and its analogs, were reduced by coculture while bacillibactin, a siderophore produced by *B. subtilis*, was significantly induced. Through further assays, we hypothesized that an iron-deficient condition modulated by bacillibactin was caused leading to a reduction in the amount of metabolite production of *Penicillium*. As these reduced compounds were antimicrobial, we speculated that the siderophore could contribute to the coexistence as a cross-protector by observing the phenotypes of the designed microbial consortia. In summary, our results propose that through iron-limitation mechanisms, bacillibactin inhibits the antimicrobial compounds and thereby indirectly protects other members within microbial communities.

Keywords: bacillibactin, siderophore, *Penicillium*, *Bacillus subtilis*

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S1.P71 Absolute configuration of unisolated epimers of pseurotin D from the marine-derived fungus *Penicillium janczewskii*

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Halogen supplements affect microbial secondary metabolism in both direct and indirect ways. During our comparative analysis on metabolic change of *Penicillium janczewskii* by different halogen supplements, we found that the addition of iodide resulted in a significant increase in pseurotin D (**1**). We deduced that iodide promoted activation of the carbon-carbon double bond in the last non- enzymatic SN2' reaction step of pseurotin D biosynthesis. The absolute configuration of **1** was deduced from the biosynthesis pathway, without spectroscopic evidence (Tsunematsu et al., 2014). Consequently, we isolated from *P. janczewskii* and determined the absolute configuration of **1** using advanced analytical technologies. Each chiral center except for C-13 was determined to be 5*S*, 8*S*, 9*R*, and 10*R* through NOESY, CD spectra, and the modified Mosher's method. Our analysis revealed that **1** exists as a mixture of diastereomers. Here, we utilized a gradient-enhanced multiplet-selective targeted-observation NMR experiment (GEMSTONE) and ultraselective heteronuclear polarization transfer (UHPT) to determine the NMR chemical shift values of each epimer (Cha et al., 2023) and applied CP3 to establish the absolute configuration.

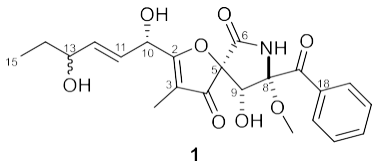

Fig. 1. Structure of pseurotin D (**1**).

Keywords: *Penicillium janczewskii,* pseurotin D, halogen, diastereomer, GEMSTONE

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S1.P72 A solution from the past: exploring the untapped potential of museum specimens to preserve secondary metabolites

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A fundamental pillar of natural products focused drug discovery is that biodiversity results in chemical diversity. To this end, marine natural products researchers have relied on sample collection trips to remote locations, searching for rare and unstudied species. The high cost of sampling trips, increased restriction of sampling permits and ecosystem degradation has resulted in multiple hurdles to acquiring new samples. One solution to these issues comes to us from the past, in the form of museum marine invertebrate collections. For hundreds of years, specimens from around the world, covering a diverse range of species, have been consolidated in museums, primarily stored in 90% ethanol. This preserving solution may be an untapped source of new natural products, from specimens with rich scientific history including varying collection depths, dates and location, high-level taxonomical assignment, and genetic studies. In collaboration with the Smithsonian Museum, this study explores the ability of this ethanol solution to preserve secondary metabolites over time from two distinct taxa: the tunicate *Synoicum* and the glass sponge *Aphrocallistes*, collected between 1945- 2016. Ethanol preserving solution was concentrated and analyzed using untargeted LC-tandem MS/MS (orbitrap). LC-MS^2 data was processed through MZmine3's targeted feature detection workflow, identifying palmerolide A (**1**) and aphrocallistin B (**2**), in samples of *Synoicum* and *Aphrocallistes,* respectively. Multiple rection monitoring (MRM) with a LC-MS QqQ was used to confirm and quantify the presence of **1** and **2**, using standards from our in-house library of marine metabolites. Patterns in chemical composition of each sample was explored by untargeted LC-IMS- QToF combined with GNPS and SIRIUS spectral libraries.

Keywords: drug discovery, mass spectrometry, metabolomics, marine natural products

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S1.P73 Discovery of a brominated Ahp-containing cyclic depsipeptide from the marine cyanobacterium *Moorena* sp. collected in Panama

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Bromine-containing natural products are recognized for their potential in drug discovery and as toxins due to their notable bioactivity. Marine organisms represent abundant reservoirs of structurally diverse halogenated natural products. With the goal of identifying novel brominated secondary metabolites from marine cyanobacteria, we surveyed several extracts using mass spectrometry and the query language (MassQL). Consequently, a brominated Ahp-containing cyclic depsipeptide was successfully isolated from *Moorena* sp. collected from the Las Perlas islands in Panama. In parallel, derivatives of this compound were identified through MS/MS molecular networking. Their structures were elucidated by a combination of MS and NMR-based methods. A genome sequence of the producing organism was also obtained, and this enabled the identification of the putative biosynthetic gene cluster responsible for the production of these new Ahp-containing cyclic depsipeptides. Curiously, no candidate gene for introducing the bromine atom was located in the cluster, suggesting that a *trans*-acting brominase may be responsible for its production.

Keywords: *Moorena* sp., brominated natural product, Ahp-containing cyclic depsipeptide

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S1.P74 The utilization of LED lamps to enhance metabolite production in plants – fagopyrins from buckwheat

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Light is one of the main signals detected by plants that influence plant development and metabolism. Metabolic processes in plants are sensitive to the ratio of blue (B) and red (R) light (Kami et al., 2010). In recent years, Light-Emitting Diodes (LEDs) have become the most prevalent light sources utilized in Controlled Environmental Agriculture (CEA), offering the capability to manipulate light spectra (Engler and Krarti, 2021). Fagopyrins (**1**) are photosensitizers present in common buckwheat (*Fagopyrum esculentum* Moench). These compounds have antifungal and antibacterial properties. There are known six forms of **1** (A-F). The primary form is protofagopyrin (**2**) which after irradiation converts to **1**. The highest content of **1** was found in buckwheat flowers (Kim and Hwang, 2020), specifically in their pistils (Hornyák et al., 2023). Our study aimed to investigate whether the LED spectrum modification may enhance the production of **1** and **2** in buckwheat pistils. The content of **1** and **2** in extracts from buckwheat pistils was analyzed using liquid chromatography-mass spectrometry (LC-MS). Common buckwheat can grow in CEA conditions using the LED lamp as the sole light source (Hornyák et al. 2022). Three LED lamps emitting wavelengths corresponding to red (R), blue (B), and red-blue (RB) light were tested. The results demonstrate that RB light stimulates the production of **1** and **2** in F form while R light had a promoting effect on production of **1** and **2** in E form in buckwheat pistils.

Keywords: *Fagopyrum esculentum*, light spectrum, photosensitizer, pistil

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S1.P75 From bugs to drugs: genomic characterisation and bioinformatic analysis of *Streptomyces* strains from the bark beetle microbiome

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Natural product research in *Streptomyces* is crucial in the battle against antibiotic resistance. Although there has been extensive research on soil-associated *Streptomyces*, there is a growing interest in exploring insect-associated strains as well. Insect microbiomes, such as those found in bark beetles, host unique *Streptomyces* with distinct production profiles that are shaped by symbiotic interactions. Studying those *Streptomyces* can reveal new biosynthetic pathways and secondary metabolites that hold promise for developing new antimicrobial substances. In this context, three novel *Streptomyces* strains (*Streptomyces* sp. ITFR-6, ITFR-16 and ITFR-21) were isolated from the microbiome of the Central European bark beetle species *Ips typographus*, collected in the Black Forest, Germany. The aim of this study was to screen the strains using a genome mining approach to identify putative biosynthetic gene clusters (BGCs) and associate them with compounds released into production media. Methods used for identifying the *Streptomyces* strains included isolating them on selective agar plates and sequencing partially conserved genes, such as 16S rRNA and GyrB. Subsequently, whole genome sequencing of the three novel strains was performed. Comprehensive genome analysis was conducted using advanced genome mining techniques, such as antiSMASH, BiG-SCAPE, MIBiG, and KEGG-KAAS-MinPath. This approach allowed exploration of the biosynthetic potential of these microorganisms at the genomic level, revealing a large number of promising biosynthetic gene clusters encoding for secondary metabolites. Crude extracts were also purified and screened for bioactive compounds, as the three novel strains have previously demonstrated antibiotic properties. In this context various aspects and new developments will be discussed.

Keywords: *Streptomyces*, genome mining, RiPPs, bark beetle, insect microbiome

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S1.P76 A microscale cultivation platform integrating OSMAC to explore metabolomic changes and antimicrobial activity of a fungal strain collection

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Antimicrobial resistance is a major global health threat. Therefore, it is imperative to discover new antimicrobials with new modes of action. In this context, natural products derived from microorganisms have proven to possess very potent activities (Dai et al., 2020). However, their cultivation is a slow process and the frequent isolation of known active compounds is a limitation when screening natural extracts. To circumvent this problem, we recently developed a drug discovery platform compatible with the OSMAC approach (Bode et al., 2002). Cultivation of fungal strains, extraction, bioassays and metabolomics are performed in a standardized 96-well plate format thus allowing high-throughput and compatibility across the screening and analytics platforms. In a proof of concept study, sixty fungal strains were cultured in triplicates in eight different liquid media. Extracts were enriched by solid phase extraction and systematically submitted to antimicrobial bioassays and metabolomic profiling. Around 1'500 fungal extracts were generated and screened for antimicrobial and antivirulence activities. The highly reproducible results exhibited hits, mostly in triplicate, which were either spread across different media or limited to a specific medium; hence highlighting the power of the OSMAC approach to increase the hit rate. The full dataset was also analyzed by UHPLC-HRMS/MS allowing for the construction of a massive molecular network which integrates the bioactivity results (Olivon et al., 2017). The integration of the massive data generated allows efficient prioritization of strains and conditions for subsequent upscaling and targeted isolation of active candidates.

Keywords: OSMAC, antimicrobials, antivirulence, metabolomics, fungi

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S1.P77 Enhanced production of hexylaconitic anhydride compounds from endolichenic fungus *Xylaria sp.* by co-culture with *Chaetomium* sp.

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Endolichenic fungi form a diverse group of microscopic fungi that live asymptomatically in the lichen thalli (Agrawal et al., 2020). In nature microorganism co-exist in the same ecological habitat interacting with each other in a symbiotic or antagonistic manner. Secondary metabolites play a key role in these interactions acting as chemical signals for communication or competition of the limited nutrients, triggering various defense mechanisms. These stress factors may increase the production of known compounds or activate silent biosynthetic pathways resulting to new compounds (Knowles et al., 2022). Therefore, by mimicking the natural environment, we studied the mixed fermentation of two endolichenic fungi, *Xylaria sp.* and *Chaetomium sp*., isolated from the lichen, *Hypogymnia tubulosa*. Co-culturing these strains led to increase the total yield of the extract and a significant change in the production of secondary metabolites of *Xylaria sp.*. HPLC-MS profiles of co-culture were different compared to the monocultures resulting in a stimulation of hexylaconitic anhydride metabolites while piliformic acid derivatives decreased significantly. These metabolites were isolated from the extract and identified by mass spectrometry and NMR analysis.

Fig. 1. Morphological interaction observed among endolichenic strains in Petri dish.

The co-culture extract showed strong nematicidal effect against the plant-parasite *Meloidogyne javanica* while the control monocultures were not active. This enhancement was mainly attributed to major production of hexylaconitic anhydride. This compound was found also to be active against the fungi (*Botrytis cinerea*, *Alternaria alternata*) and aphids (*Myzus persicae*, *Rhopalosiphum padi*). These findings suggest the potential of this metabolite, along with the co-culture extract, as promising biocontrol agents for agricultural applications.

Keywords: endolichenic fungi, co-culture, *Xylaria sp*., hexylaconitic anhydride, biopesticide

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S1.P78 Bioprospection of bioactive compounds from fungi in Cameroon: current status and outlook

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Natural bioactive compounds are considered the cornerstone for the development of high-value products with applications in medicine, agriculture and the food industry. Plants have been long studied for their bioactive constituents and the use of microorganisms as an alternative appears to be a promising strategy for offering compounds with high therapeutic potential. Among microorganisms, fungi have played an important role in the production of antibiotics and other drugs for the treatment of infectious and non-infectious diseases. The diversification of fungi and their gene clusters involved in the biosynthesis of secondary metabolites indicate their unlimited potential to produce a plethora of molecules. Although various African cultures have long used fungi as medicines, they remain relatively unexplored from a chemical standpoint. Recent scientific efforts have targeted the bioprospecting of fungi in Cameroon for the isolation of bioactive constituents (Kenla et al. 2013, Happi et al. 2015, Teponno et al. 2017, Anoumedem et al. 2020, Mountessou et al. 2023).

We will highlight the current state of chemical research on fungi from Cameroon and special attention given to their investigation under a project funded by the Alexander von Humbolt Foundation called Humboldt Research Hub- CECANAPROF.

Keywords: fungi, bioactive compounds, Cameroon, CECANAPROF

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S1.P79 Natural product biosynthesis regulation in fungi. What can system biology tells us?

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Microorganisms is an unlimited reservoir of Natural Products (NPs) that are waiting to be discovered (Carroll *et al.* 2022). To get access to a higher chemical diversity, one possibility remains to modify culture condition thus activating silent biosynthetic pathways. This strategy called "One Strain Many Compounds" – OSMAC – is however very time consuming (Romano *et al.* 2018). Thus, one may consider using alternative strategies to anticipate NP production regulation. We are currently exploring the possibility to use System Biology approaches to explore such regulation using the fungus *Penicillium rubens* as a model organism (Guzmán-Chávez *et al.* 2018). Approaches based on Genome Scale Metabolic Networks (GSMN) are widely used to interpret metabolic regulation within an organism. A GSMN represents a model of an organisms based on genome encoded biological reactions. However, still many limitations exist to be able to use of GSMN modelling. One of the main limitations lays in the absence of NP biosynthetic pathways in the available GSMN (Nègre *et al.* 2023). After manual integration of various NPs biosynthetic pathways in the available GSMN of *P. rubens* (Agren *et al.* 2013), strategies to explore biosynthetic pathways regulation was performed to explore the impact of simple culture condition modification on NP production. Such "dry laboratory" conditions are currently being explored in "wet laboratory" experiments. In conclusion, the System Biology can be applied to NP chemistry as an alternate strategy to explore NP production regulation by fungi. Further in-depth study needs to be explored to innovative approach.

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Keywords: annotation, dereplication, LC-HRMS/MS

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S1.P80 Exploration of the clusioid clade metabolome through molecular networking: In the search for new pharmacophoric scaffolds of fungal UPR inhibitors

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Exploring alternative approaches and seeking innovative solutions for crop protection is a major challenge for the next decades (Almeida et al., 2019). In this context we have developed a new strategy targeting compensatory fungal pathways, to increase the sensitivity of fungi to plant defenses and therefore controlling phytopathogenic infections (Charpentier et al., 2023). Among these pathways, the Unfolded Protein Response (UPR) appears as a promising target for the development of new antifungal drug (Joubert et al., 2011). Recently, polyhydroxylated and prenylated xanthones have been identified as potent inhibitors of the IRE1 protein, the unique effector in fungal UPR pathway (Charpentier et al., 2023). To gain a deeper understanding of structure-activity relationships of these inhibitors, phytochemistry study have been undertaken. However, these xanthones are most often isolated as complex mixtures extracted from different parts of plants belonging to the clade clusioid. Therefore, as a dereplication strategy, feature-based molecular networking (FBMN) (Nothias *et al*, 2020) was applied to map the chemical space of clusioid plant extracts and a MS/MS spectra database of 130 xanthones with different scaffolds and know active compounds, has been constituted. As will be shown this data base allowed us to annotate one FBMN as well as to explore chemical diversity of xanthones clusters in order to select nodes of interest.

Keywords: xanthones, Molecular Networking, MS/MS spectra database, UPR inhibitor

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S1.P81 Differences in chemical compounds of selected strains of *Hericium erinaceus*

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Hericium erinaceus, a medicinal mushroom, has been known in Chinese traditional medicine for its positive effects on human health. It has been used to treat a wide range of health problems or as a prevention of various diseases including Alzheimer's and Parkinson's disease, ischemic stroke or stomach disorders. *H. erinaceus* have neuroprotective properties, anticancer effects, antiglycemic, antidepressant, antimicrobial and antioxidant effects (Thongbai et al., 2015). The aim of our work was to determine the differences in the metabolome of selected strains of *H. erinaceus*. Six samples from Asia, Belgium and Czech Republic were used for the analysis. After metabolite extraction, their abundance was determined by LC/HRMS, and results were evaluated by multivariate analysis. From the reduced set of the most significant features, 13 of them were selected by comparison with published data, which were tentatively identified on the basis of MS/MS spectra and extracted ion chromatograms. By statistical comparison, we found that the strains of the genus *Hericium* show significant differences in metabolic profile, which corresponded with their origin. In the strains of Czech origin, the substances hericerin A, which has anticancer effects (Li et al., 2015), and hericenone J, which has been found to have neurotrophic activity(Ueda et al., 2008), were more represented. The biological activity of the observed strains was then tested for their anti-inflammatory effect in THP- 1 cell culture in vitro. The test proved, that *H. erinaceus* extracts inhibited the production of inflammatory markers in this model system.

Keywords: *Hericium erinaceus*, bioactive substances, biological activity, LC-HRMS

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S1.P82 Genome mining paired with stable isotope labeling allows for discovery of new cyanopeptolins in cyanobacterium *Nostoc* sp. UIC10890

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Cyanobacteria, also known as blue-green algae, are a phylum of gram-negative photosynthetic autotrophs that can be found in almost every biome of the world. Their secondary metabolites are diverse in chemical structure and many have been shown to have therapeutic relevance. They are also capable of nitrogen fixation, which influences their production of nitrogen-containing compounds such as non-ribosomal peptides (NRPs). Traditional workflows for natural product drug discovery are usually bioassay-guided to prioritize activity, however advances in technology as well as lowering costs of sequencing offer the alternative of using a genome mining-guided workflow to prioritize the discovery of novel structures and specific compound classes. The use of genome mining on a *Nostoc* sp. cyanobacterial strain, UIC10890, led to the discovery of an NRP biosynthetic gene cluster encoding for the production of a new cyanopeptolin. Cyanopeptolins (CPs) are depsipeptides characterized by the presence of 3-amino-6-hydroxy-2-piperidone in the third position, often with side chains and N-methylation occurring at other positions. ^{15}N stable isotope labeling followed by comparative metabolomics using tandem mass spectrometry (MS/MS) allowed for the detection and subsequent isolation of two new CPs from UIC10890. The structures of the CPs were confirmed through NMR spectroscopy. This poster will present the new CPs, their biosynthetic gene cluster, and the workflow used to isolate them. This research demonstrates a genetics-guided approach drug discovery and how it can be used to expedite dereplication as well as discover novel compounds.

Keywords: cyanobacteria, cyanopeptolin, genome mining, biosynthesis, mass spectrometry

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S1.P83 Diversity of *Origanum onites* essential oils in the ecological regions of Eastern Mediterranean

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Origanum onites L. is a very popular aromatic herb widely used in everyday cooking and traditional medicine since antiquity (Hanlidou et al., 2004). The native range of the species is restricted in Eastern Mediterranean; it extends from South and West Turkey, *via* South Greece and islands, up to Sicily (POWO, 2024). The essential oils (EOs) of *O. onites* have been studied for a number of potential applications in the pharmaceutical and food industry, such as antioxidant, antimicrobial, antifungal and health protective agents (Kintzios, 2002; Canli et al., 2023). Data from 37 years of scientific literature and new headspace GC-MS analyses were examined to assess the EOs diversity of wild *O. onites* plants. Information concerning the content in EO and its composition – focused on main oil components: γ-terpinene, *p*-cymene, carvacrol, thymol and linalool – were collected from different localities scattered along the total species range, and discussed in relation to six ecological regions of Eastern Mediterranean (EEA, 2024). The highest values of oil content have been recorded in the ecological region of Aegean & West Turkey sclerophyllous and mixed forest (up to 7.0% v/d.w., island of Chios). The EOs from the localities of the three Mediterranean ecological regions are characterized by the dominant occurrence of carvacrol (up to 92.7% of the total oil, island of Kos). The oil composition in the southeastern part of species range, which belongs to the more humid and colder Mediterranean-Continental ecological regions, is much more diversified, and characterized by either carvacrol, linalool and/or thymol.

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Keywords: oregano, *Origanum onites,* ecological regions, climatic regions, headspace GC-MS

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S1.P84 Comparative GC/MS analysis of essential oils from two varieties of *Vitex negundo* L.

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The genus *Vitex* L. includes several hundred species of woody plants (Islam et al., 2024). Some *Vitex* species are of significant scientific interest as they are widely used in traditional medicine of many countries. However, only fruits of *V. agnus-castus* L. are used in the conventional medicine (European Pharmacopoeia, 11th ed., 2023). Phytochemical analysis of different *Vitex* representatives is a very promising direction for further research, especially because genetic prerequisites and climatic conditions have a great influence on the biosynthesis of bioactive compounds (Shanaida et al., 2021). In this study, the gas chromatography-mass spectrometry (GC/MS) method was used to analyze the composition of essential oils from the flowering shoots of two *Vitex negundo* varieties: *V. negundo* var. *heterophylla* (Franch.) Rehd. and *V. negundo* var. *cannabifolia* L. The raw materials were harvested in 2022 under cultivation of plants in M.M. Gryshko National Botanical Garden (Kyiv, Ukraine). The GC/MS study revealed that the predominant components of *V. negundo* var. *heterophylla* essential oils were α-terpineol (13.58%), terpinyl acetate (11.37%), borneol (10.90%), 1,8-cineol (eucalyptol) (9.52%), *beta*-farnesene (8.98%), bicyclogermacrene (7.98%) and terpinen-4-ol (7.39%). The following terpenoids prevailed in the essential oil of *V. negundo* var. *cannabifolia*: borneol (58.36%), *trans*-sabinen hydrate (6.96%), eugenol (6.22%), α-terpinol (5.70%), terpinyl acetate (4.51%) and 1,8-cineol (3.61%). As the revealed predominant volatile compounds have the well-defined therapeutic properties, the essential oils of the studied *V. negundo* varieties could be considered for further investigations of their pharmacological effects.

Keywords: chromatographic analysis, flowering shoots, volatile compounds, *Vitex negundo* var. *heterophylla, Vitex negundo* var. *cannabifolia*

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S1.P85 Molecular interactions in the ocean microbiome: tracking fungal metabolites modifying *Prorocentrum lima* physiology and toxin production

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Harmful algal blooms are associated with detrimental effects on their environment and have a negative impact on associated human economic activities (shellfish farming) (Grattan et al., 2016). Toxin production is still a poorly explained phenomenon that could be related with microbial chemical induction. In this project, we worked on an original interaction model between the bloom forming toxic microalga *Prorocentrum lima*, and its co-isolated fungus *Aspergillus pseudoglaucus* (Berry et al., 2023). We observed that toxin production by the microalga was up-regulated in co- culture conditions and that the fungal crude extract yielded the same effect. Applying both a bioactivity and LC-HRMS/MS-guided strategy (including molecular networking and dereplication), fungal molecules altering *P. lima* growth and its production of diarrheic toxins were tracked. From the initial crude extract, some fractions which were mainly composed of anthraquinones and echinulin derivatives seemed to interfere with normal metabolism of the microalga. Two other fractions were shown to lower or increase toxin quantities, due to the presence of polar anthraquinone derivatives and some unknown compounds. These fractions underwent further purification work to isolate their major molecules whose structures were solved by NMR experiments. All these isolated molecules were evaluated on *P. lima* physiology. This study showed that *A. pseudoglaucus* compounds can exert different effects on *P. lima* physiology, such as increased or decreased growth, or variation of toxin concentration and/or excretion in the culture medium. Further investigations are ongoing to investigate their respective and global role in the consortium co-culture.

Keywords: *Prorocentrum lima*, *Aspergillus pseudoglaucus*, microalgal toxins, fungal natural products, chemical interactions

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S1.P86 Chemical mediation of algal microbiome across temperate and subtropical regions

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The surface microbiomes of plants and animals plays a critical role in maintaining the health of the host. As climate change warms and acidifies the ocean, alterations in the microbiome of marine algae and ideal conditions for opportunistic pathogens could lead to an unprecedented loss of algal biodiversity (Minich et al., 2018; van der Loos et al., 2019). We examined the role algal metabolites play in shaping the microbiome across two climates. Algal species, from two subtropical sites in Florida and southern California and two temperate sites in northern California, were swabbed for surface associated bacteria (SAB) and extracted (CA) or extracted and partitioned (FL). These metabolite mixtures were then screened for SAB growth-promotion or inhibition activity. Our experiments from California display site specific bacterial growth modulation. Bacteria from the same location displayed neutral activity (9% promotion, 20% inhibition) but bacteria from other sites were strongly modified by the algal extracts (27% and 43%). Chloroform partitions from Florida algae inhibited the growth of 24% of FL SAB. This demonstrates a specific relationship between these partitions and FL SAB, compared to the other two partitions. Across phyla, we observed neutral activity in 3 partitions (no strong effect on any bacteria). Comparing across the two climates, more inhibition was observed from the warmer climates suggesting the presence of stronger defenses against potential pathogens. These results demonstrate that algal chemistry plays a role in shaping SAB population structure warranting future studies to identify the metabolites responsible for the observed inhibition.

Keywords: signaling, algae, climate change, partitions, microbiome

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S1.P87 Marine actinobacterium as a source of natural products against multiple myeloma

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Multiple myeloma is a hematologic malignancy characterized by the abnormal proliferation of clonal plasma cells. Patients frequently experience relapse and develop resistance to the currently available drugs. Therefore, there is a need to find new treatment options, and the investigation of sources of natural products is of interest (Yu et al., 2022). The present study evaluated the antiproliferative activity of compounds produced by a marine actinobacterium, *Streptomyces cacaoi*, in various multiple myeloma cell lines. The fermentation conditions were first optimized using a statistical experimental design to enhance the production of bioactive secondary metabolites (Gezer et al., 2022). Subsequently, the antiproliferative activity of the EtOAc extracts was tested in RPMI 8226 multiple myeloma cells at a concentration of 45 µg/mL. Incubation temperature (25-35°C) and water type (distilled vs seawater) strongly affected the production of bioactive compounds. Extracts obtained after fermentation in distilled water at 25°C were the most active. Then, the effect of some inorganic chemicals was also evaluated. Surprisingly, KNO3, which is frequently used in media, caused a significant decrease in the antiproliferative activity of the extracts. Finally, three polyethers isolated from an extract obtained with optimized conditions showed antiproliferative activity with IC50 values in the low micromolar concentrations against the multiple myeloma cell lines RPMI 8226 and KMS-12-BM, as well as their drug-resistant counterparts. In conclusion, this study demonstrates the critical impact of fermentation conditions on producing bioactive natural products against multiple myeloma cancer cell lines.

Keywords: multiple myeloma, *Streptomyces cacaoi*, optimization, fermentation, cytotoxicity

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S1.P88 Parishin derivatives from the aerial parts of *Sedirea japonica*

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Sedirea japonica (Orchidaceae) is found in Eastern Asian countries such as Korea, Japan, and China. Their attractive beauty and ornamental value have long been appreciated. In Eastern culture, *S. japonica* was commonly given as a gift (Lee et al., 2021). However, indiscriminate harvesting led to the endangered status of *S. japonica* in Korea and Japan (Suetsugu et al., 2013). Under this circumstance, only a few phytochemical studies of this plant have been conducted. In our natural product research to discover new metabolites, eight compounds including five new parishin derivatives, sedishins A-E (**1**-**5**) and three phenolic compounds (**6**-**8**) were successfully isolated from the aerial parts of *S. japonica* through LC/MS guided isolation approach. The structures of new compounds (**1**-**5**) were characterized through one-dimensional (1D) and two-dimensional (2D) NMR (^{1}H-^{1}H COSY, HSQC, HMBC) spectral data analysis, combined with high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) analysis. All isolated compounds will be further evaluated for their biological activity.

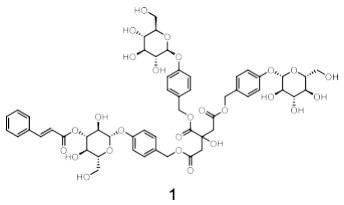

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Fig. 1. Structure of sedishin A.

Keywords: *Sedirea japonica,* Orchidaceae, parishin derivatives, sedishins A-E, structural elucidation

References

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S1.P89 Nitrobenzoyl sesquiterpenoids from a Hawaiian marine-derived fungus FM451

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Marine-derived fungi represent a vast reservoir of novel compounds, owing to their extensive chemical diversity and demonstrated ability to produce natural products with therapeutic and pharmacological potential (Hasan et al., 2015). In a screening test, the methanolic extract of a Hawaiian marine fungal strain FM451 was found to exhibit potent antimicrobial activity against *Staphylococcus aureus*. LC/MS-guided isolation of the methanolic extract of the fungus led to the isolation of six α-pyrone-derived compounds, including one undescribed compound (**1**) and five known derivatives (**2**–**5**), as well as six nitrobenzoyl sesquiterpenoids, including one unreported compound (**6**) (Fig. 1) and five known derivatives (**7**–**11**). Structural elucidation of the new compounds was achieved through extensive NMR spectroscopic methods and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data analysis. Furthermore, their absolute configurations were unambiguously determined via theoretical electronic circular dichroism (ECD) calculation and DP4+ analysis. All of these compounds will be evaluated for their antimicrobial activities against *S. aureus*.

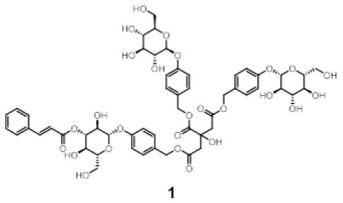

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Fig. 1. Structure of the new nitrobenzoyl sesquiterpenoid **6.**

Keywords: marine-derived fungus, nitrobenzoyl sesquiterpenoid, antimicrobial activity

References

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* Presenting author. E-mail address: huochen_0213@163.com (Chen Huo)

S1.P90 Steroid derivatives from cannabis-associated endophytic fungus *Hypoxylon perforatum*

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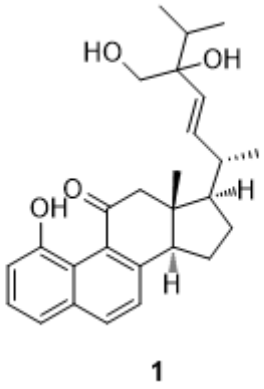
Endophytic fungi, residing within plants, serve as prolific producers of novel and structurally diverse bioactive compounds, which hold promise as potential therapeutics for addressing major global diseases (Manganyi et al., 2020). *Cannabis sativa* L., commonly known as marijuana or hemp, is a versatile plant species with a long history of human cultivation for medicinal, industrial, and recreational purposes (Bonini et al., 2018). *Hypoxylon perforatum* was isolated from *C. sativa* as an intriguing endophytic fungus. Three new steroid derivatives (**1**–**3**) were isolated and characterized from the cultivation of cannabis-associated endophytic fungus *H. perforatum* (Fig. 1). The structural elucidation of these compounds was achieved through a comprehensive approach, combining 1D and 2D NMR spectroscopy techniques (including HSQC, HMBC, COSY, and NOESY) with high- resolution electrospray ionization mass spectrometry (HR-ESI-MS) data analysis. Additionally, the determination of their absolute configurations was confidently established through theoretical electronic circular dichroism (ECD) calculations and DP4+ analysis. The investigation into the bioactivity of these newly isolated steroid derivatives is ongoing.



1

Fig. 1. Structure of new steroid derivative **1**.

Keywords: endophytic fungi, *Hypoxylon perforatum*, steroid, absolute configurations

References

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* Presenting author. E-mail address: huochen_0213@163.com (Chen Huo)

S1.P91 Sesquiterpene-phthalide hybrids from cannabis-associated endophytic fungus *Zopfiella* sp.

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Cannabis sativa is regulated as a drug in many countries due to its addictive properties. Endophytic fungi that interact with *C. sativa* plants have been investigated, which is expected to produce bioactive compounds. A total of 27 endophytic fungi were isolated from *C. sativa* and identified using ITS rDNA sequences. Among them, the endophytic fungus *Zopfiella* sp. was first isolated from *C. sativa*. LC-MS-based GNPS networking analysis has shown that *Zopfiella* sp. may produce unique compounds when grown in media containing *C. sativa* stem powder. As part of our ongoing research to discover bioactive natural products, we have isolated four new sesquiterpene-phthalide hybrids (**1** **4**) (Fig. 1), along with five known compounds (**5** - **9**) including steroids and phthalide derivatives from the cultivation of cannabis-associated endophytic fungus *Zopfiella* sp. The structures of four new compounds were elucidated through analysis of 1D and 2D (^{1}H-^{1}H COSY, HSQC, HMBC and NOESY) NMR, HR-ESIMS, optical rotation, as well as ECD calculation. All isolated compounds will be further evaluated for their biological activity.

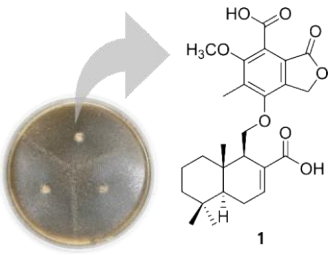

Fig. 1. Structure of new sesquiterpene-phthalide hybrids **1.**

Keywords: *Cannabis sativa, Zopfiella* sp., endophytic fungi, sesquiterpene-phthalide hybrids

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S1.P92 A new cyclic peptide and two new ellagic acid glycoside derivatives from *Jatropha podagrica* leaves

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Jatropha podagrica, a succulent plant belonging to the Euphorbiaceae family, contains various phytochemicals, which is why it has been used as a traditional medicine for analgesic, tonic, and purgative purposes (Jang et al., 2023). The genus *Jatropha* is a combination of the Greek words 'jatros' (doctor) and 'trophe' (food), implying its medicinal uses (Kumar et al., 2008). *Jatropha* species have been used to treat various diseases in Asia and Latin America and are reported to have minimal side effects (Cowan et al., 1999). As a part of our continuing research to discover bioactive natural products, we successfully isolated one new cyclic peptide (**1**) (Fig. 1) and two new ellagic acid glucoside derivatives (**2**-**3**), along with fourteen known compounds (**4**-**17**) including megastigmane glycosides, and phenolic derivatives. The structures of three new compounds (**1**-**3**) were determined through comprehensive analysis of 1D and 2D (^{1}H-^{1}H COSY, HSQC, HMBC and NOESY) NMR, HR-ESIMS, and optical rotation, as well as ECD calculation. The absolute configuration of compound (**1**) was assigned using the advanced Marfey's method. All the isolated compounds, except for compound (**14**), were identified from *J. podagrica* for the first time. All isolated compounds will be further evaluated for their biological activity.

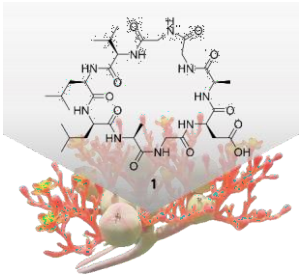

Fig. 1. Chemical structure of new cyclic peptide **1**

Keywords: *Jatropha podagrica,* Euphorbiaceae, cyclic peptide, ellagic acid glycoside, Marfey's method

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S1.P93 Discovery of new sesqui-lignans from *Salix gracilistyla* leaves and their neuroprotective effects

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Salix gracilistyla, commonly known as pussy willow, is primarily distributed along river streams in Eastern Asia, including South Korea. Various parts of *S.gracilistyla*, such as the leaves, stems, and bark, have been used in traditional medicine for treating skin diseases, wounds, and arthritis, and as a painkiller. Previous pharmacological studies on *S.gracilistyla* reported that these extracts showed anti-inflammatory activity by inhibiting NO production in LPS-stimulated macrophages (Ryu et al.,2003). NO-induced inflammation can increase oxidative stress in the brain, potentially triggering neurodegenerative disorders (Yang et al.,2013). In our ongoing research to identify new bioactive compounds from medicinal plants, the ethanolic extract of *S. gracilistyla* leaves was chemically investigated, and two new sesqui-lignans (**1**-**2**) were isolated (Fig. 1), along with 18 known compounds (**3**-**20**). The structures of the new compounds were determined by analyzing 1D- and 2D- NMR spectroscopic data and HR-ESIMS. Their absolute configuration was determined through comparison of their experimental circular dichroism (CD) data with calculated electronic circular dichroism (ECD) spectra. In this study, we evaluated the neuroprotective effects of all the isolated compounds from *S. gracilistyla* on iPSC-derived cortical neurons.

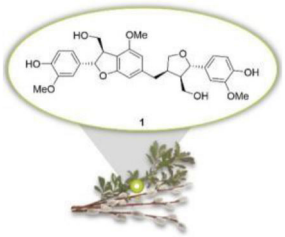

Fig 1. Chemical structure of compound **1.**

Keywords: *Salix gracilistyla*, Salicaceae, lignans, ECD, neuroprotective effects

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S1.P94 Lipopeptides from the bioluminescent bacterium *Photorhabdus luminescens*

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Photorhabdus luminescens is a Gram-negative, entomopathogenic, and bioluminescent bacterium that establishes a mutualistic relationship with entomophagous nematodes. When these nematodes invade insects, they enter the hemolymph and release *P. luminescens* from their gut (Blackburn et al., 1998). Previous pharmacological studies reported that *P. luminescens* produces antimicrobial substances with antifungal or antibacterial activity to prevent the invasion of other microorganisms, supporting the growth and reproduction of the nematodes (Bowen et al., 1998). As part of our ongoing natural product research aimed at identifying bioactive new compounds, we conducted chemical investigations on the EtOAc extract of *P. luminescens* cultivation using LC/MS, HPLC, and TLC- based analyses. This process resulted in the isolation of two new lipopeptides, photorhamides A (**1**) and B (**2**) (Fig. 1), together with six known compounds (**3**-**8**). The structures of the new compounds were confirmed by analyzing their 1D- and 2D-NMR data and HR-ESIMS. The entire peptide sequence was determined through the interpretation of HSQC, HMBC, and NOESY signals, and the absolute configuration was established using Marfey's method. The biological activities of all the isolated compounds will be further evaluated.

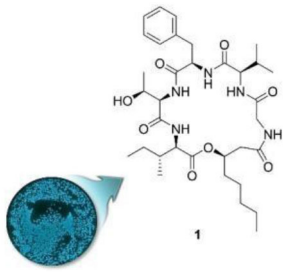

Fig 1. Structure of photorhamide A (**1**)

Keywords: *Photorhabdus luminescens*, lipopeptides, antimicrobial activity, Marfey's method

References

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S1.P95 A new phenolic glycoside from the leaves of *Salix chaenomeloides* and identification of anti- skin active compounds

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Salix chaenomeloides Kimura, commonly known as Korean King willow, is a deciduous shrub and tree belonging to the Salicaceae family. It is native to eastern Asia, including regions of Korea, China, Japan, and Russia. For thousands of years, members of the genus *Salix* spp. have been recognized as healing herbs, used for the treatment of fever, inflammation, and pain relief (Freischmidt et al., 2012). The current study aimed to investigate the bioactive natural products from *S. chaenomeloides* leaves. Phytochemical investigation of an EtOH extract of *S. chaenomeloides* leaves led to the isolation of a new phenolic glycoside, chaenomelin (**1**) (Fig. 1), together with twelve known phenolic compounds (**2–13**) from the EtOAc fraction. The chemical structure of the new compound (**1**) was established by detailed analysis of its 1D- and 2D-NMR (^{1}H-^{1}H COSY, HSQC, and HMBC), HR-ESIMS, and chemical reactions.

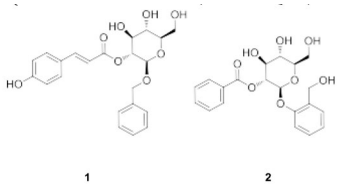

Fig. 1. Structures of compounds **1** and **2.**

The anti-skin aging effects of the isolates were examined using TNF-α-stimulated normal human dermal fibroblasts (NHDFs). Among these 13 compounds, tremuloidin (**2**) and tremulacin (**3**) inhibited TNF-α induced ROS generation ($p < 0.001$ at 100 μM). Inhibition of ROS generation by tremuloidin (**2**) and tremulacin (**3**) led to the suppression of MMP-1 secretion and protection against collagen degradation. Particularly, the observed changes in TNF-α induced responses in NHDFs by tremuloidin (**2**) were found to correlate with the inhibition of COX-2, MAPK activation, and HO-1 induction. These findings offer evidence that tremuloidin (**2**) protects skin tissues from damage induced by oxidative stress accumulation.

Keywords: *Salix chaenomeloides*, phenolic compound, chaenomelin, tremuloidin, anti-skin activity

References

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S1.P96 Transcriptome mining, immunochemistry, and MSMS *de novo* sequencing – phosphorylated peptides from marine sponge *Stryphnus fortis*

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Stryphnus fortis (order: Tectractinellida, family: Ancorinidae) is a deep-sea sponge from the North Atlantic (Cárdenas, 2010). We could probably find cysteine-rich peptides in this sponge as it is closely related to the genus *Asteropus* from which such peptides – asteropsins – were isolated (Takada et al., 2006). Phosphorylation is one of proteins' most common types of posttranslational modifications (PM). However, it is rarely observed in cysteine-rich peptides and its physiological relevance remains elusive. Phosphorylation complicates the sequencing. It is difficult to observe in mass spectrometry as it is unstable and undergoes a process called beta elimination resulting in a loss of water (Tinette et al., 2007). It is also not apparent in standard NMR. In the current study, we describe the discovery of phosphorylated peptides from the marine sponge *S. fortis*. Moreover, it is an example of the application of transcriptome mining, immunochemistry, and mass spectrometry for the detection and characteristics of phosphorylation. In the initial experiments, we found two peptides with MW of 3330.385 Da and 3516.472 Da, named later named *P*-stryphnines A, and B. We established that they contain 6 cysteines. Knowing these characteristics and assuming similarity to asteropsins, we mined the transcriptome for sequences fitting the general pattern. These resulted in finding sequences matching theoretical MW -18 Da (Fig. 1). We confirmed the sequence and ruled out other PMs. The phosphorylation was detected using anti-phosphoserine/threonine antibody in a dot-blot experiment and the modification was pinpointed to S10 by MSMS *de novo* sequencing marine sponges, posttranslational modification.

Fig. 1. Sequence alignment of *P*-stryphnines A, and B *Keywords:* knottin,

References

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S1.P97 Distribution of cyanochelins, cyanobacterial beta-hydroxy aspartate lipopeptides capable of iron chelation

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Deficiency of biologically accessible iron e.g. in the arid-soil environment is one of the limiting factors for thriving of the microorganisms inhabiting such areas. Cyanobacteria as an important contributor in terms of primary production and excretion of bioactive compounds in the arid-soil- crust communities developed a coping mechanism, the secretion of siderophore compound enabling the ferric ions chelation. Cyanochelins, lipopeptides bearing two β-hydroxy-aspartate residues as chelating agents, were recently discovered in three phylogenetically distant cyanobacterial strains (Galica et al., 2021). Genome mining for biosynthetic gene clusters though indicates their widespread presence. Our metagenomic (Illumina) and secondary metabolite research (HPLC-MS) on environmental soil-crust samples discovered novel cyanochelin compounds and their putative producers from the genus *Gloeothece* (Microcystaceae), as well as novel producers of known cyanochelin B from genus *Phormidesmis* (Leptolyngbyaceae). Research on our isolated producers and additional strains from algae collections showed wider distribution of cyanochelin gene clusters in genomes of those two groups of common inhabitants of soil-crust consortia. The production of another novel cyanochelin compound was confirmed e.g. in cyanobacteria in genus *Myxacorys* (Leptolyngbyaceae) and putative responsible biosynthetic gene clusters were identified. The intriguing feature of cyanochelins is the ability to undergo UV-dependent photolysis, release the bioavailable ferrous ions and presumably provide them to the microbial community, which together with their distribution suggest their potential ecological importance. Bioinformatic analyses of our metagenomes, targeted at cyanochelin transporters, discovered that the recipients could occur among both cyanobacterial and other bacterial taxa. In ongoing cocultivation experiments, characterisation of cyanochelin-dependent cohabitants can tell us more about processes of iron cycling in the soil- crusts of arid regions.

Keywords: arid soils, cyanobacteria, cyanochelins, iron, siderophores

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S1.P98 Syringin and phillygenin - natural compounds with a potential role in preventing of foam cells formation in human atherosclerotic plaque

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The presentation will cover an explanation of the biological properties of two natural compounds: syringin and phillygenin in inhibit cholesterol-induced differentiation of macrophage into foam cells (CD36 receptor) and increase cholesterol efflux from macrophage (ABCA1 transporter) (Chistiakov et al. 2016). Syringin is a phenylpropanoid glycoside isolated from the bark of *Syringa vulgaris*, (Oleaceae). Phillygenin is a lignan obtained mainly from the fruits and flowers of *Forsythia x intermedia* (Oleaceae). In previous studies, both compounds showed strong anti-inflammatory and antioxidant properties (Filipek, et al. 2019). Syringin and phillygenin were isolated from plants at the Department of Pharmaceutical Biology of the Medical University of Warsaw. Human monocyte- derived macrophages were incubated (24h) with syringin or phillygenin (10 mM, 20 mM, 50 mM) and cholesterol (20 mM). Oil-red-O staining showed that phillygenin and syringin in dose-dependent manner significantly ($p<0.001$) reduced lipid deposits in macrophages. Cytometric analysis showed that for syringin, the greatest decrease in CD36 receptor expression was determined, from 20% (10 μM) to 80% (50 μM) compared to the cholesterol-stimulated control ($p<0.001$). Phillygenin inhibited the expression of the CD36 receptor, from 5% (10 μM) to 25% (50 μM), compared to the stimulated control ($p<0.05$). The CD36 receptor down-regulation pathway was PPR-g-dependent ($p<0.05$). Western Blot analysis showed that phillygenin caused a statistically significant ($p<0.001$) increase in the expression of the ABCA1 receptor by 2.5-fold (10 μM), 3-fold (20 μM) and 4-fold (50 μM), respectively, compared to the cholesterol-stimulated control. Also, in the case of syringin, a statistically significant increase (50%) in the expression of ABCA1 transporter proteins was observed. The ABCA1 transporter activation pathway was HO-1-dependent ($p<0.05$).

Keywords: syringin, phillygenin, foam cells, CD36, ABCA1

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S1.P99 Deciphering monoterpene indole alkaloid metabolism in *Heliconius* butterfly: a MS-based computational chemistry to describe plant-host interactions

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French Guiana renowned for its rich tropical biodiversity, notably exhibits a remarkable interplay between its Amazon vegetation and preserved fauna. Within this intricate ecosystem, butterflies of the Heliconius genus establish a distinct association with select plant species (See passion vines, Morrison et al., 2023). Extracts from Heliconius butterflies provided a unique dataset for developing a metabolite identification workflow through computational chemistry. Employing liquid chromatography–high-resolution tandem mass spectrometry, a molecular network was constructed. Thanks to the Lotus library (Rutz et al., 2022), in-silico spectral libraries were generated, highlighting compounds found in Heliconius like the notable presence of harmalan (**1**). Employing this library failed to produce results, but upon including **1** in the metadata folder, we surprisingly observed its clustering with three Monoterpene Indole Alkaloids (MIAs). Specifically targeting one of them, ajmalicine (**2**), identified with Sirius and In Silico DataBase (ISDB), characteristic fragments of this molecule were identified and a high cosine score similarity during annotation was observed. Additionally, **1** comprises a MIAs' characteristic 144.0808 m/z fragment shared with **2** (Nakabayashi et al., 2020). The presence of such molecules, although absent in Passiflora plants (Yang Ning et al., 2023), leads us to consider the sequestration of metabolites through the caterpillar's nutrition on a different plant Consequently, we investigated native plants from Guyana known to contain MIAs, and identified *Uncaria guianensis* and Rauvolfia tetraphylla as potential host plants. This fascinating prospect of such interaction also suggest a possibility of de novo biosynthesis.

Fig. 1. Structure of harmalan **1**, ajmalicine **2** and a photo of *Heliconius erato*

Keywords: *Heliconius butterflies,* molecular networking, plant-host interactions, MIAs

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S1.P100 Aporphine alkaloids from *Phaeanthus lucidus* Oliv. and their α-glucosidase inhibitory activity

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Phaeanthus lucidus Oliv. is a medium-sized tree distributed in Southern Thailand and Peninsular Malaysia. To our knowledge, there have been no reports on phytochemical investigations and biological activities from *P. lucidus*. This presentation will cover the isolation, structure elucidation, and α-glucosidase inhibitory activity of compounds isolated from the twig and leaf extracts of *P. lucidus*. In addition, molecular docking simulations of α-glucosidase inhibition of active compounds were included in this presentation. The twig and leaf extracts of *P. lucidus* were fractionated by various column chromatography to yield 14 compounds and seven of them were identified as new aporphine alkaloids (Teerapongpisan et al., 2023; Teerapongpisan et al., 2024). Their structures were elucidated using extensive NMR spectroscopy and HRESIMS. Compounds **1**-**3** were analyzed and resolved by chiral HPLC to yield the (*Ra*) and (*Sa*) atropisomers, whose absolute configurations were determined by ECD calculations. Compound **1** showed the best α-glucosidase inhibitory activities with IC50 values of 6.7 μM.

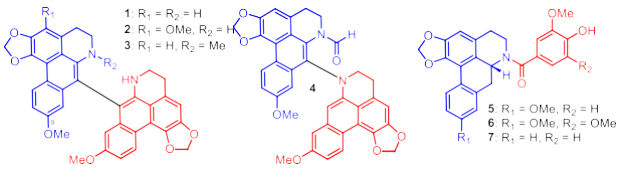

Fig. 1. Structures of new compounds isolated from *P. lucidus*.

Keywords: *Phaeanthus lucidus,* Annonaceae, α-glucosidase inhibitory, Aporphine alkaloids

References

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S1.P101 Tychonemazole, a linear depsipeptide, from the cyanobacterium *Tychonema bourrellyi*

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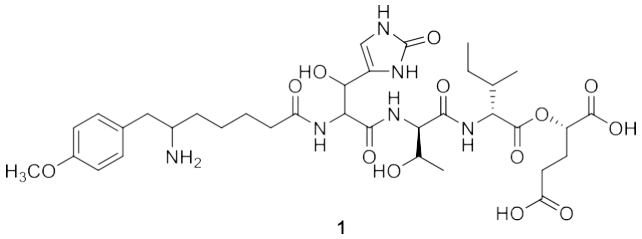
Many sponge-derived compounds are structurally similar to compounds isolated from cyanobacteria. Some are synthesized by symbiotic cyanobacteria in sponges, but some are derived from different bacteria. Citronamides A and B were isolated from the sponge *Citronia astra*, which contains many symbiotic cyanobacterial cells (Carroll et al., 2009). During our OSMAC approach for cyanobacteria, we discovered similar compounds to citranamides from the cyanobacterium *Tychonema bourrellyi*. Tychonemazole (**1**) was found as a major compound even in a culture condition without iron supplementation. Therefore, we attempted to isolate **1** by LC-MS-guided isolation approach. Analyses by 2D NMR including 1,1-ADEQUATE revealed a structure of linear depsipeptide, **1** containing Ile, Thr, glutaric acid, hydroxy oxo-histidine, an amino methoxyphenyl heptanoic acid moiety. The latter two moieties are unique residues in the compounds related to citranamides. A configurational analysis of these moieties is under progress.



1

Fig. 1. Structure of tychonemazole **1.**

Keywords: cyanobacteria, sponge, 1,1-ADEQUATE, oxo-histidine, OSMAC

Reference

Carroll, A.R., Duffy, S., Avery, V.M., 2009. Citronamides A and B, tetrapeptides from the Australian sponge *Citronia astra*. J. Nat. Prod. 72, 764-768.

* Presenting author. E-mail address: okino@ees.hokudai.ac.jp (Tatsufumi Okino)

S1.P102 Isocoumarin derivatives from cannabis-associated endophytic fungus *Phialemonium* sp. SUNP1021

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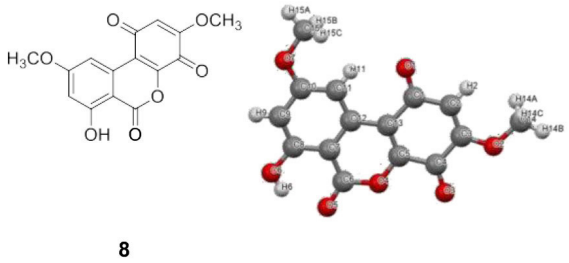
Cannabis sativa is renowned for its psychoactive metabolites. Because of its regulation in Korea, our attention shifted to the endophytic fungus *Phialemonium* sp. (Cephalothecaceae), isolated from *C. sativa* since we anticipated that this fungus would produce novel metabolites, as determined by LC/MS analysis. *Phialemonium* sp., a well-known endophytic fungus (Su et al., 2016) has not been extensively investigated for its metabolites. The chemical investigation of the methanol extract of *Phialemonium* sp. cultivated in cannabis powder dextrose agar (CDA) resulted in the isolation and identification of 12 isocoumarin derivatives (**1**–**12**), facilitated GNPS analysis. The planar structures of novel isocoumarins (**1**–**8**) were established by comprehensive analysis of 1D and 2D (^{1}H-^{1}H COSY, HSQC, and HMBC) NMR spectra and high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) data. Their absolute configurations were fully established by computational methods, including DP4+ analysis and ECD calculations. Especially, the chemical structure of compound **8** was validated through X-ray crystallography. The biological activities of isolated compounds will be further evaluated.



8

Fig. 1. Structure of compound **8** and its X-ray structure.

Keywords: *Cannabis sativa*, cannabis-associated endophytic fungus, *Phialemonium* sp., Cephalothecaceae, isocomarin

References

Su, L., Deng, H., Niu, Y. C., 2016. *Phialemoniopsis endophytica* sp. nov., a new species of endophytic fungi from Luffa cylindrica in Henan, China. Mycol. Prog. 15, 1-7.

* Presenting author. E-mail address: jseyun12@gmail.com (Se Yun Jeong)

S1.P103 Novel polyketide metabolites, skeletocutins M–Q, from *Tyromyces chioneus* and their antibacterial activity against *Staphylococcus aureus*

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Tyromyces chioneus (Polyporaceae) is widely distributed throughout temperate boreal pine forests across Asia, Europe, and North America (Chander et al., 2018). The extract of *T. chioneus* has been reported to possess potent antimicrobial activity against pathogenic microorganisms and notable antioxidant properties (Manjusha et al., 2020). Nonetheless, the bioactive metabolites of *T. chioneus* have not been thoroughly investigated. The skeletocutins from wood-inhabiting Basidiomycete, *Skeletocutis* sp. (Polyporaceae) have been reported to show antimicrobial activity against Gram- positive bacteria and hepatitis C virus (Chepkirui et al., 2019). As part of our extensive research on Korean wild mushrooms, a chemical investigation of ethyl acetate extract of *T. chioneus* cultivated in potato dextrose broth (PDB) resulted in the isolation and identification of 8 polyketide metabolites (**1**–**8**). The chemical structures of novel compounds (**1**–**5**), skeletocutins M–Q, were fully established via detailed analysis of 1D and 2D (^{1}H-^{1}H COSY, HSQC, and HMBC) NMR spectra and HR-ESIMS data.

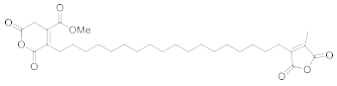

Fig. 1. Structure of compound **4**

The antibacterial activity of isolated polyketide metabolites (**1**–**8**) against *Staphylococcus aureus* was assessed by determining the inhibitory effect of PepZ leucine aminopeptidase which is crucial for the survival of *S. aureus* inside the host cell. All the tested compounds inhibited the PepZ leucine aminopeptidase of *S. aureus*. Specifically, compounds **4** and **5** displayed the most potent inhibition of PepZ. These findings demonstrate that skeletocutins identified from *T. chioneus* can selectively kill intracellular *S. aureus* with minimal perturbation of normal microbiota.

Keywords: *Tyromyces chioneus,* skeletocutins, antibacterial activity, leucine aminopeptidase, *Staphylococcus aureus*

References

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S1.P104 praca wycofana

S1.P105 Usnic acid enantiomers in the lichen-forming fungal family Parmeliaceae

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Enantiomers of usnic acid in lichens have potent antimicrobial activity, and the lichens in the genus *Usnea* (Parmeliaceae, Ascomycota) containing the (+)-usnic acid have been used as herbal medicines (Xu et al., 2016). However, there are multiple genera (e.g. *Flavocetraria*, *Alectoria*, etc.) in the family Parmeliaceae producing usnic acid, but they were not selected for medicinal uses. We hypothesize that they may produce the other enantiomer – (-)-usnic acid that might be less favorable than the (+)- usnic acid in bioactivities. The study aimed to characterize the usnic acid enantiomeric composition in the lichen-forming fungal family Parmeliaceae in a fungal phylogenetic context. A validated chiral HPLC method was sued for the enantiomeric separation of enantiomers (Xu et al., 2022), and fungal phylogenetic tree was constructed using three fungal genetic markers (i.e. ITS, MCM7 and RPB1).

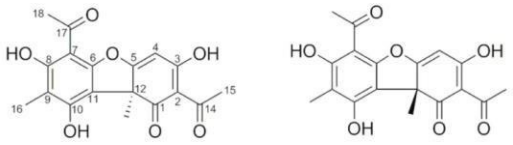

Fig. 1. Structures of usnic acid enantiomers: (-)-usnic acid (left) and (+)-usnic acid (right)

Our preliminary data show interesting phylogenetic patterns of usnic acid enantiomeric production: the cetrarioid clade is rich in (-)-usnic acid, while the parmelioid clade in (+)-usnic acid. We also performed phylogenetic analyses on the biosynthetic genes and proposed the biosynthetic pathways for both enantiomers. Ongoing antimicrobial assay tests indicate that the (+)-usnic acid is more potent than (-)-usnic acid in antibacterial activity.

Keywords: usnic acid, stereoisomers, lichens, Parmeliaceae

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S1.P106 The potential of Amazonian microorganisms in drug discovery

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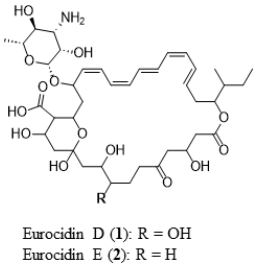
The discovery of antibiotics from soil microorganisms in the mid-20th century revolutionized medicine, with recent research underscoring continued interest in natural products, exemplified by the discovery of the antibiotic teixobactin (Ling et al, 2015). In parallel, the Amazon's rich and untapped biodiversity faces threats from climate change and deforestation, stimulating further studies. As part of our continuous interest in microbial natural products from different Brazilian biomes, we have collected soil specimens from the Amazon Forest in the Anavilhanas National Park. Ninety Actinobacterial strains were isolated, and 24% of them showed bioactivity in antimicrobial assays against *Candida albicans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Among these, Actinomycetota AMYS3.17 was selected for chemical studies, leading to the annotation of eurocidins D (**1**) and E (**2**). (Fig. 1). Eurocidins have been relatively underexplored in research, with only eurocidins D (**1**) and E (**2**) being extensively characterized. Molecular networking analysis (GNPS) (Wang et al, 2016) indicates that bacterial strain AMYS3.17 can produce over 20 compounds resembling eurocidin, including novel analogues putatively annotated through mass spectrometry. This highlights the significant potential of this microorganism for discovering new polyene compounds.



Eurocidin D (**1**): R = OH
Eurocidin E (**2**): R = H

Fig. 1. Structures of eurocidin D (**1**) and eurocidin E (**2**)

Keywords: Actinomycetota, soil, microorganisms, natural products, Amazon Forest

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* Presenting author. E-mail address: mtpupo@fcfrp.usp.br (Mônica T. Pupo)

S1.P107 From kitchen to lab: unveiling methyl 5-vinylnicotinate and other compounds formation during cooking with olive oil

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Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Greece

The current study investigated the fate of the phenolic compounds found in extra virgin olive oil (EVOO) during common Mediterranean cooking practices, specifically sautéing and frying. A range of plant-based (onion, garlic, pepper, mushroom, carrot) and animal-based (cheese, bacon, egg) foods were fried in varying food-to-oil ratios to assess this impact. Key findings include the formation of a rare pyridine alkaloid, methyl 5-vinylnicotinate, identified through ^{1}H-NMR and MS techniques for the first time as a food ingredient. The compound was isolated from the olive oil matrix after cooking using low-pressure adsorption chromatography and preparative thin-layer chromatography. Further, a potential formation mechanism was proposed, involving the reaction of EVOO's phenolic compounds oleomissional or/and oleokoronal with nitrogen sources in the food, catalyzed by heat (Fig. 1). The semi-synthesis of methyl 5-vinylnicotinate was also performed using two methods: one involving the reaction of the dry polar extract of extra virgin olive oil with ammonium hydroxide, and the other one using pure oleomissional, extracted from olive tree leaves, also reacting with ammonium hydroxide. The synthesized compound was purified and isolated, highlighting a novel synthetic methodology. Additionally, hydroxytyrosol acetate and tyrosol acetate were produced during the above reaction and were correlated for the first time with cooking of EVOO.

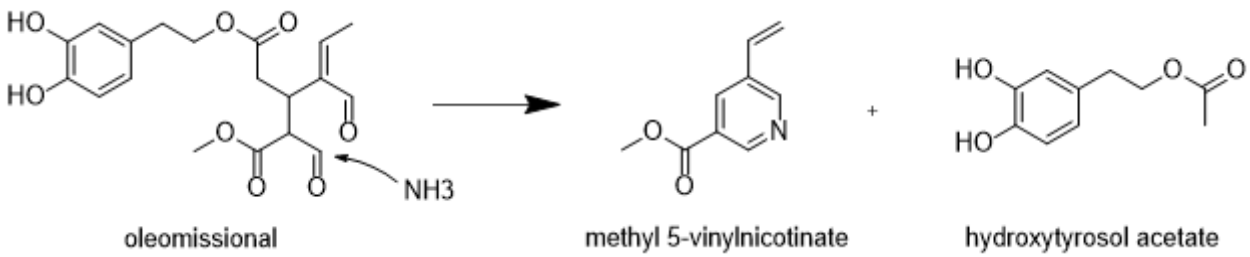

Fig. 1. Methyl 5-vinylnicotinate and hydroxytyrosol acetate formation during frying with EVOO rich in oleomissional.

This research provides crucial insights into the chemical transformations occurring in Mediterranean cooking with EVOO, opening avenues for understanding the health impacts of these culinary practices. Future research will focus on evaluating the potential benefits or risks associated with methyl 5-vinylnicotinate consumption.

Keywords: EVOO, methyl 5-vinylnicotinate, Mediterranean cuisine, olive oil phenols

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S1.P108 C15 acetogenins and sesquiterpenes with anti-inflammatory activity from red algae of the genus *Laurencia* collected in the Red Sea

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[c] *Red Sea Research Center (RSRC), King Abdullah University of Science and Technology (KAUST), 23955-6900 Thuwal, Saudi Arabia*

Among red algae, the genus *Laurencia* (Rhodomelaceae), comprising approx. 140 accepted species distributed in tropical, subtropical and temperate coastal waters, is one of the richest sources of new secondary metabolites in the marine environment (Harizani et al., 2016). Up to now, almost 1,200 secondary metabolites, mostly halogenated C15 acetogenins and terpenes often displaying unprecedented carbon skeletons, have been reported from *Laurencia* sp. and mollusks of the genus *Aplysia* that feed on them. Among the isolated metabolites, a high number has displayed antiviral, anti-inflammatory, antibacterial, antifungal, cytotoxic, antiproliferative, antifeedant, ichthyotoxic, antifouling and insecticidal activity (MarinLit, 2024). In the framework of our investigations, aiming for the isolation of new bioactive marine metabolites, we studied the chemical profiles of two populations of *Laurencia* collected from Hurghada reef in Egypt and Rose reef off Thuwal in Saudi Arabia. Algal specimens were extracted with mixtures of CH2Cl2/MeOH and the organic residues were submitted to a series of chromatographic separations, leading to the isolation of 32 different secondary metabolites, including 12 new C15 acetogenins and 5 new sesquiterpenes. The structures and the relative configurations of the isolated natural products were established on the basis of extensive analysis of their NMR and MS data, while the absolute configuration was determined by single-crystal X-ray diffraction analysis. The anti-inflammatory activity of the isolated compounds was evaluated by measuring suppression of nitric oxide release in TLR4-activated RAW 264.7 macrophages in culture.

Keywords: marine natural products, *Laurencia* sp., red algae, anti-inflammatory activity

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S1.P109 Diterpenes featuring the spatane, seco-spatane and prenylcubebane carbocycles from the invasive brown alga *Rugulopteryx okamurae*

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Brown algae of the family Dictyotaceae, widely distributed in the tropical and subtropical regions of the Atlantic, Pacific and Indian Ocean, in the Sea of Japan, as well as in the Mediterranean Sea, are a prolific source of natural products. Approximately 700 secondary metabolites, mainly sesquiterpenes, diterpenes and meroterpenoids, have been isolated from taxa of this algal family, exhibiting a wide spectrum of biological activities, such as cytotoxic, antibacterial, antifungal, antiviral, antifouling, antiparasitic and anti-inflammatory activity (MarinLit, 2024). *Rugulopteryx okamurae* is an invasive member of this family, causing severe environmental and economic problems on the western Mediterranean coasts. Therefore, research is mandated in order to reduce the biomass and transform a problem into an opportunity for obtaining new valuable products. In this context, we investigated the chemical profiles of two populations of *R. okamurae* collected from deep and shallow waters from the coasts of Cadiz. Following extraction of the algal biomass, the organic residues were submitted to numerous chromatographic separation steps to afford up to now 25 metabolites, mostly diterpenes featuring the spatane, seco-spatane and prenylcubebane skeletons. Among them, 7 diterpenes are new natural products. The structures and relative configurations of the isolated metabolites were elucidated on the basis of extensive analyses of their NMR and MS spectroscopic data. The evaluation of the bioactivity of the isolated compounds is currently ongoing.

Keywords: marine natural products, *Rugulopteryx okamurae*, brown algae, bioactivity

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S1.P110 Metabolic and biosynthetic potential of microbes isolated from North American snake venom

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Animal venoms are a well-established source of pharmacologically active peptides, which has led to a long-standing assumption that venom glands are sterile micro-environments (Calveteet al., 2009). However, advances in genome sequencing and bioinformatics tools have stimulated renewed interest in exploring venom microbiota (Esmaeilishirazifard et al., 2022). Moreover, new efforts seek to probe the diversity of venom-derived microbes and their evolutionary responses (i.e., antimicrobial compound production) to life in venom-associated organs (Ul-Hasan et al., 2019). Due to extreme conditions, including low nutrient availability, low pH, and the presence of antimicrobial peptides, venom microbes are hypothesized to harbor uniquely untapped biosynthetic potential as an evolutionary response to their hostile environment. We collected venom samples from rattlesnake species found in the Southwestern United States. Venom was collected via a standard sterile technique. We obtained control samples of oral microbiota to compare community composition. Liquid venom and oral swabs were frozen prior to transport on dry ice. Metagenomic DNA was extracted and sequenced via Illumina short-read sequencing. Following this, metagenome-assembled genomes (MAGs) were binned and annotated using an in-house bioinformatic pipeline. Additionally, venom samples were diluted in sterile H2O before plating on agar plates and in liquid culture in varying nutrient media. Resulting mixed bacterial cultures with distinct morphological differences suggested many microbial lineages inhabiting snake venom. Genomic DNA from individual colonies was isolated for Illumina short-read sequencing. Using bioinformatics tools, we mined assembled genomes of our bacterial isolates to predict biosynthetic gene clusters (BGCs) that encode production of specialized metabolites.

Keywords: microbiome, venom, genomics, metabolomics, blood-clotting

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S1.P111 Functionalized diterpenes featuring the cembrane skeleton from soft corals of the genus *Sarcophyton* collected in the Red Sea

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On a global scale, coral reefs are regarded as the most spectacular and diverse marine ecosystems on the planet, hosting hundreds of thousands of species, many of which are currently undescribed. Among them, the coral reefs of the Red Sea are considered the best-developed reefs in the western Indian Ocean. Soft corals of the genus *Sarcophyton* (Sarcophytidae) have been proven to be a prolific source of bioactive natural products, having afforded more than 850 metabolites to date (MarinLit, 2024). They are especially rich in sesquiterpenes, diterpenes, steroids and ceramides, exhibiting a wide range of biological activities, such as antimicrobial, antiviral, antidiabetic, anti-inflammatory, neuroprotective and antitumor activities (Elkhawas et al., 2020). In the course of our investigations targeting the detection and isolation of new marine metabolites with bioactivity, we explored the chemical profiles of two morphologically distinct populations of *Sarcophyton* collected by SCUBA diving from the coral reefs near Al Lith on the south-west coast of Saudi Arabia. After exhaustive extraction of the fresh animal tissues with mixtures of CH2Cl2/MeOH, the organic residues underwent laborious chromatographic separations to yield 38 different secondary metabolites, mostly cembranoids, in pure form. Among them, 7 are new natural products. The structures and the relative configurations of the isolated natural products were determined on the basis of thorough analysis of their NMR and MS data. When possible, the absolute configuration was established by single-crystal X-ray diffraction analysis. The evaluation of the biological activity of the isolated compounds is currently in progress.

Keywords: marine natural products, *Sarcophyton* sp., soft corals, bioactivity

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S1.P112 Neuroprotective activity of *Swertia iberica* (Gentianaceae) on BV-2 microglia cells

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The *Swertia* L., belonging to the Gentianaceae family, includes 166 species worldwide (http-1), and 2 species growing in Türkiye (http-2). *Swertia* exhibits a various pharmacological activities, including anticancer, antipyretic, analgesic**,** antidiabetic, antibacterial, antioxidant, hepatoprotective, and neuroprotective. *Swertia* species contain xanthones, iridoids/secoiridoids, flavonoids, alkaloids, andterpenoids (Kshirsagar et al., 2019). The aerial parts of *S. iberica* were extracted with methanol and partitioned by *n*-hexane, *n*-butanol, and water, respectively. The *n*-butanol extract was chromatographed over a polyamide column eluting with stepwise H2O–MeOH gradient to obtain fractions. Alzheimer's disease, a progressive neurodegenerative disease, affects learning and memory resulting from cholinergic dysfunction. Scopolamine has been employed to induce Alzheimer's disease-like pathology *in vivo* and *in vitro* through the alteration of cholinergic systems (Puangmalai et al., 2017). *S. iberica* extracts were applied to BV-2 microglia cells at 200 µg/mL concentrations to 3.12 µg/mL. Sodyum valproate was used as a positive control. BV-2 cells were pretreated with extracts and fractions. 1 hour later, scopolamine (4mM) was applied. After 24 hours of incubation, the cell viability was evaluated using the MTT method. All tested extracts showed neuroprotective effects but, the water extract was found to be significant at the doses of 12.5-50 µg/mL ($p<0.01$). The isolation and structure elucidation studies to determine the active compounds are ongoing.

Keywords: *Swertia iberica*, cytotoxicity, Gentianaceae

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S1.P113 Tyrosinase inhibitory and cytotoxic activities of two *Inula* species (Asteraceae)

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The *Inula* L. genus, belonging to the Asteraceae family, includes 128 species worldwide (http-1) and 32 taxa in Türkiye (http-2). *n*-hexane, ethyl acetate, methanol extracts of *Inula ensifolia* L. (Basionym: *Pentanema ensifolium* (L.) D.Gut.Larr.) and *Inula montbretiana* DC. were prepared from the aerial parts. The tyrosinase inhibition properties of the extracts were assessed following the method described by Likhitwitayawuid and Sritularak (2001) with slight modifications. Extracts were tested at 1 mg/mL and 0.5 mg/mL concentrations. Notably, at a concentration of 0.5 mg/mL, significant tyrosinase inhibition was observed solely in the methanolic extract of *I. montbretiana*. This extract exhibited a remarkably high tyrosinase inhibition activity (16.40 ± 0.48 %), compared to the positive control kojic acid (16.52 ± 0.9 %). Also, at a concentration of 1 mg/mL, the most potent tyrosinase inhibition was observed in the methanolic extract of *I. ensifolia* species (23.10 ± 2.49 %). The methanol extracts of the plants were evaluated in terms of their cytotoxic activity on MCF-7 and MDA-MB-231 breast cancer cell lines using the resazurin reduction assay (Kuete et al., 2013). The methanolic extract of *I. montbretiana* exhibited a higher cytotoxic activity profile on MDA-MB-231 and MCF-7 breast cancer cells with IC50 values of 73.28 and 237.04 *m*g/mL, respectively, compared to those of *I. ensifolia.* The IC50 value of the methanolic extract of *I. ensifolia* on MDA-MB-231 cells was 393.06 *m*g/mL, while it did not inhibit cell viability of MCF-7 cells at the tested concentrations (31.25 to 500 *m*g/mL).

Keywords: *Inula,* tyrosinase, cytotoxicity, activity, Asteraceae

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S1.P114 Linking soil chemistry to Parkinson's disease pathogenesis: novel neurodegenerative compounds from Actinomycetes

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With the increase in the global average age there has been a corresponding rise in age-related diseases such as Parkinson's Disease. One of the known risk factors for sporadic PD is the rural lifestyle, although the driving environmental factors are not fully understood. Prior research has demonstrated that an extract of rural soil-dwelling *Streptomyces venezuelae* resulted in neuronal death in *Caenorhabditis elegans* through disruption of mitochondrial complex-I and formation of reactive oxygen species (Caldwell et al., 2009). We have isolated three distinct small-molecular weight metabolites from the *S. venezuelae* neurodegenerative fraction via bio-guided fractionation using a *C. elegans* neurodegeneration model. The metabolite structure will be known by Summer 2024. These metabolites will be screened on human IPSC mid-brain dopaminergic neurons later this spring as well as a human blood-brain barrier transwell model. High-resolution MS/MS data will be uploaded to the Global Natural Products Social Molecular Networking (GNPS), to determine their presence in other samples. We also determined that the actinomycete metabolite piperafizine A has the ability to cross the human blood-brain barrier and cause dopaminergic neuron death within *C. elegans*. We are further working on the identification of other neurodegenerative compounds from rural soils (such as fungal prenylated diketopiperazines), as well as screening a collection of previously established neurodegenerative *Streptomyces* strains from Alabama soils to create an MS/MS library (Watkins et al., 2016). This project seeks to uncover a potential contributor to the incidence of PD within rural communities due to specialized metabolites within the soil.

Keywords: Parkinson's disease, *Streptomyces*, blood-brain barrier, IPSCs, neurodegeneration

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S1.P115 Isolation and bioactivity evaluation of sesquiterpenes from soft corals of the genus *Lemnalia* collected in the Red Sea

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Over the last decades, soft corals have been proven to be a rich source of biologically active compounds, featuring a wide range of chemical structures. Soft corals of the genus *Lemnalia* (Neptheidae) are particularly rich in sesquiterpenes and nor-sesquiterpenes featuring various carbon skeletons, including the nardosinane and neolemnane carbocycles, that have demonstrated a broad spectrum of biological activities, such as cytotoxic, anti-inflammatory and antiviral (MarinLit, 2024; Wu et al., 2018). In the framework of our ongoing research towards the isolation of new bioactive marine metabolites, we investigated the chemistry of two populations of *Lemnalia* collected by SCUBA diving from the coral reefs of Al Jadir and Shi'b Sulaym near Al Lith on the south-west coast of the Kingdom of Saudi Arabia. Exhaustive extraction of the animal tissues with mixtures of CH2Cl2/MeOH afforded the organic residues that were subjected to several chromatographic steps to allow up to now for the isolation of 48 different sesquiterpenes, among which 6 are new natural products. The structures of the isolated compounds were determined on the basis of thorough analysis of 1D- and 2D-NMR and MS data. The metabolites isolated in sufficient amounts were evaluated in vitro in human tumor and non-cancerous cell lines for a number of biological activities, including their cytotoxic, anti-inflammatory, anti-angiogenic, and neuroprotective activities, as well as for their effect on androgen receptor-regulated transcription.

Keywords: marine natural products, *Lemnalia* sp., soft corals, biological activity

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S1.P116 Production of natural products by using plant cell cultures as chemical factories

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Reliable production of plant-based natural products can be challenging. Numerous issues have an impact on supply chains associated with natural harvest. Plant to plant variety in yields and profiles, endangered plant species which are difficult to cultivate, and climate changes are some of the limitations to be considered. In contrast, plant cell culture is known to be an attractive alternative supply solution. Plant cell culture technology is an excellent tool to enrich specific structures out of closely related compounds thus reducing impurities in a significant manner. Furthermore, and superior to synthetic biology approaches, plant cells can produce combinations of molecules thereby allowing matching with extract profiles identified in the whole plant. In fact, it has been shown that the production of secondary metabolites with plant cell fermentation can not only be economically attractive, but at the same time allows the supply of the substance of interest in high quality. Independently from the natural environment a plant cell fermentation process, the supply needs for active compounds can be fulfilled in terms of reliability, consistency, quality and quantity. Examples of successful production of natural products through plant cell culture technology will cover a diversity of secondary metabolites, including thapsigargin, ingenol mebutate, paclitaxel and QS-21. Presenting these case studies will also include confirmation of bioequivalence of plant cell culture-derived QS-21 profile to the product isolated from the bark extract (Lv et al., 2024). Furthermore, we will highlight the use of paclitaxel purified from *Taxus chinensis* cell culture cultivated at the 75000 L scale in pharmaceutical development (Dizerega et al., 2024).

Keywords: plant cell culture, paclitaxel, thapsigargin, ingenol mebutate, QS-21

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S1.P117 Synergistic approaches in natural products discovery: metabolomic, genomic, and bioactivity- guided isolation of new compounds from the cyanobacterium *Phormidium* sp. LEGE 15488

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Cyanobacteria are a diverse group of photosynthetic prokaryotes explored for their potential to produce bioactive compounds with diverse applications. However, discovering natural products in cyanobacteria is challenging due to slow growth rates for biomass production and low secondary metabolites yields. More recently, the development of genomic and metabolomics tools has increased the efficiency of natural products research, and the combination of traditional and new methodologies delivers more successful results. *Phormidium* sp. LEGE 15488 (strain from the Blue Biotechnology and Ecotoxicology Culture Collection, LEGE-CC) was identified as a producer of novel bioactive compounds during a previous cyanobacterial natural products screening, which combined anticancer assays with untargeted metabolomics dereplication (Ferreira et al., 2021). Its genome was sequenced and analysed with antiSMASH, revealing numerous Nonribosomal Peptides/Polyketides biosynthetic gene clusters. Herein, we will present the genome mining and the isolation of novel bioactive metabolites from *Phormidium* sp. LEGE 15488. After large-scale cultivation, the compound isolation was guided by mass spectrometry and bioactivity results. The structure of a novel pseudospumigin- like compound was elucidated by 1D and 2D NMR experiments, along with LC-HRESIMS/MS. At 10 µM this compound reduced 53% of appetite in the zebrafish feeding assay with fluorescent labelled *Paramecia bursaria*, which is an interesting pathway for obesity treatment. This work emphasizes the significance of integrating multiple strategies to discover novel cyanobacterial natural products with potential therapeutic applications.

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Keywords: cyanobacteria, metabolomics, genomics, pseudospumigins, obesity

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S1.P118 Peptide toxins from ribbon worms (*Nemertea*)

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This presentation will cover the field of ribbon worm peptide toxins. The ribbon worms, Nemertea, is a phylum of mostly marine animals, comprising some 1350 species. They vary in size from millimeters to 50 meters; some of them are the top predators/scavengers in their habitats. Ribbon worms secrete toxin-loaded mucus for protection and use an eversible proboscis to hunt prey. The mucus from some species has been investigated chemically. Some contain small molecule toxins (tetrodotoxin, TTX (Asakawa et al. 2003)), while others contain peptide toxins (nemertides a-c, parborlysins 1-7, toxin-A, and neurotoxins B I-IV (Göransson et al. 2019)). The peptide toxins have been attributed two main functions: nemertides a-c and neurotoxins B I-IV exhibit ion channel activity, while toxin-A and parborlysins are cytolytic. With increasing ribbon worm genetic data available in scientific databases (two genomes, and more than 100 transcriptomes), the number of putative peptide toxins and toxin families in ribbon worms is rising rapidly. Here, we present the current status of peptide toxins in ribbon worms.

Keywords: Nemertea, ribbon worm, peptide toxin, marine

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S1.P119 Exploring the pharmaceutical potential of the Antarctic fungus *Epicoccum nigrum* isolated from *Kallymenia antarctica* seaweed

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The Earth's last frontiers, the poles, host extreme climatic conditions, triggering organisms to develop survival strategies, including biosynthesizing unique chemical compounds. Exploring organisms in these regions offers potential for discovering novel bioactive pharmaceutical compounds (Santos et al., 2021). In this context, we studied *Epicoccum nigrum*, an endophytic fungus isolated from the Antarctic seaweed *Kallymenia antarctica*. Our objectives involved the chemical and biological analysis of secondary metabolites produced by *E. nigrum*, including isolation/elucidation of the extract's major constituents, also assessing their biological potential. For this purpose, *E. nigrum* was cultured on parboiled rice and seawater. Ethyl acetate was used for extraction. Fractionation by liquid chromatography yielded nine fractions (A-I). All samples were tested for antifungal, antiparasitic and photoprotective activities. Four compounds were isolated and identified: epicoccarine A (**1**), epicoccarine B (**2**), beauvericin (**3**) and triterpene S19159 (**4**). Fractions E-G showed significant activity against fungal species, as did **1**, **3** and **4**. Fractions C-I and compound **1** exhibited high toxicity against *T. brucei* parasites but also human HEK293 cells, presenting low viability. Fractions F and G also displayed photoprotective potential, absorbing UV radiation with no degradation after UVA exposure. However, they showed phototoxic potential. One compound (pending identification) exhibited broad UV absorption with the advantage of no phototoxicity. Further study will involve co- cultivation with Actinomycetes to explore the potential for producing new compounds when challenged. Preliminary tests suggest that *E. nigrum* produces colorful compounds in proximity to other organisms. In conclusion, this work indicates that *E. nigrum* has potential as a source of diverse natural products with promising bioactivities.

Keywords: marine natural products, *Epicoccum nigrum*, photoprotection, antifungal, antiparasitic

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S1.P120 Antiparasitic activity of the antarctic fungus *Aspergillus unguis* metabolites against *Trypanosoma brucei*

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The Antarctic exhibits extreme climatic conditions prompting organisms to evolve unique survival mechanisms, such as the synthesis of distinct chemical compounds. Investigating polar organisms presents significant opportunities for uncovering innovative bioactive pharmaceutical agents (Teixeira et al., 2019). Considering this, we suggested investigating *Aspergillus unguis*, which was obtained from the Antarctic seaweed *Palmaria decipiens*. The study aimed to conduct chemical and biological analyses of secondary metabolites generated by *A. unguis*, including the identification and characterization of the primary constituents of the extract. Additionally, biological activity against *Trypanosoma brucei* was evaluated. To achieve this, *A. unguis* was cultivated on parboiled rice and seawater. Ethyl acetate was employed for extraction, and subsequent fractionation via liquid chromatography resulted in the isolation of nine fractions (A-I). The full sample collection (4 mg) was tested for antiparasitic activity, and until now, one compound from D fraction was isolated and identified as ergosterol (**1**). According to the results, the crude extract and fractions C, D, E, F and H were promising against *Trypanosoma brucei,* ranging between 97 and 100% inhibition. Furthermore, the crude extract and the C and D fractions retained very high cell viability in HEK293 cells (CC50 µg/mL >10). Fraction D was submitted to GC-MS analyses which demonstrated that ergosterol was the main compound present in the fraction. Given the above, the most promising fractions will be further separated, and the compounds isolated and identified. In summary, this study highlights the potential of *A. unguis* as a source of diverse natural products with promising bioactivities against the parasitic organism *Trypanosoma brucei.*

Keywords: marine natural products, Antarctic, *Aspergillus unguis*, *Trypanosoma brucei*, antiparasitic

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S1.P121 Unlocking nature's pharmacy : *Iris pseudacaros* from Ireland as potential source for antimicrobial and anti-inflammatory compounds

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Iris pseudacorus, commonly known as Yellow Iris or Yellow Flag Iris, holds significant botanical and ethnopharmacological importance, particularly in Irish folklore. This flowering plant species, prevalent in Irish Boglands, is renowned for its diverse phytochemical composition, including flavonoids, isoflavonoids, triterpenes, phenolics, and alkaloids. Various studies have highlighted its pharmacological potential, showcasing activities such as antiviral, antibacterial, and anti-tumor properties. In this study, part of a broader project on unlocking nature's pharmacy from bogland species in Ireland, we focused on profiling *I. pseudacorus*. Through the analysis of 16 dried plant materials (leaves, stems, and roots) using HPTLC, LC-MS, and NMR, we aimed to identify unique compounds and explore their biological activities. HPTLC bioautography assays combine chromatographic separation with in situ biological activity determination, allowing the direct and rapid isolation of active compounds in complex plant extracts. Our investigation led to the isolation of three antimicrobial compounds - syringic acid, luteolin-7-O-glucoside, and liquiritigenin - from *I. pseudacorus* leaf and root extracts, guided by HPTLC bioautography. These compounds exhibited antimicrobial activity against *S. aureus* and were further evaluated for their anti-inflammatory and cytotoxic properties. Overall, our findings contribute to understanding the pharmacological potential of *I. pseudacorus* and underscore the significance of utilizing traditional botanical resources for drug discovery and development.

Keywords: *Iris pseudacorus,* HPTLC bioautography, antimicrobial and anti-inflammatory activity

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S1.P122 Antitrichomonal activity of secondary metabolites of *Alternaria* sp., a fungal endophyte of alpine Edelweiss

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Sexually transmitted infections (STIs) are considered a major global health concern, caused by bacteria, viruses or parasites. (Rowley et al., 2019). Among them, Trichomoniasis, with an estimated annual incidence of 300 million cases worldwide, is one of the most common sexually transmitted infections, caused by the unicellular protozoan parasite *Trichomonas vaginalis.* Resistance of the parasite to the existing therapies has increased the quest for the discovery of novel antitrichomonal substances (Natto et al., 2021). *Alternaria* species exhibit saprophytic, endophytic or pathogenic lifestyles. They have been studied thoroughly due to the production of toxins and unusual secondary metabolites, classified into the groups of nitrogen-containing compounds, quinones, and pyranones, displaying significant pharmacological activities against human pathogens (Lou et al., 2013). This investigation aimed to isolate and identify the bioactive metabolites of *Alternaria* sp., an endophytic fungus isolated from the leaves of alpine edelweiss (*Leontopodium alpinum*), and assessment of their antitrichomonal activities. Bioprospecting of *Alternaria* sp. resulted in purification of two known pyranone derivatives, alternariol (**1**) and alternariol-9-methyl ether (**2**), one unknown pyranone-type derivative (**3**), two new perylene quinone derivatives (**4**) and (**5**) and two unknown diastereomers of tetramic-acid containing metabolites (**6**) & (**7**). The structural characterization was performed using 1D- & 2D-NMR and HRMS, and their absolute configurations were established using electronic circular dichroism (ECD). Pure compounds were evaluated against *T. vaginalis,* all of which illustrated moderate to promising bioactivities. According to the results obtained, *Alternaria* sp. is considered a promising source of unexplored compounds with potential antitrichomonal activity.

Keywords: *Alternaria, endophytes,* metabolomics, structural characterization, *Trichomonas vaginalis*

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S1.P123 Isolation and structural elucidation of bioactive cycloartane glycosides from *Astragalus glycyphyllos*

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Species belonging to the *Astragalus* genus (Fabaceae family) have been used for millennia for treating various diseases, including cancer. Compounds isolated from plants belonging to this genus have been shown to exert significant anticancer activity in vitro and in animal models (Graziani et al., 2019a). Cycloartane saponins isolated from *Astragalus boeticus* have been shown to preferentially inhibit cell growth in colorectal cancer cell models resistant to epidermal growth factor receptor inhibitors (Graziani et al., 2019b). In this study, two further *Astragalus* species, namely *Astragalus hamosus* and *Astragalus glycyphyllos*, were taken into consideration as potential sources of bioactive compounds. To speed-up the discovery process, the plant material was subjected to a preliminary screening using a metabolomics approach. NMR-based profiling showed the presence of cycloartanes only in the *A. glycyphyllos* samples. The plant material of the latter was therefore extracted and separated using chromatographic techniques, to obtain the pure compounds. Structural elucidation of these compounds was carried out by extensive 1D and 2D-NMR analyses. Based on the knowledge acquired through NMR profiling, it was possible to design a custom-made time- and resource-saving isolation procedure, consisting only of a few chromatographic steps. It was, therefore, possible to rapidly isolate and characterize nine cycloartane glycosides, which were identified as cycloastragenol and cyclocanthogenin derivatives. Six of the isolated compounds also contained an unusual oxalyl moiety and, to the best of our knowledge, eight of them were reported for the first time. Ongoing studies are dedicated to the evaluation of the anticancer potential of the compounds.

Keywords: *Astragalus glycyphyllos,* Fabaceae, metabolomics, NMR, cycloartane glycosides

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S1.P124 Screening marine bacterial compounds for adhesion blockers of enteropathogenic *Escherichia coli*

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Due to the rise of antimicrobial resistance and little recent progress in the discovery of new antimicrobials, novel chemical structures for treating bacterial infections are desperately needed. In the past, secondary metabolites of bacteria with larger genomes are more likely to carry biosynthetic gene clusters, and led to the discovery of most antibiotics. Furthermore, many compounds inhibiting virulence have been similarly discovered in screening collections of natural products and microbial metabolites. Therefore, we studied the antibacterial and virulence inhibiting activity of marine actinobacteria gathered on an earlier research cruise around Svalbard, in the Arctic Sea. For the first time, these bacteria were successfully cultivated and grown in liquid media and were then processed by extraction and fractionation using FLASH chromatography. The bioactivity of these fractions was then assessed against the human intestinal pathogen, enteropathogenic *Escherichia coli* (EPEC), utilizing both classic growth inhibition and virulence related assays. We have previously set up an EPEC effector translocation assay and a cell culture-based coinfection model using high-content microscopy. EPEC uses the type three secretion system to inject virulence-causing molecules (effectors), which ultimately lead to abnormal actin condensation, often called actin pedestals, and to the effacement of microvilli from the intestinal epithelium. Inhibiting these processes could effectively inhibit the virulence of the pathogen without largely damaging normal flora, or causing harsh selectional pressure on the pathogen, ultimately leading to antibiotic resistance.According to the screening results, several of the fractions reduced the readouts of these assays in a concentration- dependent manner. Some of these acted by suppressing growth, but some fractions, however, were able to diminish EPEC pathogenicity in our assays without inhibiting growth through as-yet undiscovered mechanisms of action. Work is on-going to determine the composition of the fractions as well as the precise mechanism of action by which these phenotypic changes occur.

Keywords: enteropathogenic *Escherichia coli*, marine actinobacteria

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S1.P125 NMR-based metabolomics approach and anti-proliferative activity of *Olea europaea* products

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Olive (*Olea europaea* L.), belongs to the Oleaceae family, own a significant economic value, as well as nutritional and medicinal properties. Today, 98% of olive are cultivated in Mediterranean area (Antoniou and Hull 2021) and virgin olive oil represent a key food of the Mediterranean diet. However, the value of olive tree extends to its products as olives and leaves. Employed in traditional medicine since ancient times, recent research has highlighted the anticancer properties of olive extracts, attributed to their unique chemical composition rich in specialized metabolites (Scognamiglio 2012). Polyphenols, secoiridoid glycosides, and triterpenoids isolated from olive demonstrate efficacy across various *in vitro* and *in vivo* cancer models. In this way, this work aims to characterize the molecular and functional effects of 21 selected cultivars of *Olea europaea* on three cancer cell lines belonging to a group of "poor prognosis cancers": acute myeloid leukemia (AML), Glioblastoma (GBM) and Pancreatic adenocarcinoma (PA) for which to date there are no evidence in literature. A new screening method combining a NMR-based metabolomic approach (Esposito 2021) with MTT assays was employed to identify olive drupe crude extracts with anti-proliferative properties. The most active extract(s) will be purified to obtain enriched fraction and/or pure compounds for evaluate the involvement of several molecular players in the so-called alternative regulated cell death (RCD) pathways. This presentation will cover analyses of 21 cultivars of olives collected in the experimental agricultural company of Campania Region "Improsta". Dried drupes have been extracted directly with phosphate buffer in D2O and methanol-d4 (1:1) assisted by ultrasound and an aliquot was directly analyzed by Nuclear Magnetic Resonance (NMR). With the same methodology (1.67 g dried drupes), and after hexane extraction and SPE purification, in order obtain enriched in specialized metabolites, have been prepared crude extracts for cell viability test. The results highlighted that Oliviello and Racioppa cultivars are very rich in verbascoside, Grossale Oliva Bianca in oleocanthal, Romanella and Marinese cultivars in DHPEA-EA. Study of modulation of cell proliferation and cell death operated by crude extract of olive showed different effect on three cancer cell lines selected. In conclusion, this study increases the knowledge about *Olea europaea* cultivars diversity in term of metabolite profiling and bioactivity.

Keywords: *Olea europaea,* NMR, metabolomics, cytotoxicity, DNA fragmentation

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S1.P126 Potential nematicidal agents from a bioactive fraction of the fungus *Trichoderma parceramosum*

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Endophytic fungi are a category of fungi that reside inside the tissues of host plants without causing evident damage; this symbiotic relationship can be beneficial for both participants. One of the main advantages for plants is that endophytic fungi provide protection against pathogenic organisms (Selim et al., 2012). For example, fungi can produce substances that inhibit the growth of pathogens or directly compete with them for resources, reducing the incidence of diseases (Rajesh et al., 2016). *Trichoderma* spp. secretes a range of specialized metabolites, many of which have demonstrated a broad-spectrum of biological properties in *in vitro* assays. For example, the koningines, polyketides obtained from *Trichoderma koningii*, in an *in vitro* assay, exhibited strong activity against plant pathogens (Ba-Yi et al., 2015). In the latter years, the potential of *Trichoderma* metabolites against parasitic nematodes has also been highlighted (Moo-Koh et al., 2022). In this context, the focus of this work is the evaluation of unexplored *Trichoderma parceramosum* as a potential source of nematicidal agents. The hydroalcoholic extract of *T. parceramosum* solid culture was extracted first with *n*-hexane and successively with dichloromethane. This latter extract, through chromatographic techniques, afforded eight fractions which were tested for their nematicidal activity against *Meloidogyne incognita,* one of the most damaging pests in vegetable crops worldwide. From more active fractions, phenolic compounds and heterocyclic lactones have been isolated. The structural characterization of the isolated compounds was performed by 1D-NMR and 2D-NMR investigations.

Keywords: endophytic fungi, *Trichoderma parceramosum,* NMR, nematicidal activity, *Meloidogyne incognita*

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S1.P127 Antimicrobial secondary metabolites of *Trochila* sp., a new endophytic fungus of *Lilium carniolicum*

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Endophytes are group of microorganisms, which protect their host plants against various abiotic and biotic stress factors. These microorganisms are underexplored sources of new biologically active natural products against not only phytopathogens, but also against bacteria, fungi, viruses, and protozoans that affect human and animals (Strobel et al., 2003). In a screening campaign for the discovery of antimicrobial natural products against a human pathogen *Trichomonas vaginalis* and a phytopathogen *Botrytis cinerea*, we have identified a fungal hit, *Trochila* sp., an undescribed species isolated from leaves of *Lilium carniolicum*, an endemic *Lilium* species found in the alpine region of Austria. In this study we aimed for dereplication, and identification of secondary metabolites produced by a rice culture of *Trochila* sp., using Feature-Based Molecular Networking of the GNPS platform, as well as SIRIUS for in silico annotations. By inspecting the network clusters and annotations results, we were able to dereplicate and perform targeted isolation of two dibenzofuran derivatives, usnic acid and isousnic acid (**1**-**2**), two unknown and two previously reported pyridine type alkaloids, (**3**-**6**), and a known dibenzoquinone, oosporein (**7**), and annotated potentially new metabolites through analysis of the MN clusters. The structures of the isolated compounds were elucidated using 1D and 2D-NMR spectroscopy and their absolute configurations established utilizing DP4+ probability and circular dichroism calculations. Ultimately, the biological activity of isolates was evaluated against three aforementioned pathogens. Our results shed new light on the metabolite profile of the genus *Trochila* as a promising source of bioactive natural products.

Keywords: *Trochila,* endophytes, metabolomics, structural characterization, *Trichomonas vaginalis*

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S1.P128 Seasonal variation of dimeric flavonoids in ginkgo (*Ginkgo biloba* L.) leaves

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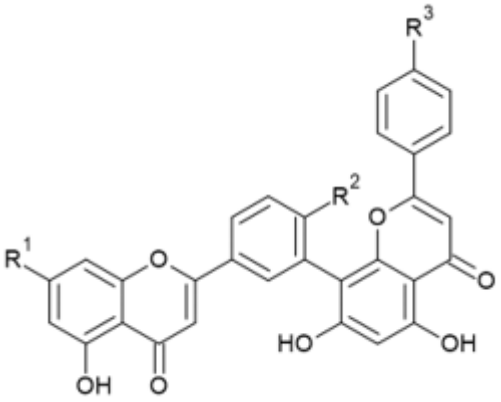
Ginkgo (*Ginkgo biloba* L.) is a well-known medicinal plant that is rich in various bioactive compounds. To date, 110 different flavonoids have been identified in ginkgo, including flavonoid dimers known as biflavonoids. Biflavonoids have recently gained much attention as potential molecules with biological activity such as antiviral and antimicrobial activity as well as effective molecules for the treatment of neurodegenerative and metabolic diseases and anti-cancer molecules. As part of our ongoing project, we developed a method for the separation and quantification of biflavonoids in ginkgo leaves using HPLC-DAD (Kovač Tomas et al., 2023). The biflavonoids present in ginkgo leaves are amentoflavone, bilobetin, isoginkgetin, ginkgetin and sciadopitysin (Fig. 1).



	R¹	R²	R³
amentoflavone	OH	OH	OH
bilobetin	OH	OMe	OH
isoginkgetin	OH	OMe	OMe
ginkgetin	OMe	OMe	OH
sciadopitysin	OMe	OMe	OMe

Fig. 1. Structure of ginkgo bioflavonoids.

During the 2022 season, from May to November, we collected leaves from ginkgo trees every month, 7 stages in total. The leaves were freeze-dried and we analysed the content of individual biflavonoids. In the period from May to November, we observed an increased accumulation of biflavonoids, for most of them by more than 300%, indicating that fall leaves are richer in biflavonoids than green leaves. Only in the case of amentoflavone we did not detect an increase in content. A particularly large increase in biflavonoids occurred between the leaves collected in June and July, which could indicate extensive biosynthesis of biflavonoids during this period. Further investigations will reveal possible factors related to the biosynthesis of biflavonoids.

Keywords: *Ginkgo biloba* L., biflavonoids, seasonal variation

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S1.P129 Efficient production of some bioactive depsides by *Aronia* × *prunifolia* (Rosaceae) microshoot agitated *in vitro* cultures as the effect of precursor feeding strategy

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Aronia × *prunifolia* (Marsh.) Rehd. (Rosaceae) is rich source of phenolic acids (PhAs) - antioxidants important in phytotheraphy and also in phytocosmetology (Szopa et. al., 2017). (PhAs) could be produced in high amounts in plant *in vitro* cultures, also in established by our team microshoot cultures of *A.* × *prunifolia* (Ekiert et al., 2022). The aim of the present study was the testing of precursor feeding strategy on the production of (PhAs) in agitated cultures of *A.* × *prunifolia.* The cultures were maintained in Murashige-Skoog medium (1mg/l BAP and 1mg/l NAA) (Murashige et al., 1962). Precursors of simple (PhAs) - phenylalanine, cinnamic acid, benzoic acid and of depsides - caffeic acid at five concentrations (0.1, 0.5, 1.0, 5.0 and 10.0 mmol/L) were fed into the medium at point "0" (culture initiation) and independently on the 10th day of growth cycle. After the 20th day of growth cycle, the contents of compounds were determined in methanolic extracts of biomasses by HPLC (Ellnain-Wojtaszek et al., 1999). All extracts contained 4 depsides (chlorogenic, noeochlorogenic, rosmarinic and cryptochlorogenic acids) and 4 simple (PhAs) - protocatechuic, vanillic, caffeic and syringic acids. Chlorogenic and neochlorogenic acids, dominated in all extracts (max. 388.39 and 263.54 mg/100g d.w., respectively). The total content of all compounds was the highest after the addition at point "0" of cinnamic acid (5 mmol/l) and caffeic acid (10 mmol/l) which caused a 2.68- and 2.49-fold increase in the contents of (PhAs) - 603.03 and 558.48 mg/100g d.w., respectively. The obtained results documented efficacy of the precursor feeding strategy in enhancing the production of bioactive depsides in microshoot agitated cultures of *A.* × *prunifolia* and have a potential application value.

Keywords: *Aronia* × *prunifolia*, *in vitro* cultures, biogenetic precursors, depsides, HPLC

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S1.P130 Acridone alkaloids and lignans from the leaves of *Luvunga scandens* (Rutaceae)

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Luvunga scandens (Roxb.) Buch. Ham. is a species with a restricted range can be found in tropical rain forests in Vietnam and Thailand. This species is one of just twelve in the world that belong to the genus *Luvunga*, which has a wide range of chemical components such as coumarins, triterpenes, flavonoids, and alkaloids; in addition, exhibiting a variety of biological properties as cytotoxicity, antibacterial, anti-inflammatory, etc (Nguyen et al., 2017); (Nguyen et al., 2021). In the ongoing pursuit of novel phytochemistry and bioeffects chemicals from genus *Luvunga*, we made collections of *L. scandens* from Vietnam. Together with 13 known acridone alkaloids (Fig. 1) and 19 known lignans, the ethanol extract of the leaves delivered a previously unknown acridone alkaloid and 9 new lignans.

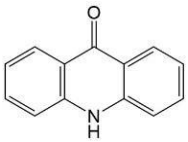

Fig. 1. Structural framework of acridone alkaloid

The structures of isolated compounds were identified by detailed spectroscopic data, including 2D- NMR (COSY, HMQC, HMBC, and NOESY), HRESIMS, X-ray crystallographic analysis, and comparison with reported data (Tran et al., 2019). All new isolates' radical scavenging activity, anti- inflammatory and cytotoxicity activities were evaluated.

Keywords: *Luvunga scandens,* Rutaceae, cridone alkaloid, lignan, cytotoxicity

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S1.P131 *Schisandra henryi* bioreactor grown *in vitro* culture as a valuable raw material – biotechnological, phytochemical and biological studies

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Schisandra henryi C. B. Clarke is endemic to the Yunnan Province in China and is a plant species known in traditional Chinese medicine (Szopa et al., 2019). *In vitro* culture of *S. henryi* microshoot propagation (acc. to Jafernik et al., 2020) in Plantform bioreactors (temporary immersion systems, TIS) was investigated. The phytochemical analyses of dibenzocyclooctadiene lignans, aryltetralin lignans and neolignans were carried out using UHPLC-MS/MS, and HPLC-DAD methods for qualitative and quantitative determinations. The dominant compounds were isolated using CPC chromatography. The highest content of compounds was found in microshoots extracts (max. 543.99 mg/100g DW). The major compound was schisantherin B (390.16 mg/100g DW). The anti- inflammatory activity tests (COX-1, COX-2, sPLA2 and LOX-15 inhibition), showed the strong activity against COX-1 and -2 (for 177 mg/mL inhibition percentage was 76% and 66%, respectively). The studies showed also the high antioxidant power (FRAP, CUPRAC and DPPH assays), and good antiproliferative activities (Jurkat, MCF-7, HT-29 and HeLa lines) of extracts. Antimicrobial studies against gram+ and gram– bacteria and fungi proved especially high activity towards *H. pylori* (MIC and MBC 0.625 mg/mL). To sum up, the bioreactor grown *in vitro* culture of *S. henryi* may be a cost-effective, innovative alternative to the rare naturally grown medicinal-raw material.

Keywords: plant biotechnology, *in vitro* cultures, PlantForm temporary immersion system, dibenzocyclooctadiene lignans, pharmacological activity

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S1.P132 The phenolic profile and antioxidant potential of different types of *Schisandra henryi in vitro* cultures

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Schisandra henryi C. B. Clarke is an endemic species, used as medicinal plant in Asia, but it is practically unknown in European and American countries. The small number of studies on the *S. henryi* has been focused on the phytochemical profiling and biological activity (Szopa et al., 2019). The aim of the study was to investigate the polyphenol profile and to assess the antioxidant power of the extracts from the *in vitro* cultures of *S. henryi*. Under the study the different types of *in vitro* cultures were tested: agar microshoot and callus, microshoot in bioreactor Plantform, agitated microshoot and suspension cultures (Jafernik et al., 2020). The UHPLC-DAD-ESI-MS^3 and HPLC- DAD methods were used for the analyses of extracts. The total content of polyphenols (TPC) was measured using the Folin-Ciocalteu method. The antioxidant potential was assessed using 2,2- diphenyl-1-picrylhydrazine hydrate (DPPH), ferric reducing antioxidant power (FRAP) and ferrous ion chelating (FIC) assays. In the extracts the procyanidins, phenolic acids (neochlorogenic acid, caffeic acid, protocatechuic acid) and catechin were estimated. The highest content of phenolic compounds was found for microshoot agar culture (max. total content: 229.87 mg/100 g DW) and agitated culture (max. total content: 22.82 mg/100 g DW). The max. TPC was equal 1240.51 mg GAE/100g DW (agar microshoot culture). The highest antioxidant potential was indicated for agar microshoot culture (90.08% of inhibition and 59.31 nM/L TEAC, respectively). To sum up, the performed studies are innovative and showed that extracts from *S. henryi* microshoot cultures are the valuable source of polyphenolic compounds, which are characterized by a very strong antioxidant potential.

Keywords: plant biotechnology, *in vitro* cultures, phenolic compounds, antioxidant potential

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Jafernik, K.; Szopa, A.; Barnaś, M.; Dziurka, M.; Ekiert, H. *Schisandra henryi* C. B. Clarke in Vitro Cultures: A Promising Tool for the Production of Lignans and Phenolic Compounds. *Plant Cell Tissue Organ Cult* 2020, 143, 45–60.

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S1.P133 Isolation and characterisation of stigmasterol and β-sitosterol from *Starchytarpheta jamaicensis* (L) Vahl (Verbennaceae) leaves of Eastern Nigeria

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Stachytarpheta. jamaicensis (L.) Vahl (Verbenaceae) is a potent medicinal plant commonly used in treatment of infections in West African (**1**). This study was to isolate and characterize the bioactive constituents from the leaves of *Stachytarpheta jamaicensis.* The powdered leaves were extracted with dichloromethane: methanol (1:1) using cold maceration method. Column chromatography of the crude extract lead to a number of fractions. TLC fingerprinting and the spraying reagent (Concentrated H2SO4 and vanillin in methanol) were used to identify the fraction containing phytosterols. The isolation and purification afforded white crystalline powder which was subjected to physical, chemical and spectral identification by IR, 1H-NMR, 2D-NMR and 13C-NMR. The compound was identified as a mixture of stigmasterol and β-sitosterol (Fig. 1).

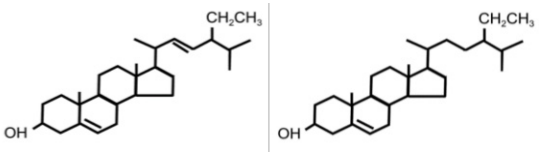

Fig. 1: Structures of stigmasterol and β-sitosterol.

Stachytarpheta jamaicensis contains stigmasterol and β-sistosterol which are responsible for the various therapeutic activities of the plant.

Keywords: *Stachytarpheta jamaicensis*, stigmasterol, β-sistosterol, characterization

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S1.P134 UHPLC-HRMS profiling of sulphated constituents in different plant parts of *Salvadora persica*

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Salvadora persica L. (Salvadoraceae) is traditionally used for oral health. Chewing sticks prepared from its roots and twigs are a widespread traditional oral hygiene device in Middle Eastern, African and Asian countries, and the leaves are used to alleviate gum problems (Khatak et al, 2010; Ahmad and Rajagopal, 2014). The plant is known to contain flavonoids, phenolic acids, volatile oil, alkaloids, and some sulphur-containing compounds (Aumeeruddy et al, 2018). In our previous work, we detected numerous sulphated constituents in *S. persica* leaf extracts (Kobetitsch et al., 2024). This study aimed to systematically profile sulphated constituents in methanolic *S. persica* leaf, twig and root extracts. Analysis was performed by UHPLC-QExactive-MS/MS, and sulphates were tracked on the basis of the MS/MS-fragment *m/z* 96.960 $[HSO4]^-$. Around 60 peaks with sulphate moiety were detected. Almost all of them displayed an additional *m/z* 241.002 $[hexosylsulphate\text{-}H2O]^-$ fragment, indicating glycosylsulphate structures. The profile of the root extract was dominated by the only detected glucosinolate glucotropaeolin, which also occurred in the leaves and twigs at lower levels. 15 peaks were annotated as aromatic (phenyl, benzyl-, phenethyl-) glycosylsulphates; two among them that occurred in all three extracts were annotated as isomers of the known benzylglycosylsulphate salvadoside (Kamel et al., 1992). 24 peaks that displayed 19 C-atoms in their molecular formula were annotated as megastimane glycosylsulphates, detected for the first time in our previous work (Kobetitsch et al., 2024). They displayed highest levels and variety in leaves, followed by stems and roots. Their isolation and structural elucidation are under progress.

Keywords: *Salvadora persica*, UHPLC-HRMS, secondary metabolites

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S1.P135 Koninginins DS A-C, novel polyketides from *Trichoderma koningii*, a soil fungus collected from medicinal herbal garden

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Most of sources of foods, health supplements and natural medicines consumed by humans are cultivated in the soil. So, beneath the ground, soil fungi may influence bioactive metabolites (or their biosynthesis pathway) of medicinal plants or microorganisms through their interactions. Based on this assumption and curiosity, we collected soil samples from herbs garden in Duksung Women's University where various medicinal plants having used for Korean traditional therapeutics grow every year. Totally, 10 fungi were isolated from the soil sample of herb garden, which are expected to produce bioactive compounds with novel scaffolds. Among these, the first strain (DS1-1) was identified as *Trichoderma koningii*. *Trichoderma* is one of the most common genera of soil fungi including various species known for their biological effects such as anti-cancer, anti-microbial and biological control agents (Cutler et al., 1989). Among this genus, *T. koningii* has been known as rich sources of koninginins (polyketides) and peptaibols, which have been reported various biological activities (Lang et al., 2015). As part of our continuing study for the discovery of soil fungi-derived novel bioactive molecules, three new koninginins, koninginins DS A-C (**1**-**3**), together with 6 known koninginin derivatives (**4**-**9**) and two known peptaibols (**10**-**11**), were isolated from the EtOAc extract of *T. koningii* fermented on oat-based cereal, Cheerios^{Ò} (Fig. 1). Their relative structures were determined by *J* value and chemical shifts comparing with previously reported known molecules, and biosynthetic consideration. The elucidation of the absolute chemistry is remained, so we will establish by comparison experimental ECD spectrum with calculated one.

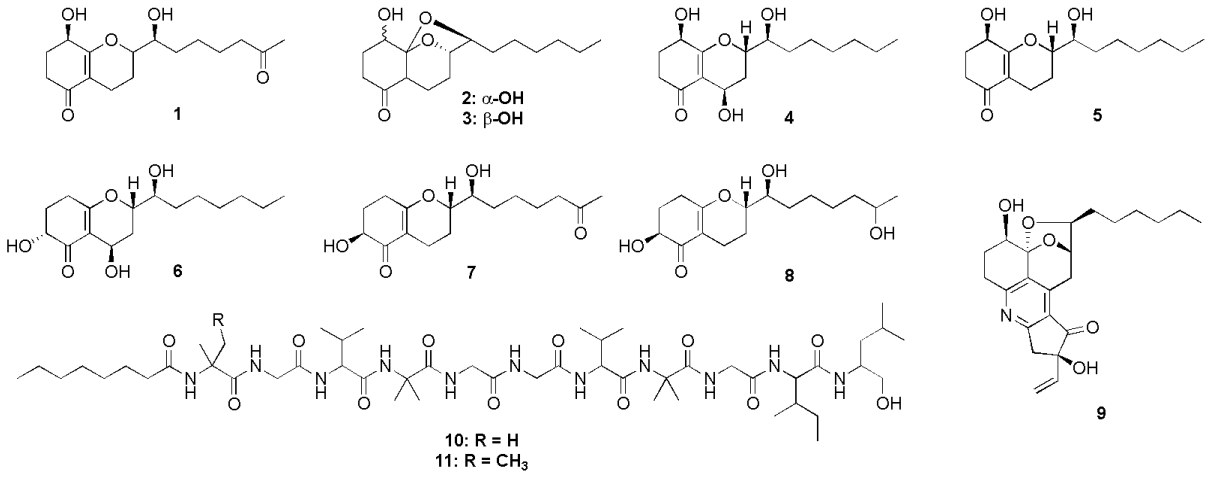

Fig. 1. Structures of isolated metabolites **1**-**11** from *T. koningii*

Keywords: soil fungi, *Trichoderma*, *T. koningii*, koninginins, peptaibols

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S1.P136 Anti-parkinson pyridone alkaloids from an endolichenic fungus, *Thielavia sp.* CNC14

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4-Hydroxy-2-pyridone alklaoids have attracted much attention in the scientific community due to their distinctive structures as well as diverse biological effects. In an effort to discover this class of compounds from fungi, endolichenic fungal extracts were screened by analysis of LC-UV-MS profiles, which afforded a strain, *Thielavia sp.* CNC14 with characteristic UV pattern for 4-hydroxy- 2-pyridone alkaloids using our in-house library. This result prompted us to culture the strain in large scale, followed by several steps of chromatographic methods, which led to the isolation of four new (**1**–**4**) and ten known (**5**–**14**) compounds. The structures of the isolated compounds were elucidated by spectroscopic methods such as NMR and MS together with ECD calculation. Stereochemistry of the new compounds (**1**–**4**) were established using ROESY and comparison of their electronic circular dichroism spectra with those of calculated data. Interestingly, compounds **1** and **2** were cyclized in opposite direction to that of tolypyridone A (**5**) and predicted to be biosynthesized from reduced tolypyridone C (**7**) through hetero-Diels-Alder reaction. Moreover, compounds **4** and **5** had reduction and dehydration in comparison to compound **7**. Among the isolated compounds, **4** significantly protected neuronal cells against treatment with MPP^+, a Parkinsonian neurotoxin, in an *in vitro* Parkinson's disease model.

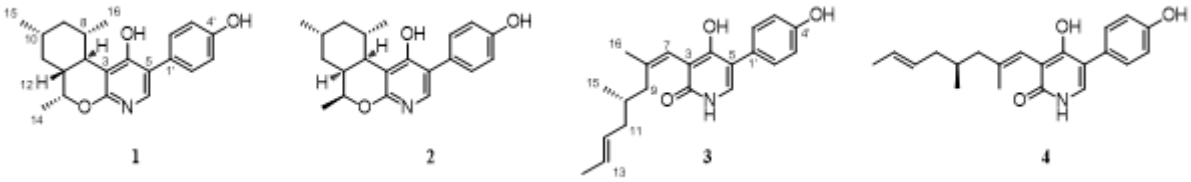

Fig. 1. Structures of isolated new compounds **1**–**4**

Keywords: endolichenic fungi, 4-hydroxy-2-pyridone alkaloid, anti-parkinson

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S1.P137 The anticancer activity and DNA synthesis inhibition potential of *Teucrium sandrasicum* and Teucrium divaricatum subsp. graceum

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This presentation will cover the evaluation of the cytotoxic activities of different extracts (methanol, ethanol, water and n-hexane) prepared from *Teucrium sandrasicum* O.Schwarz and *Teucrium divaricatum* subsp. *graceum* (Celak.) Bornm. *T. sandrasicum*, one of the species belonging to the genus *Teucrium*, is a species endemic to Turkey. *T. sandrasicum*, a perennial plant that grows in the serpentine regions of Muğla Sandras Mountain, is used among the people in our country as a diuretic, diaphoretic, tonic, antipyretic, antidiabetic, antispasmodic and cholagogic (Aksoy-Sagirli et al., 2015). *T. divaricatum* subsp. *graecum*. is distributed on rocky, stony, gravelly, calcareous and arid slopes. It is popularly known as 'Böceotu" in Turkey (Guner et al., 2012). *Teucrium* is used for the treatment of coughs, abdominal pain complaints, diseases of the urinary tract, gallbladder, and kidneys, for fevers, colds and stomach disorders, as well as for an external cicatrisant (Formisano et al., 2010; Asghari et al., 2020). There is a lot of interest in the discovery and development of herbal raw materials that can efficiently cause apoptosis and have cytotoxicity in tumor cells. In previous studies, extracts and compounds of different species within the genus *Teucrium* have been evaluated for cytotoxicity and anticancer activity in various cell lines (Stankovic et al., 2011). The cytotoxic effects of different extracts of the plants were evaluated MTT assay against different cell lines including C6, A549, MCF-7, and NIH3T3. Methanol extract of air-dried aerial parts of *T. divaricatum* subsp. *graceum* (TDM) exhibited significant cytotoxicity (IC50=17.09 µg/mL) against MCF-7 cell line. BrdU (5-bromo-20-deoxy-uridine) cell proliferation method was applied to analyze the effects of this extract on the proliferation of MCF-7 cells. Three concentrations, IC50/2, IC50, and 2xIC50, were studied. It was determined that the percentage of DNA synthesis inhibition of TDM extract increased depending on the concentration and incubation time.

Keywords: *Teucrium,* cytotoxicity, DNA synthesis inhibition, Lamiaceae

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S1.P138 Kronopolitides A-D, a new family of 36-membered polyol macrolides with antiparasitic activity

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The neglected tropical diseases Leishmaniasis and American Trypanosomiasis (Chagas disease) caused by the parasites *Leishmania* spp. and *Trypanosoma cruzi*, respectively, cause thousands of deaths worldwide, and in recent years they have also emerged as a health concern in developed countries. New therapeutic solutions are required due to increasing resistance and adverse effects of existing treatments (No, 2016; Pérez-Molina et al., 2018). Natural products (NPs) have been historically a rich reservoir of new bioactive compounds, originating new drugs used today in clinical practice (Newman et al., 2020). With the aim of discovering new NP scaffolds with novel mechanism of action (MoA) against *Leishmania* and *T. cruzi*, 40K representative microbial extracts from MEDINA's NP collection have been tested. The acetone extract of a strain identified by 16S rRNA as *Streptomyces kronopolitis* (CA-143054) displayed activity against *T. cruzi*. Bioassay-guided isolation using SP207ss resin column and preparative reversed-phased HPLC led to the isolation of four novel polyketide macrolides, kronopolitides A-D. The planar structures of the new family of 36- membered polyol macrolides were determined using a combination of 1D and 2D NMR data, HRMS, and ESI-qTOF-MS/MS. The absolute configuration of their chiral centers was established using NOE, *J*-based configuration analysis, comparison with NMR data of structurally related macrolides, and the bioinformatic analysis of the biosynthetic gene cluster. The antiparasitic activity, measured as the number of host cells infected with parasite in their cytoplasmatic region, displayed IC50 values ranging from 0.16-1.27 µM for *T. cruzi*, being kronopolitide D the most potent compound. To characterize their MoA, the new macrolides will be profiled in an innovative high content imaging screen parasite painting assay.

Keywords: *Streptomyces kronopolitis*, *Trypanosoma cruzi*, *Leishmania*, polyketide macrolides, kronopolitides

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S1.P139 A correlative metabologenomics approach for the elucidation of the biosynthetic pathway of 3-(3-Furyl)alanine-containing endolides produced in *Stachylidium bicolor*

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The marine sponge-derived fungus *Stachylidium bicolor* 293 K04 is a prolific producer of bioactive natural products, particularly the rare 3-(3-furyl)-alanine containing cyclic tetrapeptides known as endolides. Leveraging molecular networking integrated with targeted MassQL queries we've recently unveiled the intricate amino acid makeup of numerous endolides, hinting at their biosynthesis through a combinatorial process (Berger et al., 2024). The production of 3-(3-furyl)-alanine moieties in rhizonin A was recently proven to originate from oxidative cleavage and rearrangement of tyrosine in the endofungal symbiont *Mycetohabitans endofungorum*, catalyzed by a dioxygenase (RhzB, Ehinger et al., 2023). This revelation raises intriguing questions about the potential bacterial contribution to endolide production, especially considering reports of *Burkholderia contaminans* within *S. bicolor* (Almeida et al., 2018). Despite our efforts, confirming this hypothesis has proven elusive. On the contrary, after sequencing and assembly of the genome of *Stachylidium bicolor* 293 K04 we found the putative biosynthetic gene cluster for endolide production. Furthermore, while feeding experiments with the fungus were generally successful (incorporation of ^{13}C-labeled phenylalanine, and leucine in respective endolides) in our case 3-(3-furyl)-alanine did not show ^{13}C- enrichment in LC-MS experiments after feeding ^{13}C-labeled-tyrosine, suggesting an alternative biosynthesis. Feeding experiments with [U-^{13}C] glycerol, however, point to a biosynthesis of the unusual amino acid via the shikimate pathway and a putatively new cyclic precursor intermediate (El Maddah et al., 2016) Through a correlative metabologenomics approach, we're tirelessly working to unravel the enigma surrounding the origin of endolides—whether they stem from fungal or bacterial sources.

Keywords: *Stachylidium bicolor,* cyclic tetrapeptide, 3-(3-furyl)alanine, biosynthesis

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S1.P140 Discovery and structure elucidation of novel antagonistic cyclic lipopeptides for the control of fungal agricultural pathogens

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Novel antifungal cyclic lipopeptides (CLPs) produced by a *Pseudomonas tensinigenes* strain have been found to be related to Amphisin (**1**), and its family of CLPs (Gotze, S. et al., 2017) with applications as biopesticides. While pest and pathogen management products help ensure crop quality and yield in conventional farming, the widespread over-use of synthetic pesticides have been found to have many detrimental non-target effects. These include decreasing soil fertility, contaminating water systems and triggering the development of fungicide-resistant pathogen strains. Biopesticides, which consist of microorganisms and their natural products (NPs) are a less harmful alternative to synthetic pesticides as the NPs already exist in the environment with developed degradation pathways, eliminating the environmental concerns associated with synthetic products. A microbial biopesticide screening study of using thin-layer chromatography direct bioautography (TLC-DB) assays resulted in the discovery and subsequent structure elucidation of **2**, and **3.** Work involving the discovery and structure elucidation of these compounds by TLC-DB, mass spectrometry and nuclear magnetic resonance will be discussed.

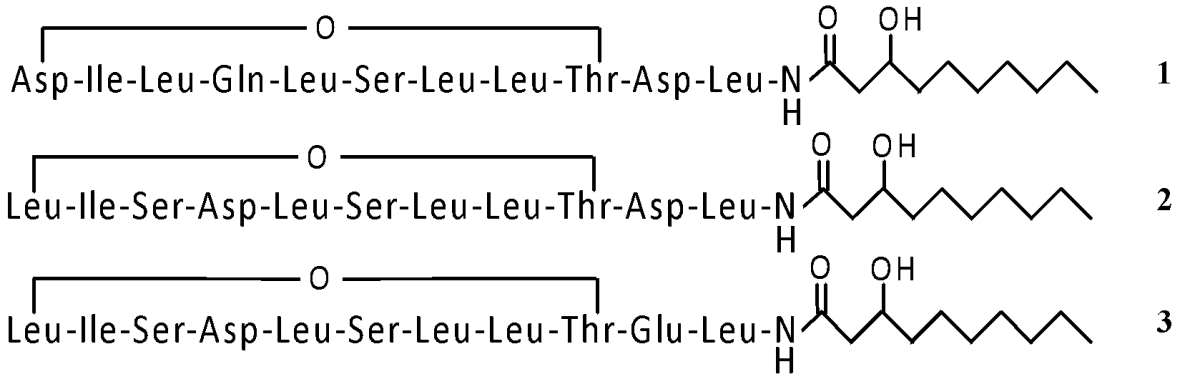

Figure 1. The proposed structures of two novel biopesticide candidates, **1**, and **2**, elucidated by nuclear magnetic resonance and mass spectrometry.

Keywords: Cyclic lipopeptides, biopesticides, NMR, Mass spectrometry, structure elucidation.

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S1.P141 Hydrogen sulfide is a key mediator in the antioxidant effects of osthole on mouse corpus cavernosum and liver

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Osthole is a major bioactive coumarin found in *Cnidium monnieri* and is also isolated from *Prangos* species traditionally used as aphrodisiacs in Türkiye(Albayrak et al., 2023). Erectile dysfunction(ED), affecting 52% of men, is exacerbated by risk factors like diabetes and hypertension, which cause endothelial dysfunction by increasing oxidative stress(Agarwal et al., 2006). Given the ineffectiveness of PDE-5 inhibitors in cases of endothelial dysfunction, there is a need for treatments independent of the endothelium. Hydrogen sulfide(H2S), produced from L-cysteine mainly in the liver, enhances erectile function endothelium-independently. Osthole demonstrates antioxidant effects by increasing antioxidants(catalase, etc.) and activating the Keap1/Nrf2 pathway, similar to H2S mechanisms. Therefore, we aimed to investigate the osthole-H2S relationship for the first time. We examined the effects of osthole(30μM,30min) on H2S production by measuring real-time H2S synthesis using an H2S microsensor in the presence/absence of pyrogallol(0.1mM,5min)-induced oxidative stress in mouse corpus cavernosum(MCC) and liver(ML). Additionally, we evaluated the role of H2S in the antioxidant effects of osthole by measuring reactive oxygen species(ROS) using chemiluminescence assays(luminol/lucigenin) in MCC/ML. Osthole increased L-cysteine-induced H2S synthesis in healthy MCC/ML(P<0.01,ANOVA,n=6) and inhibited the pyrogallol-induced reduction in H2S production in MCC/ML(P<0.05,ANOVA, n=6). Osthole decreased the pyrogallol-induced increase in ROS levels(P<0.01,ANOVA,n=6), these effects were inhibited by the H2S synthesis inhibitor aminooxyacetic acid(P<0.05,ANOVA,n=6). These results indicate that osthole protects MCC/ML from oxidative stress by increasing H2S synthesis. Osthole could be an effective drug candidate, especially in oxidative-stress-induced endothelial dysfunction-related ED and liver diseases.

This project is supported by the Ege University Office of Scientific Research Projects(Grant number:23603).

Keywords: oxidative stress, hydrogen sulfide, erectile function, corpus cavernosum, liver

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S1.P142 Isoimperatorin induces relaxation in mice corpus cavernosum via increasing hydrogen sulfide (H2S) synthesis

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Prangos sp. roots have been used traditionally as aphrodisiac in Anatolia. Hydrogen sulfide(H2S), as a novel multi-targeted gasotransmitter with antioxidant, anti-inflammatory, and vasorelaxant effects, plays a regulatory role in erectile function(Srilatha et al., 2007). Previously, we showed that chloroform extracts of *Prangos heyniae* roots (PHC) induced relaxation on mice corpus cavernosum(MCC) through H2S(Alan et al., 2022). However, the active compound responsible for the relaxant effect of *P. heyniae* remained ambiguous. Thus, we aimed to investigate whether the major compound has a relaxing effect on MCC and the role of the H2S mechanism in its effects. Previously, we determined isoimperatorin(ISO), a furanocoumarin, as the major compound in PHC by HPLC(Arzuk et al., 2023) and then the compound was isolated and identified in another study using column chromatography, NMR, and LC-MS(Albayrak et al., 2023). The relaxant effect of ISO was tested in MCC strips by using a myograph in the presence/absence of H2S synthesis inhibitor aminooxyaceticacid(AOAA,10^{-2}M). Furthermore, time-dependent H_2S formation was measured in L-cysteine(L-cyst,10mM)-stimulated conditions in the presence/absence of AOAA in MCC homogenates by H2S sensor. ISO (10^{-7}-10^{-4}g/mL) caused relaxation in MCC(P<0.001,Anova,n=4-6). AOAA inhibited ISO-induced relaxations(P<0.001,Anova,n=6-7). ISO significantly increased L- cyst-stimulated H_2S formation in the MCC homogenates(P<0.01,Anova,n=6-8) and this augmentation was significantly inhibited by AOAA(P<0.001,Anova,n=4-5). Our study demonstrated that ISO induces relaxation on MCC through H2S pathway for the first time. As a major compound of PHC, ISO could be responsible for the relaxant effect of this plant. ISO could be a potential drug candidate as an H2S inducer for the treatment of ED.

Keywords: *Prangos heyniae*, isoimperatorin, erectile function, hydrogen sulfide

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S1.P143 Purple willow (*Salix purpurea* L.) varieties as a source of valuable herbal raw material with a high content of salicylic glycosides

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Purple willow (*Salix purpurea* L.) bark is considered as valuable herbal raw material, which is characterized by analgesic, antipyretic, anti-inflammatory, antirheumatic, antibacterial and antimicrobial properties. The medicinal properties arise from the unique composition of therapeutic substances, such as salicylic glycosides (SG), flavonoids, phenolic acids, and tannins (Wichtl 2004, Sulima and Przyborowski 2019). Currently, the bark used in the production of natural aspirin, referred to medicinal products manufactured from willow bark, is primarily sourced from various randomly growing forms in highly diverse and uncontrolled conditions. This results in the material's heterogeneity and contamination. Therefore, field cultivation of selected willow varieties under strictly controlled conditions appears to be a simple and effective solution to the above problem (Bubner et al. 2018, Sulima et al. 2021). In this study we conducted a qualitative and quantitative chromatographic analysis of three registered varieties of purple willow (Aspi, Aspira, Cortexa) with the use of UHPLC methods. In the plant material, approximately 40 different polyphenolic compounds were detected, of which six were identified as SG. The presence of salicin and salicortin was confirmed through parallel analyses using standard substance testing. Salireposide, tremulacin, and disalicylortin 1 and 2 were identified based on the comparison of their spectral data. The obtained results showed the high potential of all studied varieties of *S. purpurea*. A high concentration of SG was observed in the bark of ASPIRA (145.47 ± 4.18 mg g^{-1}), ASPI (133.22 ± 4.41 mg g^{-1}) and CORTEXA (104.93 ± 1.69 mg g^{-1}). In this study, the content of SG in leaves, wood and young shoots was also examined. The results indicated the possibility of using whole, several months old shoots as a herbal raw material of *S. purpurea*. This is a completely novel proposal that provides an opportunity for easier acquisition of larger quantities of herbal raw material from purple willow varieties cultivated on arable lands.

Keywords: *Salix purpurea,* herbal raw material, salicylic glycosides

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S1.P144 Exploring myxobacteria for bioactive compounds against malaria and neglected tropical diseases

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Tropical parasitic diseases such as malaria, human African trypanosomiasis, Chagas disease, and leishmaniasis are major health hazards affecting more than a billion people worldwide, mainly in developing countries. Currently available drugs are problematic given their limited efficacy, serious adverse effects and emergence of drug resistance. Thus, there is an urgent need for new antiparasitic drugs. Natural products, particularly those deriving from microbial origins, still play a dominant role in lead discovery and development against neglected tropical diseases. In this work, we aimed to systemically identify and isolate new scaffolds of myxobacteria secondary metabolites that could serve as potential leads for antiparasitic drug development. A subset of our library of extracts and pure compounds driven from myxobacteria was screened for *in vitro* antiparasitic activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi, Leishmania donovani,* and *Plasmodium falciparum*. Here we communicate the results of using different approaches that combine bioactivity- and structure-guided isolation as well as metabolome mining for the characterization of bioactive molecules. Also, we report the remarkable antiprotozoal activity of some myxobacterial compounds that are described for the first time.

Keywords: myxobacteria, trypanosomatids, malaria, bioactivity-guided isolation

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S1.P145 Dereplication of hidden saponins in bioactive extracts

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Saponins have been highlighted for their biological properties, including anticancer, antibacterial, antifungal, or anti-inflammatory. They are formed by a complex two-part structure, a hydrophobic core named aglycone and a hydrophilic moiety constituted by a sugar chain linked together by a *O*- glycosidic bond (Durán et al., 2021). Nevertheless, the purification of these compounds is still a challenge due that they appear as complex mixtures with similar structurally related forms. Thereby, a dereplication strategies by combination of NMR spectra with UPLC-QTOF/MS^{E} and HMAI method it is proposed and it allowed the identification of the main saponins of *Agave* enriched fractions (Simonet et al., 2021). This strategy has allowed the dereplication of four fractions of *Agave* plants to led the identification of a total of 26 saponins, 14 of which have already been described and 12 of which are proposed as new saponins. Some of this saponins have structural isomers in their aglycone as well as in their sugar chain and these differences, not observed by UPLC/MS analysis, could be identified by NMR data.

Keywords: steroidal saponins, *Agave*, dereplication, HMAI

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S1.P146 Understanding the chemistry of the genus *Amylostereum* in its multilateral symbioses

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The genus *Amylostereum*, with many species tragically known as forest pathogens, especially in the southern hemisphere, live in multilateral symbioses with their corresponding, exclusive siricid woodwasps infesting coniferous trees causing white rot and subsequent pine tree diebacks (Slippers et al., 2012).

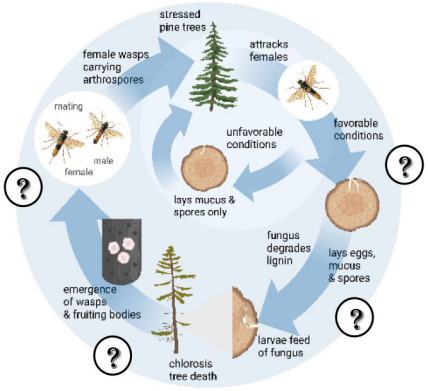

Fig 1. *Amylostereum* and its symbiotic lifestyle (created with biorender.com; based on Ryan & Hurley, in: Slippers et al. 2012).

To unravel the chemistry of this multilateral symbiosis we deployed, among other, an ecomimetic cultivation and OSMAC (one strain – many compounds) approach to activate silent gene clusters in multiple species of the genus *Amylostereum*, combined with untargeted metabolomics. The effect of isolated secondary metabolites on corresponding woodwasp parasites was investigated. Results showed a variety of decorated (nor-)sesquiterpenoids and lanostane-type triterpenoids in *A. chailletii*, *A. areolatum* and *A. laevigatum*, hinting to a closer relation to species of the Echinodontiaceae.

Keywords: *Amylostereum,* siricid woodwasp, sesquiterpenoids, white rot

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S1.P147 *In vitro* evaluation of proliferative activity of Turkish endemic *Prangos hulusii* S.G. Şenol, H. Yıldırım & Ö. Seçmen and its major compounds

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Prangos sp. roots have been used as an external wound-healing agent in Anatolian traditional medicine(Kaval et al., 2014). Therefore, we obtained *n*-hexane(PH), chloroform(PC), *n*-butanol(PB), aqueous fractions(PW), and total extract(80% ethanol,PT) of the *P. hulusii* roots. We isolated and identified oxypeucedanin, isoimperatorin, and osthole as three major compounds using chromatographic(column chromatography, HPLC) and spectroscopic(^{1}H-NMR, LC-MS) methods.

We evaluated the potential wound-healing effects of these extract/fractions(50 and 100 µg/mL,24 and 48h.) and three major compounds(50 and 100 µM,24 and 48h.) on CCD-34Lu cells through cell proliferation assay(MTT). The activity results are shown in Table 1.

Table 1. The proliferative effects of *P. hulusii* root extracts and its major compounds

Extracts	50 µg/mL(Final%)	100 µg/mL(Final%)
n-hexane	218±8%*	17±4%*
Chloroform	198±5%*	141±24%*
n-butanol	115±7%	118±3%
Aqueous	119±2%	115±4%
Total	147±14%*	177±24%*
Compounds	**50 µM(Final%)**	**100 µM(Final%)**
Oxypeucedanin	117±6%	114±8%
Isoimperatorin	106±11%	111±6%
Osthole	126±20%	164±31%*
Control(DMSO,0.5%)	100±8%	
Doxorubicin(20 µg/mL)	76±7%*	

CCD-34Lu cell proliferation activities of tested materials after 24 h of incubation.
Values are the mean±SD from three independent experiments (n:3).

(*) significantly different($p<0.05$) than the solvent control(0.5%DMSO).

n-hexane, chloroform fractions, and total extract showed higher proliferative effects than other fractions. *n*-hexane fraction demonstrated a hormetic effect, evidenced by an increase(218%) in proliferation(50 µg/mL) and a decrease(17%) in proliferation(100 µg/mL). Osthole showed the highest proliferative activity among the tested compounds. These results suggest that apolar fractions of *P. hulusii* roots and osthole have the potential to be used as wound healing agents.

Keywords: *Prangos hulusii,* Apiaceae, cell proliferation, MTT, furanocoumarins

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S1.P148 Spectral data mining of museum-sourced soft corals collected in the 19th century for natural product discovery

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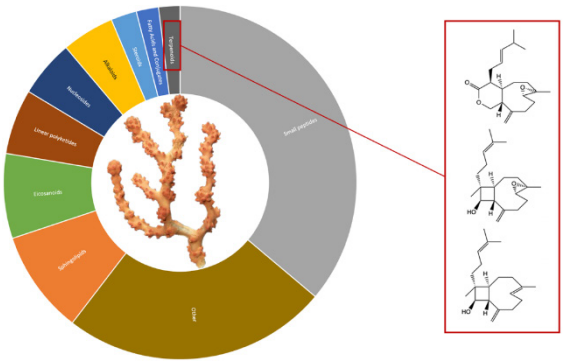
Conventional marine natural product workflows rely on collection trips to the sea for the retrieval of sponges, corals, and other invertebrates. However, an alternative source of samples was newly realized: museums of natural history. Within museums are holdings of tens of thousands of invertebrates preserved in ethanol – valuable extracts for natural product chemists. In this study, museum-sourced marine invertebrate extracts were subjected to high-resolution accurate mass (HRAM) mass spectrometry, NMR, and spectral data mining techniques to profile the metabolome of the preservative extracts, providing a novel, targeted pipeline for the discovery of new natural products from an unconventional source. This newly established spectral data mining workflow is a combination of selective NMR experiments, HRAM mass spectrometry, and SIRIUS (MS^2 annotation tool). This method allowed for 'turnkey chemical profiling', by which the compound class composition of marine invertebrates is described from the crude extract (**Figure 1**), allowing for streamlined isolations of natural products through targeting both reported and unreported ion masses belonging to compound classes of interest.



Paragorgia arborea extracts, originally collected from the 1800's and supplied by the Smithsonian's National Museum of Natural History, were among the various invertebrates subjected to this described workflow. The presence of xenicane diterpenoids was detected by SIRIUS via *de novo* predictions based on fragmentation pattern and binary molecular fingerprinting, leading to the isolation and purification of unreported natural products. This technique demonstrates the utility of museum-sourced samples as well as the benefit of spectral data mining of crude extracts for targeted natural product discovery.

Keywords: marine natural products, data mining, mass spectrometry

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S1.P149 Influence of flavonoid 3',8" dimerization on antioxidant, neuroprotective, anti- hyperpigmentation and anti-diabetic activities: comparative *in vitro* analysis of acacetin and isoginkgetin

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Flavonoids are the most extensively researched group of specialized plant metabolites that play a crucial role in various plant-environment interactions, with their biological activity greatly influenced by their molecular structure. They may occur as dimers, known as biflavonoids. Although over 600 biflavonoids have been described so far, their function and biosynthetic pathways in plants are not fully understood (He et al., 2021). As well it is unknown how dimerization influences the biological activity of flavonoids. We conducted a direct comparison of the biological activities of acacetin and its 3',8" dimer, isoginkgetin (Fig. 1). We assessed their radical scavenging activity utilizing DPPH, as well as their inhibitory effects on the enzymes acetylcholinesterase, tyrosinase, α-amylase, and α- glucosidase.

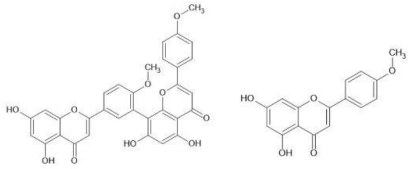

Fig. 1. Chemical structure of a) isoginkgetin and b) acacetin.

Both compounds exhibited weak antioxidant activity when measured by DPPH, up to a concentration of 1 mg/mL. Notably, isoginkgetin demonstrated significantly stronger inhibition of acetylcholinesterase compared to acacetin, registering at 41.99 ± 3.68% and 19.86 ± 0.78%, respectively, at a concentration of 100 μM. Additionally, the dimer isoginkgetin displayed nearly double the inhibition potency against α-amylase and α-glucosidase compared to acacetin, suggesting potential antidiabetic properties. However, acacetin exhibited increased tyrosinase inhibition activity compared to the dimer isoginkgetin. Our findings suggest that 3',8"-dimerization could potentially enhance the neuroprotective properties and antidiabetic activities of flavonoids. Conversely, monomers may be better suited for addressing hyperpigmentation disorders.

Keywords: acacetin, 3',8"-biflavones, isoginkgetin

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S1.P150 Development of quality criteria for *Vitex pinnata* Leaf as an herbal medicine

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Vitex pinnata L. a shrub tree from the Lamiaceae family, is widely used in traditional medicine in Asian countries and Western Europe (Kamal *et al*., 2022), with its leaf used to treat hypertension, fever, and gastrointestinal diseases (Islam *et al*., 2024). The present study aims to establish the standard parameters for proper identification and quality control of *V. pinnata* dried leaf through botanical (macroscopic and microscopic) identification and phytochemical (TLC, colorimetry, and UV-visible spectroscopy) assessments of the leading chemical classes of secondary metabolites. Macroscopically, dried leaves showed pale grey to yellowish color, 3/5 foliate with acuminate apex and ovate/ elliptic shape, leaflet with 6-16 cm long and 2-8 cm wide, petiolule 2-3 mm long and petiole 3-10 cm long. Microscopically, the leaf exhibited the presence of capitate glandular trichomes, non-glandular trichomes, spheroidal trichomes, and thick and prominent midribs. Stomata were found to be of anomocytic type and densely distributed. Chemical characterization revealed the presence of phenolic compounds (phenolic acids, flavonoids, condensed and hydrolysable tannins) and terpenoids (iridoids) among the main classes of secondary metabolites of the leaf 70% hydroethanolic extract. Total phenol and flavonoid contents were calculated as 206.5±14.2 mg gallic acid equivalent/g dried extract and 92.2±5.2 mg catechin equivalent/g dried extract, respectively, and luteolin derivatives were detected among the main marker compounds. The data obtained will contribute to establishing botanical and chemical quality criteria for identifying and authenticating *V. pinnata* leaf as a possible herbal substance.

Keywords: *Vitex pinnata*, leaf, herbal medicine, botanical identification, chemical characterization

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S1.P151 Monographic characterization of *Vitex peduncularis* Leaf as an Herbal Medicine

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Vitex peduncularis Wall. ex Schauer, a deciduous shrub, typically reaches heights of 6-12 meters and is noted for its slender branches and aromatic foliage. This species has been recognized for its medicinal properties, particularly in treating joint pain, diabetes, fever, and skin disorders, as emphasized in Islam et al.'s 2024 study. The current research focuses on establishing monographic parameters for accurately identifying and quantifying the dried leaf of *V. peduncularis*. This involves botanical (macroscopic and microscopic) identification and phytochemical evaluation (including Thin Layer Chromatography, Colorimetry, and UV-visible spectroscopy) of the principal chemical classes of secondary metabolites. The dried leaf is macroscopically characterized by its green to yellowish color, being 3-foliate with an acute apex, and a lanceolate/elliptic shape. Each leaflet measures 5-20 cm in length and 2-5 cm in width, with a petiolule 1-2 cm long and a petiole extending 3-11 cm. Microscopically, the leaf demonstrates the presence of capitate glandular trichomes and non-glandular trichomes. The stomata are of the anomocytic type and are densely distributed. Chemical profiling of the leaf's 70% hydroethanolic extract revealed the presence of flavonoids, condensed tannins, and terpenoids (notably iridoids) as the main classes of secondary metabolites. The total phenolic and flavonoid contents were quantified as 90.9 ± 4.7 mg of gallic acid equivalent per gram of dried extract and 42.9 ± 1.4 mg of catechin equivalent per gram of dried extract. Additionally, apigenin derivatives were identified among the main marker compounds. This study contributes valuable data towards establishing botanical and chemical quality standards for the authentication of *V. peduncularis* leaf as herbal medicine.

Keywords: *Vitex peduncularis*, leaf, herbal medicine, botanical identification, chemical characterization

Reference:

Islam, Z., Caldeira, G.I., Caniça, M., Islam, N., Silva, O., 2024. *Vitex Genus* as a Source of Antimicrobial Agents. Plants 13, 401.

Kamal, N., Mio Asni, N.S., Rozlan, I.N.A., Mohd Azmi, M.A.H., Mazlan, N.W., Mediani, A., Baharum, S.N., Latip, J., Assaw, S., Edrada-Ebel, R.A., 2022. Traditional Medicinal Uses, Phytochemistry, Biological Properties, and Health Applications of *Vitex* sp. Plants (Basel) 11, 1944.

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S1.P152 Identification of ergot alkaloids and lysergic acid derivatives in Kykeon, a traditional concoction of the Eleusinian Mysteries

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To explore traditional preparations of ancient Greece, we revisited the theory regarding the ceremonial use of kykeon, a sacred concoction used during the Eleusinian Mysteries (Wasson et. al, 1978). Beside the hallucinogenic properties attributed to the drink, kykeon is mentioned by Homer to be offered as a tonic to soldiers in battle, as well as for wounds' treatment (Homer, Iliad, 635-641). Kykeon was a mixture consisting mainly of barley flour, water, and herbs. *Claviceps purpurea* is a fungus known for infecting cereals while producing sclerotia containing ergot alkaloids and probably is the psychoactive agent responsible for kykeon's properties. Our aim was to support the hypothesis that ancient Greeks had discovered a way of hydrolysing ergot alkaloids into lysergic acid amide and lysergic acid. Following a traditional formulation, a concoction utilizing lye was prepared and monitored by NMR. *C. purpurea* sclerotia were powdered and boiled in a lye solution. After 30 min, ^{1}H-NMR spectra revealed that the peak at 9.6 ppm of the amide linkage of ergocryptine connecting the lysergic acid moiety and the peptide disappeared, and the occurrence of the characteristic peaks at 8.03 and 6.94 ppm indicating the presence of ergine and lysergic acid (Fig. 1).

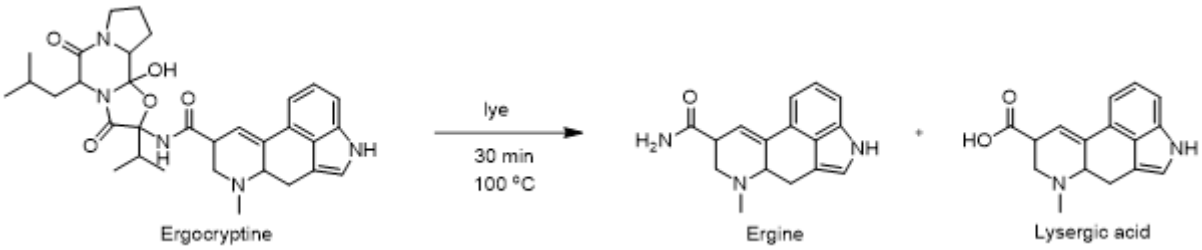

Fig. 1. Conversion of ergocryptine in ergine and lysergic acid with lye solution treatment

UPLC/Q-TOF-MS analysis in positive ionization mode confirmed the presence of ergine (*m/z* 268.14), lysergic acid (*m/z* 269.12) and ergocryptine (*m/z* 576.31). The transformation of ergot alkaloids was confirmed into the traditional concoction prepared with lye and support the hypothesis that the hallucinogenic properties of kykeon can be attributed to the use of ergot.

Keywords: Eleusinian Mysteries, kykeon, *Claviceps purpurea*, ergocryptine, lysergic acid

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S1.P153 Molecular networking-based dereplication and structural elucidation of compounds derived from liquid culture supernatants of marine-sourced *Streptomyces* strains

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Marine environments are a treasure trove of microbial diversity teeming with actinomycetes, renowned as producers of chemically diverse bioactive metabolites. Marine actinobacterial compounds—including amphiphilic biosurfactants and siderophores, studied within the EU's Horizon2020 SECRETed‡ project—offer a promising avenue for potentially novel therapeutic and/or industrial applications. In this work, a liquid-liquid extraction of culture supernatants of ten marine- derived *Streptomyces* strains was conducted with ethyl acetate, followed by UPLC-ESIHRMS analyses of the yielded extracts. The acquired spectral data were subjected to a molecular networking- based dereplication workflow to: (a) cluster compounds based on their spectral and structural similarities, (b) generate annotations through matching against curated spectral libraries, and (c) perform a chemical classification of the annotated natural products. A multi-step data processing, parametrisation, and visualisation in several chemoinformatic/computational environments (MZMine3 (Schmid et al., 2023), GNPS (Wang et al., 2016), R (Posit team, 2023), and Cytoscape (Shannon et al., 2003)) were involved in this process, including manual workflow validations. The extracts underwent Sephadex® LH-20-based fractionation and the fractions were further purified and analysed via UPLC-ESIHRMS and NMR to structurally elucidate the compounds of interest, pinpointed as potential targets through the dereplication. A diverse range of N-containing compounds were identified, including carboxylic and amino acid derivatives, cyclic and linear peptides, various alkaloids, alongside compounds with both hydrophobic and hydrophilic moieties, suggesting an amphiphilic nature thereof and, hence, their potential function as biosurfactants and/or siderophores.

Acknowledgments: European Union's Horizon2020 Research and Innovation Programme (Grant Agreement No. 101000794).

Keywords: marine *Streptomyces*, UPLC-HRMS, molecular networking, NMR structural elucidation

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S1.P154 Src tyrosine kinase inhibitory activity of selected flavonoids

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The aim of the present study was to investigate various flavonoids for their inhibitory effect on Src tyrosine kinase, as this enzyme plays an important role in multiple cellular processes and is over- activated in both cancer and inflammatory cells (Martellucci et al., 2020; Brian and Freedman, 2021). Inhibiting the activity of Src tyrosine kinase is an important strategy to prevent chronic inflammation and thus the development of cancer and other inflammation-related diseases. Twenty flavonoids in the form of aglycones were analysed using a time-resolved fluorescence resonance energy transfer (TR-FRET) assay, with staurosporine serving as a positive control (Vladimir-Knežević et al., 2023). The tested flavonoids belonged to the structurally diverse types of flavonoids, including flavones, flavonols, flavanones, isoflavones, flavan-3-ols and chalcones. Among them, luteolin was found to be the strongest Src tyrosine kinase inhibitor (IC50 = 9 mM), followed by baicalein, myricetin, fisetin and diosmetin (IC50 = 27-38 mM). The IC50 values obtained for the other flavonoids were in the range of 104-708 mM or the inhibitory effect was not detected at a concentration of 2000 mM. In this study, the relationship between the structure and activity of the tested flavonoids was discussed and the most effective candidates were selected for further investigation.

Keywords: Src tyrosine kinase inhibitory activity, flavonoids, luteolin

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S1.P155 Green extraction of bioactive volatile terpenes from Croatian *Salvia* species

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Salvia L. is the largest genus within the Lamiaceae family and includes many medicinal plants used in traditional and modern medicine. In addition to *S. officinalis* L., the most important representatives of this genus growing wild in Croatia are *S. fruticosa* Mill., *S. glutinosa* L., *S. sclarea* L. and *S. verticillata* L., which have not yet been sufficiently studied (Mervić et al., 2022). The aim of this study was to isolate volatile terpenes from selected *Salvia* species using environmentally friendly techniques such as hydrodistillation and supercritical carbon dioxide (sCO2) extraction and to determine their composition and biological activity. The leaves of *S. officinalis* and *S. fruticosa* proved to be very rich sources of essential oil, containing 22 mL/kg and 28 mL/kg, respectively, while 0.3-0.8 mL/kg of essential oil was isolated from the other *Salvia* species by hydrodistillation. A total of 143 compounds were identified by GC/MS analysis, accounting for 87.34-99.62% of the oil content. The main constituents of the tested oils were α-thujone, 1,8-cineole, camphor, γ-gurjunene, caryophyllene oxide and germacrene D. The essential oils of *S. fruticosa* and *S. officinalis* showed antioxidant and acetylcholinesterase inhibitory activity, with α/β-thujone and 1,8-cineole being most responsible for the effects. To obtain the highest yield of the extract with the highest content of the most bioactive volatile terpenes, the sCO2 extraction process was optimised. The extraction yield varied between 0.60% and 4.51%, depending on the applied pressure (100-220 bar) and temperature (40-60°C). A pressure of 220 bar and a temperature of 40°C were optimal parameters for the extraction of α/β-thujone and 1,8-cineole.

Keywords: *Salvia* species, essential oils, sCO2 extracts, neuroprotective effect

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S1.P156 Anti-*Fusarium* activities of microemulsions of essential oils of *Artemisia annua* L. (Asteraceae) and *Salvia fruticosa* L. (Lamiaceae) to be used as pre-harvest treatment of fruits

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The study covers the analysis, formulation and biological evaluation of two essential oils (EOs), *Artemisia annua* L. (Asteraceae) and *Salvia fruticosa* L. (Lamiaceae). Both EOs have been reported for their bactericidal and fungicidal activities (Santomauro et al, 2016; id., 2018; Jaradat et al., 2022). Their activity could be optimized if nanoencapsulated in drug delivery systems to decrease their volatility, improving the stability, water solubility, and efficacy. Composition of essential oils was obtained by GC-MS analysis. The main constituents characterized the *Salvia* EO were the oxygenated monoterpenes (65.1%) with 1,8-cineole (38.5%) and camphor (7.8%). Main constituents of *Artemisia* EO were monoterpenes (ca. 88%) with camphor (25.2%), 1,8-cineole (20%), and artemisia ketone (12.5%). The microemulsions were developed using vitamin E acetate as oil (8.22%), Cremophor RH 40 and Labrasol ALF as surfactants (14.8%) in a 1:1 ratio. The formulation was selected using a pseudo-ternary diagram. The microemulsion was loaded with *S. fruticosa* and *A.annua* EOs at a concentration of 10 mg/mL with 100% recovery using HPLC. Antifungal activity was carried out using a microplate dilution method using amphotericin B® as a positive control, while sterile Sabouraud broth was the negative control. Twenty microliters of *Fusarium verticilloides* subcultured in Sabouraud agar to 1.0 x 10^5 spores/mL were added to the wells. The plate was sealed and incubated for 24 hours at 28°C. *Fusarium* ufc per mL resulted 2,4x10^7. Microemulsions containing 1.8, 1.6, 1.4, 1.2 and 1.0 mg of EOs were added and incubated in the for 5 days. The results are the average of two tests repeated in triplicate. MIC values were determined when a logarithms reduction of 4 was found. The two microemulsions displayed a similar activity towards *Fusarium* spp., being the MIC of *S. fruticosa* 1.2 mg and that of *A. annua* 1.4 mg. The unloaded microemulsions had no activity. These two formulations could be very useful for the in-field application of the tested EOs in order to treat *Fusarium* infections.

Keywords: essential oils, microemulsions, antifungal activity, *Fusarium verticillioides*

Acknowledgments: This research was funded by the project EthnoHERBS (H2020-MSCA-RISE-2018, Grant Agreement No. 823973)

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S1.P157 Antibacterial and cytotoxic activities of bark and branches extracts from *Lafoensia replicate* Pohl (Lythraceae)

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Lafoensia replicate Pohl (Lythraceae) is an endemic tree from Cerrado vegetation in central Brazil. The bark of this species is used in the treatment of liver and kidney diseases, general inflammations, wound healing, gastritis, high blood pressure, headache, and stomach pain (Sobrinho et al, 2005). In this study, the antibacterial (*Staphylococcus aureus* and *Escherichia coli*) and cytotoxicity (human non-tumor keratinocyte cell line) activities of bark and branches extracts from *L. replicata* were evaluated and the chemical composition was analyzed by chromatography coupled to mass spectrometry with an electrospray ionization system (LC/ESI-MS/MS-negative mode). The extraction was carried out by maceration at room temperature with hexane (HE), followed by ethanol extraction (EE). The activities were performed by the broth microdilution technique (Santos, 2022; Riss et al. 2013). The extract shows MIC against *S. aureus* of 6.25, 0.78, 0.39 and 12.5 µg/mL for EE-bark, EE-branches, HE-bark and HE-branches, respectively. The MIC against *E. coli* was 0.39, 6.25, >800 and 50 µg/mL, for EE-bark, EE-branches, HE-bark and HE-branches, respectively. In general, EE-bark and EE-branches showed better results against the bacteria evaluated. The cytotoxicity of these extracts was evaluated and IC50 of the EE-bark and EE-branches were 407.90 and 376.33 µg/mL, respectively. The composition annotated by LC/ESI-MS/MS for these extracts showed variated hexahydroxydiphenols (HHDP), (*epi*)gallocatechin, variated punicalins, gallic acid, punicalagin, flavogallonic acid, terflavin A, isorhamnetin hexoside, quercetin hexoside, ellagic acid and hexoside kaempferol. This study showed that *L. replicate* is a natural source of compounds with antibacterial activity and low cytotoxicity.

Acknowledgments: CNPq, FAPEMIG and CAPES.

Keywords: *Lafoensia replicate,* cytotoxicity, antibacterial, tannin, HHDP

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S1.P158 Phytochemical composition and biological activities of *Pleroma granulosum* (Melastomataceae) leaf extract

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Pleroma granulosum is a widely distributed tree and widely used in square decoration due to its exuberant flowering. This species has been little explored scientifically. Therefore, this study investigated the antioxidant and antifungal activities and the chemical composition of *P. granulosum* leaf extracts. Extracts were obtained by maceration in hexane (HE) followed by ethanol (EE). Antifungal activity was evaluated by the broth microdilution method (CLSI, 2012). The antioxidant activity was analyzed by DPPH, ABTS and FRAP methods (Quaresma et al., 2020, Malta, Liu, 20014). The composition was carried out by liquid chromatography coupled to electrospray ionization source mass spectrometry (LC/ESI-MS/MS). The antioxidant activity by the DPPH method showed EC50 of 6.07±0.28 and 206.08±25.50 μg mL^{-1} for EE and HE, respectively. The ABTS method presented 3068.00±123.60 and 24.10±6.50 μmol ET (Equivalente Trolox) g^{-1} for EE and HE, respectively. The FRAP method showed 736.09±7.97 and 180.41±2.21 μmol ET g^{-1} for EE and HE, respectively. In all methods, greater activity was observed for EE. The antifungal activity showed MIC of 2.93, 1.46, 11.72 and 1.46 μg mL^{-1} for the EE against *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis*, respectively. HE presented MIC>3000 μg mL^{-1} against all fungi. Therefore, EE presented better activities compared to HE. Therefore, the composition of EE showed several phenolic compounds, including flavonoids, phenolic acids and tannins. The results demonstrate that the extract of *P. granulosum* is a source rich in phenolic compounds with high antioxidant and antifungal activity. Acknowledgments: CNPq, FAPEMIG and CAPES.

Keywords: *Pleroma granulosum,* antioxidant, antifungal, tannin, phenolic

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S1.P159 Combining a 'multi-bioassay screening' approach with metabolomics for in depth exploration of four Andean-Patagonian fungi

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Finding compounds that are both bioactive and structurally novel is arguably the most desired and challenging goal in natural products research. Bioguided fractionation is considered the gold standard for isolating bioactive compounds, despite some drawbacks. These drawbacks include time- consuming and costly lab work as well as the substantial production of noxious chemical waste, among others. Additionally, bioactivity often takes precedence over structural novelty in bioguided fractionation, resulting in the isolation of bioactive but already-known compounds. Recent advancements in mass spectrometry-based bioinformatics have facilitated the organization and visualization of large datasets, such as specialized metabolites from natural extracts. These tools, relying on spectroscopic data, are particularly suitable for profiling and prioritizing compounds to identify structural novelty. Furthermore, metabolomics platforms are generally flexible enough to incorporate various layers of additional information into their processes. In theory, this flexibility enables the identification of compounds with desired characteristics based on analytical, taxonomical, experimental conditions, or bioactivity data. Based on these principles, we present a multilateral approach that combines standard bioactivity screening with metabolomics guidance. This methodology is applied for the first time to four Basidiomycetes fungi from the Andean-Patagonian region of Chile: *Stereum hirsutum*, *Stereum greslebinii*, *Aleurodiscus vitellinus*, and *Anthracophyllum discolor.* These strains were grown and extracted under various conditions to promote chemical diversity through experimental variations. The resulting panel of extracts underwent a 'multi-bioassay screening' against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, HCT-116 cells, and zebrafish. Additionally, a feature-based molecular network integrating taxonomy, experimental variations, and bioactivity data was generated to identify compounds with unique characteristics within the dataset.

Keywords: fungi, Basidiomycetes, bioactivity, metabolomics, antimicrobials

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S1.P160 Diketopiperazines from the marine sediment-derived bacterium *Streptomyces hoynatensis*

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Cyclic dipeptides also known as diketopiperazines (2,5-DKPs), or dipeptide anhydrides, are among the most common peptide derivatives found in nature (Prasad 1995). They have been obtained from a variety of natural resources, including marine organisms (Huang et al. 2010). The interest in DKPs is high because they exhibit not only antimicrobial, antitumor, and antiviral activities (Huang et al. 2014) but also activities such as a relatively rare treatment of ischemic brain injury (Guan 2008), anti- Alzheimer's (Turkez et al. 2020) and inhibition of microtubule polymerization (Ding et al. 2020). *Streptomyces hoynatensis* isolated from Black Sea sediments, produced a new diketopiperazine, Hoynatenamide (**1**) along with six known diketopiperazines (**2-7**) (Fig. 1). The structures of the compounds were determined based on 1D- and 2D-NMR spectroscopic analysis, and mass spectrometry data. The absolute configuration of compound **1** was deduced by traditional hydrolysis, derivative formation, and chromatographic analyses compared with standards. Among the compounds tested, compound **2** showed the best activity against HeLa cancer cell lines and **3** against MCF-7 cell lines with an IC50 value of 19.4 and 32.4 μM, respectively. Except for compound **1**, all of the compounds showed mild antifungal activity against *C.albicans* with an IC50 value of 64 μg/mL.

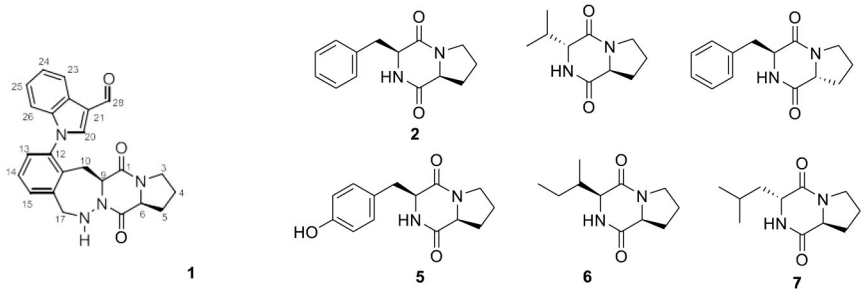

Fig. 1. Structure of the isolated diketopiperazines.

Keywords: diketopiperazines, *Streptomyces*, microbial secondary metabolites, antimicrobial activity, antiproliferative activity

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S1.P161 The phenolic acids production in stationary and agitated shoot cultures of *Aronia melanocarpa, A. arbutifolia* and *A.* × *prunifolia*

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Aronia melanocarpa (Michx.) Elliott (black chokeberry), *A. arbutifolia* (L.) Pers. (red aronia) and *A.*× *prunifolia* (Marsh.) Rehd. (purple aronia) are known crop and medicinal plant species of North American origin (Kokotkiewicz et al. 2010). Different plant *in vitro* culture systems (e.g., stationary and agitated cultures) have a great influence on the accumulation of bioactive metabolites in the cultivated biomasses. The aim of this study was to compare the productivity of *Aronia* sp. shoots cultivated in stationary and agitated cultures. The object of interest were phenolic acids - the metabolites with anticancer, anti-inflammatory and other activities important in phytotherapy and cosmetology. The cultures were cultivated on three variants of Murashige-Skoog medium (MS) supplemented with cytokinin, 6-benzyladenine (BA) and auxin, 1-naphthaleneacetic acid (NAA) in the following concentrations [mg/L]: variant I - 0.5 and 2.0; variant II - 2.0 and 2.0; variant III - 3.0 and 1.0. In the methanolic extracts from biomasses, 19 compounds (derivatives of cinnamic acid, benzoic acid and depsides – chlorogenic, neochlorogenic and rosmarinic acids) were estimated by an LC-DAD method (Ellnain and Wojtaszek, 1999). The studied *in vitro* cultures produced as the main metabolites three depsides: neochlorogenic acid, chlorogenic acid and rosmarinic acid. The maximal amounts of these compounds were higher in shoots cultivated as agitated cultures in comparison with stationary cultures and reached respectively 82.00, 105.45 and 134.24 mg/100g DW (*A. melanocarpa*), 25.48, 175.94 and 132.49 mg/100g DW (*A. arbutifolia*), and 70.91, 260.34 and 225.26 mg/100g DW (*A.* × *prunifolia*). The studied *Aronia in vitro* cultures could be proposed as an innovative source of the chosen phenolic compounds.

Keywords: plant biotechnology, *in vitro* cultures, phenolic acids, *Aronia* species

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S1.P162 Meroterpenoids from *Centrapalus pauciflorus* leaves with antiproliferative activity

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Centrapalus pauciflorus (Willd.) H.Rob (Asteraceae) is an annual or sometimes short-lived perennial herb with tropical African origin. In the African traditional medicine, its leaves are used as tea or cooked into porridge to ease chest pain and stomach-ache (Chhabra et al., 1989). Previous phytochemical studies of *C. pauciflorus* revealed the presence of fatty acids, coumarins, flavonoids, phenolic acids, sesquiterpenes, and triterpenoids (Maroyi, 2000). In continuation of our ongoing study on *C. pauciflorus* (Saidu et al., 2023; Krstić et al., 2023), the present paper reports the isolation, and structure determination of twelve previously unreported meroterpenoids from the chloroform extract of the leaves besides the known ethuliaconyzophenone. Solvent-solvent partition, OCC, VLC, and HPLC were used for isolation of the compounds. The structures were determined using extensive NMR spectroscopy and HRESIMS. The compounds proved to be hybrid molecules of monoterpenes with 5-methylcoumarin, 5-methylchromone, or acetophenone. New carbon skeletons, dimeric meroterpenoids, and the first meroterpenoid containing a monoterpene unit fused with both a coumarin and an acetophenone part are among the structural novelties. Nine isolated compounds were tested for antiproliferative activity against human adherent malignant cell lines of gynecological origin using the MTT assay, and six of them exhibited moderate activity (IC50 10 – 25 μM) against MCF-7, HeLa, and A2780 cells.

Keywords: *Centrapalus pauciflorus*, meroterpenoids, centrapalus coumarin, pauciflorin, X-ray

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S1.P163 Volatiles derived from Philippine plant species exhibited antimicrobial activity, antioxidative and cytotoxicity using a newly developed microplate-based assay

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Essential oils derived from Philippine plant species were evaluated for bioactivity using a newly developed microplate-based bioassay. It has been known that several volatile plant-derived products exhibit considerable bioactivities, however, results of conventional microplate-based assays used to evaluate such highly shifting vapor products can sometimes yield erratic and unusual results. With the aim to demonstrate such phenomenon, antimicrobial, antioxidant, and cytotoxic activities of three Philippine essential oils (*Alpinia elegans*, *Cinnamomum iners*, and *Xanthostemon verdugonianus*) were evaluated in a series of experiments including both ethylene vinyl acetate (EVA) Capmat sealed and nonsealed microplates. The results clearly illustrated that vapor transition to adjoining wells causes false-positive results of bioassays performed in nonsealed microtiter plates. The microplate layout and a duration of the assay were demonstrated as the key aspects defining level of the results affected by the vapors of volatile agents. Additionally, we reported biological activities and chemical composition of essential oils from *A. elegans* seeds and *X. verdugonianus* leaves, which were, according to our best knowledge, were analyzed for the first time. Considering our findings, certain modifications of conventional microplate-based assays are necessary (e.g., using EVA Capmat as vapor barrier) to obtain reliable results when biological properties of volatile agents are evaluated.

Keywords: broth microdilution, essential oil, microtiter plate, supercritical CO2 extract, volatilization

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S1.P164 Metabolomics-driven discovery of new ophiobolin-type sesterterpenes from marine-derived fungus *Pleosporales* sp. and their antimicrobial activities

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In the quest to discover new bioactive chemical entities from nature, a bottleneck is natural product isolation, which can be overcome by integrating several layers of data into multi-informative approaches (Martínez-Aldino et al., 2023). A combination of taxonomy, nontargeted metabolomics, and bioactivity information has led to the selection of a marine-derived Pleosporales fungus i.e. *Uzbekistanica* sp. to isolate biologically active new natural products. The strain was prioritized based on the hypothesis that investigating poorly studied and highly metabolic taxa could lead to the isolation of new natural products. A non-targeted metabolomics investigation of the fungal extract using molecular networking indicated the presence of a terpenoid cluster with several unidentified compounds within (Wang et al., 2016). The isolation of the targeted compounds then led to new Ophiopholin-type sesterterpenes (**1**-**8**). Their structures were determined by extensive spectroscopic analysis and the absolute configurations were further determined by ECD assessment. The antimicrobial effects of these compounds were tested against several pathogenic bacteria and a fungal pathogen. Compounds **5**, **6**, and **8** exhibited inhibitory activity against *Streptococcus agalactae* with MIC values of 85, 70, and 70 µM, respectively. In addition, **2** showed moderate activity against *Candida albicans* at 100 µM concentration.

Keywords: pleosporales, *Uzbekistanica*, sesterterpenoids, bipolarolides, ophiobolin

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S1.P165 Molecular networking of actinobacteria sourced from thai environments reveals the presence of metabolites with antimicrobial properties

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Actinomycetes, particularly from mangrove and marine sediments in Thailand, are relatively unexplored for their potential antimicrobial properties. The aim of this study was to isolate actinobacteria using different media and evaluate the antimicrobial potential of the isolated actinobacteria against methicillin resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and the chloroquine-resistant *Plasmodium falciparum* strain K1 (Septama et al 2022; Phuwajaroanpong et al 2022). Methanolic crude extracts of isolates demonstrated significant in vitro inhibitory activities against chloroquine-resistant *P. falciparum* lactate dehydrogenase (pLDH), agar-overlay and microdilution bacterial assays. The active isolates were further subjected for metabolite profiling using LCMS aided by a global molecular networking platform . We identified numerous active and known compounds, such as Geldanamycin and elaiophylin. Additionally, we also performed the identification of the selected actinobacterial isolates using 16S rRNA sequencing. Further studies are needed to determine the complete chemical structures and explore the biosynthetic gene clusters. Lastly, our research aimed to provide sustainable solutions for antimicrobial drug resistance, highlighting the potential of actinobacteria-derived compounds in global health initiatives.

Keywords: Microbial natural products, antibiotics, actinobacteria, antimicrobial resistance, drug discovery

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S1.P166 MSMS-based molecular networking and fragmentation patterns reveal new insights into the astins chemodiversity from the fungal endophyte *Cyanodermella asteris*

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Among the endless number of biologically active cyclopeptides encountered in nature, the plant-derived family of astins represents an interesting case of cyclic peptides with promising anti-tumor and immunomodulating bioactivity. Astins were first isolated from the root extracts of the flowering plant *Aster tataricus* L.f. (Asteraceae) which has a long history of use in traditional Chinese medicine (TCM) as antitussive and expectorant remedy (Yu et al., 2015). Chemically, astins are macrocyclic 16-membered pentapeptides which may contain unusual non-proteinogenic (L-Abu, L-*allo*-Thr, b- Phe, L-Ava, and mono- or dichlorinated L-Pro) and proteinogenic (L-Ser and L-Pro) amino acids. Up to the present, sixteen derivatives (Astin A-I and K-P) from the astin series of cyclopentapeptides have been characterized as natural-occurring peptides (Xu et al., 2013). Recently it was shown that three members of astin family can be produced by the newly discovered fungal endophyte *Cyanodermella asteris* (Schafhauser et al., 2019). In this study, mass spectrometry-based molecular networking and detailed inspection of the fragmentation trees uncovered the idea that *C.asteris* can produce many more astins including proline desaturated derivatives, aminovaleric acid-containing analogues, formylated and acetylated derivatives and some lipopeptide astins. These findings give new information on astin biosynthesis in fungi.

Keywords: astins, fungi, molecular networking, high-resolution MS

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S1.P167 Phytochemicals and Enzyme inhibition potential of *Rydingia* and *Moluccella* Species in Iran

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Three plants of the genus *Otostegia* Benth (Lamiaceae): *O. persica* (Burm.) Boiss., *O. aucheri* Boiss.; and *O. michauxii* Briq (Mozaffarian, 2003) are renamed to: *Rydingia persica* (Burm.f.) A.-C. Scheen & V. A. Albert and *Rydingia michauxii* (Briq.) A.-C. Scheen & V. A. Albert and *Moluccella aucheri* (Boiss.) A.-C. Scheen, based on their molecular and morphological studies (Sheen and Albert, 2009). We have isolated and identified several diterpenoids and flavonoids with α-glucosidase and acetylcholine esterase activities. *ent*-Labda-8(17),13-dien-18-oic acid-15,16-olide, its glycoside ester, and a trimethoxy flavone: 5, 4'-dihydroxy-6,7,3'-trimethoxy flavone (Eupatorin) were isolated from

R. michauxii. 6ß-acetoxy-9α-hydroxy-7-oxa-labd-13-ene-15,16-olide,5-hydroxy-7,4-dimethoxy- flavone and genkwanin were reported from *M. aucheri* (Doorandishan et al., 2021). Finally, four labdane diterpenoids: 15,16-epoxy-3α, 7β, 9α-trihydroxylabdan-13-(16), 14-dien-6-one; 15,16- epoxy-3α, 7α, 9α-trihydroxylabdan-13-(16),14-dien-6-one;9,13,15,16-diepoxy-3α,7β,15α(β)- trihydroxy-labdan-6-one were characterized in an ethyl acetate fraction of an ethanol extract of the aerial parts of *R. persica*. The structures of the compounds were elucidated by spectral data analyses of their ^{1}H and ^{13}C NMR and ESIMS spectroscopy. The *in vitro* and in *silico* α-glucosidase and acetylcholine esterase activity of the isolated compounds are reported in this presentation.

Acknowledgments: The study was financially supported by the National Institutes for Medical Research Development (NIMAD), Tehran, Iran (grant number: 988197).

Keywords: *Otostegia, Rydingia persica, Rydingia michauxii, Moluccella aucheri,* labdane diterpenoids

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S1.P168 Phenanthrenes from the aerial parts of *Juncus tenuis*

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Phenanthrenes are a promising group of natural small molecules, with great structural variety and noteworthy pharmacological (e.g., antiproliferative, antibacterial, and anti-inflammatory) activities (Bús et al., 2018; Tóth et al., 2018). Although, Juncaceae is a relatively small plant family with approximately 500 plant species worldwide, almost 20% of currently known natural phenanthrenes were described from species belonging to this family. Especially members of the *Juncus* genus are rich sources of phenanthrenes. Various *Juncus* species are used mainly in traditional Chinese medicine for the treatment of numerous disorders (e.g. fidgetiness, insomnia, painful urination, pharyngitis, and aphtha). Medulla Junci, the dried stem pith of *J. effusus*, is official in the Pharmacopoeia of the People's Republic of China. In continuation of our search for phenanthrenes from plants belonging to the family Juncaceae, *Juncus tenuis* was investigated. The dried and ground plant material was extracted with methanol. After evaporation, it was subjected to solvent–solvent partition using *n*-hexane, chloroform, and finally ethyl acetate. Phenanthrenes were enriched in the chloroform phases; therefore, it was fractionated by vacuum liquid chromatography, gel filtration on Sephadex LH-20 gel, and in the last step by HPLC to obtain pure compounds. The structure elucidation of the compounds was carried out by extensive NMR spectroscopic analysis, and HRMS experiments as well as by comparison of spectroscopic data with literature values. The results allowed the identification of 17 phenanthrenes, among them 14 monomers and three dimers from *J. tenuis*. Four compounds are previously undescribed natural products.

Keywords: *Juncus tenuis*, Juncaceae, phenanthrenes

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S1.P169 Discovery of anti-inflammatory cyanobacterial secondary metabolites targeting the Keap1/Nrf2 pathway

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Chemical investigation of marine cyanobacterial samples collected from Florida Keys resulted in the discovery of two secondary metabolites belonging to two different structural classes. The planar structures of both compounds were elucidated using a combination of 1D and 2D NMR spectroscopy and mass spectrometry. The structures feature an α,β-unsaturated carbonyl system, a key motif required for the activation of the Keap1/Nrf2–ARE pathway, a cytoprotective pathway that controls the activation of antioxidant genes and phase II detoxification enzymes. The compounds were screened in ARE-luciferase reporter gene assay using stably transfected HEK293 cells and found to induce Nrf2 activity up to 10 μM. Due to the established crosstalk between Nrf2 and NF-κB pathways, the anti-inflammatory potential of both compounds was investigated in LPS-induced mouse macrophages (RAW264.7 cells), a commonly used model for inflammation. The compounds significantly upregulated *Nqo1* (Nrf2 target gene) and downregulated *iNos* (NF-κB target gene) resulting in a significant reduction of nitric oxide (NO) levels. Furthermore, we performed RNA- sequencing and demonstrated the transcriptional activity of both compounds on a global level and identified canonical pathways and upstream regulators involved in inflammation, immune response, and certain oxidative stress-underlying diseases such as multiple sclerosis.

Keywords: marine cyanobacteria, inflammation, RNA sequencing, immune response, multiple sclerosis

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S1.P170 A terpenoids-enriched fraction of *Inula viscosa*: its chemical composition and anti-cancerous effects against human lung cancer cells

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Inula viscosa has been a heavily used plant in traditional Mediterranean and Middle Eastern medicine to handle various illnesses. *I. viscosa* has been shown to have anticancerous effects against a variety of cancers, but its effects against lung cancer have been under limited investigation. The aim of the present study is to examine the potential anticancerous properties of methanolic or aqueous extracts of stems and leaves of *I. viscosa* against human lung cancer A549 cells. *I. viscosa* leaves methanolic extracts (IVLM) showed the highest reduction in viability of A549 cells among all the extracts. IVLM also reduced the viability of a panel of human cancer cell lines. Fractionation of IVLM by liquid-liquid separation using hexane, ethyl acetate and dichloromethane (DCM) solvents revealed that *I. viscosa* DCM fraction (IVL DCM) displayed the most notable reduction in viability of A549 cells (IC50= 28.68 ± 3.83µg/mL at 48 h), and was not cytotoxic to normal human embryonic fibroblasts (HDFn). This led to a detailed assessment of IVL DCM phytochemical constituents using GC-MS analysis which revealed 22 metabolites, highlighting an enrichment in terpenoids like 9,19-cyclolanostan-3-ol acetate, betulin, longifolene and lupeol, known for their pro-apoptotic, pro-autophagic and anti-metastatic functions. Furthermore, analysis of proliferation, autophagy, and apoptosis markers by Western blotting indicated that IVL DCM reduced proliferation (reduction of proliferation marker Ki67 and induction of proliferation inhibitor proteins P21 and P27) and induced intrinsic apoptotic pathway (P38/P53/BCL-2/BAX/ Caspase 3) in A549 cells. IVL DCM also reduced the migration of A549 cells, potentially through reduction of FAK activation. Future identification of anticancer metabolites of IVL DCM may emphasize its terpenoids as anti-lung cancer agents, and confirm *I. viscosa* as a resource of herbal anticancer agents.

Keywords: *Inula viscosa,* lung cancer, A549 cells, phytochemical analysis, cytotoxicity

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S1.P171 Molecular identification and metabolite profiling of endophytes isolated from *Bambusa vulgaris* var. *striata* shoot

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Endophytic fungi have been reported to produce many plant-derived biochemicals. In this study, endophytic fungi were isolated from *Bambusa vulgaris* var. *striata* shoot and the biocompounds in the ethanolic extract of the endophytes were investigated. Three endophytes comprising of 2 strains of *Candida albicans* and 1 strain of *Fusarium oxysporum* were characterized by 18S rRNA gene and internal transcriber spacer region sequence analysis. GC-MS analysis revealed 18, 30 and 36 biocompounds in the ethanolic extracts of *C. albicans* strain KY101911.1, MK805514.1 and *F. oxysporum* respectively, with variations in the concentration as well as the type of the phytoconstituents in these endophytes. However, the main biocompounds in each of the endophytes were the 9-octadecenoic acid, methyl ester (E)- (18.58%), 11,14-octadecadienoic acid, methyl ester (18.77%), hexadecanoic acid ethyl ester (21.66%) and butyl 9,12-octadecadienoate (12.15%) for *C. albicans* strain MK805514.1, KY101911.1, and *F. oxysporum* respectively. Generally, some of the compounds occurred in fairly large amounts while others occurred in trace amounts. However, the phytoconstituent with the largest concentration was hexadecanoic acid ethyl ester in the extract of *C. albicans* strain MK805514.1. Seven compounds (hexadecanoic acid, ethyl ester; hexadecanoic acid, methyl ester; ethyl stearate; 11,14-octadecadienoic acid, methyl ester; 9-octadecenoic acid, methyl ester (E)-; n-propyl 9,12-octadecadienoate; ctadecanoic acid, 17-methyl-, methyl ester) were similalry revealed in each of the endophytes but in varying quantities. These findings indicate that *B. vulgaris* var. *striata* shoot is a huge reservoir of micro-organisms that could be useful for the development of novel drugs with therapeutic properties (Khiralla et al., 2015).

Keywords: bamboo shoot, endophytes, *C. albicans*, *F. oxysporum*, phytochemicals

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S1.P172 *Annulohypoxylon stygium,* an endophyte from the marine red alga *Asparagopsis taxiformis* is a source of antiviral binaphthalenes

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Naphthalene derivatives with unique structures are widely found in *Annulohypoxylon* species. Truncatones A-D have been isolated from *A. leptascum* and *A. truncatum* and showed cytotoxic activity against mouse fibroblast cells (Sudarman, 2016). A strain of *A. stygium* was isolated from the red alga *Asparagopsis taxiformis* and grown on red rice solid medium for 20 days. After extraction with MeOH and solvent removal, the MeOH extract was dissolved into MeOH:H2O (4:1) and defatted with *n*-hexane. The hydromethanol fraction was evaporated to yield 1.2 g of extract, which was subjected to subsequent MPLC (C18) and Sephadex LH-20 fractionation steps to yield verrucosapyrone B and diketopiperazines along with asperlone B (**1**), obtained as an amorphous red powder. Asperlone B showed a molecular formula C20H12O8, determined by ESI-QqTOF-MS in positive mode, and was isolated for the first time as a fungal metabolite from the genus *Annulohypoxylon*. Its structure was established by NMR and ESI-QqTOF-MS data, and comparison with the literature (Xiao, 2015).

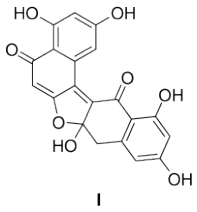

Fig. 1. Stucture of asperlone B.

Molecular docking analysis of asperlone B evidenced effectiveness in modulating the biomacromolecular target of the Mayaro virus, confirmed by the release of -7.0 kcal/mol, mainly due to the resonance effect inherent to its aromatic rings, which allowed PI-type interactions with the amino acids lysine-138 and alanine-256, as well as Van der Waals interactions with further amino acids in the reaction site. Once this ligand is complexed in the hydrophobic site, the virus capsid bonds are no longer effective, thus interrupting its cycle. Such results confirmed marine endophytic fungi as a relevant source for potential antiviral metabolites.

Keywords: marine fungi, asperlones, endophytic fungus, *Annulohypoxylon,* molecular docking

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S1.P173 Antibacterial anthraquinone produced by the fungal endophyte *Botryosphaeria rhodina*, isolated from the mangrove *Xylocarpus granatum* J. Koenig

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The exploration of the secondary metabolites of fungus originating from mangroves, known for their notable biological activity and promise in pharmaceuticals, has attracted substantial attention (Deshmukh et al., 2020). Our previous investigation focused on isolating endophytic fungi from mangrove vegetation with antimicrobial characteristics. Among these fungi, Botryosphaeria rhodina was identified from the mangrove *Xylocarpus granatum* J. Koenig. (Linda et al., 2024).

In the continuum of our research focused on deriving antibacterial agents from endophytic fungi associated with mangroves, this study aims to isolate these agents from the fungi. To this end, solid- state fermentation was employed to yield an extract rich in antibacterial compounds, which was then subjected to vacuum liquid chromatography using a solvent system of n-hexane, ethyl acetate, and methanol, resulting in ten distinct fractions. From fraction 6, an orange precipitate was obtained, which was further purified through re-crystallization to produce orange crystals. Subsequent elucidation and comparison of spectroscopic data confirmed the compound as macrosporin, an anthraquinone derivative (**1**), as depicted in Figure 1.

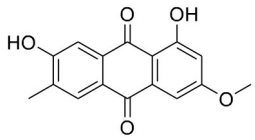

Fig. 1. Structure of macrosporin **1**

Compound **1** demonstrated remarkable activity against a range of pathogenic bacteria, with Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values between 25 and 100 µg/mL. This data underscores the potency of macrosporin as an antibacterial agent, instilling confidence in the potential of our findings. Furthermore, *in silico* analyses of **1**'s antibacterial mechanism revealed a strong affinity for the bacterial receptor's active and allosteric sites, enabling it to disrupt cell wall integrity and inhibit protein synthesis.

Keywords: antibacterial, anthraquinone, *Botryosphaeria rhodian*, *in vitro*, *in silico*

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S1.P174 Elucidating phytochemical profiles and antibacterial potential of *Helminthostachys zeylanica* (L.) through untargeted LC-HRMS metabolomics analysis

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Helminthostachys zeylanica (L.) Hook. is renowned for its traditional therapeutic uses in managing diabetes mellitus and other illnesses in China, Malaysia, and Indonesia (Ridhasya et al., 2019). During our previous investigation into the antibacterial properties of several extracts derived from the roots of this species, we discovered that dichloromethane and ethyl acetate extracts showed inhibitory effects against various pathogenic bacteria (Army et al., 2023). This study employs LC-HRMS for untargeted metabolomic analysis of *H. zeylanica*'s various parts (root, stem, leaf) to elucidate its chemical profile and antibacterial potential. We identified diverse secondary metabolites, highlighting the plant's complex chemistry. The antibacterial properties were assessed using the Kirby-Bauer disc diffusion method and further quantified by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Our research findings suggest that extracts from *H. zeylanica* demonstrate moderate antibacterial activity, particularly against Gram-positive bacteria. However, their effectiveness is lower compared to chloramphenicol. Advanced metabolomic profiling techniques were used, including Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA). This analysis revealed distinct metabolic patterns in different sections of plants, highlighting the diverse phytochemical contents. These investigations helped identify promising antibacterial metabolites. The study affirms the existence of distinct bioactive chemicals in *H. zeylanica*. However, their effectiveness against Gram- negative bacteria is limited compared to conventional antibiotics. This comprehensive metabolomic method improves our comprehension of the pharmacological properties of *H. zeylanica*.

Keywords: LC-HRMS, metabolomics, *Helminthostachys zeylanica*, antibacterial activity, secondary metabolites

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Ridhasya, F. E., Teruna, H. Y., Hendra, R., Almurdani, M., 2019. Natural Antidiabetic of Tunjuk Langit (*Helminthostachys zeylanica*) Rhizome Extracts. Pharmacol. Clin. Pharm. Res. 4, 67-70.

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S1.P175 Sesquiterpenoids and hexanorcucurbitacin from *Aquilaria malaccensis* agarwood with anti- inflammatory effects by inhibiting STAT1/AKT/MAPK/NLRP3 pathway

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Seven undescribed compounds **1-7**, including four sesquiterpenoids, one azulene-type, one indene- type, and one rare hexanorcucurbitacin, together with eleven knowns ones (**8-16**), were isolated from the agarwood chips of *Aquilaria malaccensis* (Fig. 1.). The structures of isolated compounds were elucidated by extensive spectroscopic methods such as mass spectrometry, UV, IR, NMR spectroscopy. The precise stereo-chemical configurations of new compounds were determined by calculated ECD spectra data, as well as a single-crystal X-ray diffraction analysis. This study demonstrates the phytochemical investigation of the extract of *A. malaccensis* and the pharmacological activities of seven previously undescribed compounds. The isolated compounds **1- 7** were evaluated by estimating the levels of nitric oxide (NO), TNF-α, and the expression of enzyme iNOS, and COX-2. Among them, a rare hexanortriterpenoid (**7**) derived from a cucurbitane-type triterpenoid showed the significantly attenuated neuro-inflammatory effects via STAT1/AKT/MAPK/NLRP3 signaling pathway on the mechanistic studies.

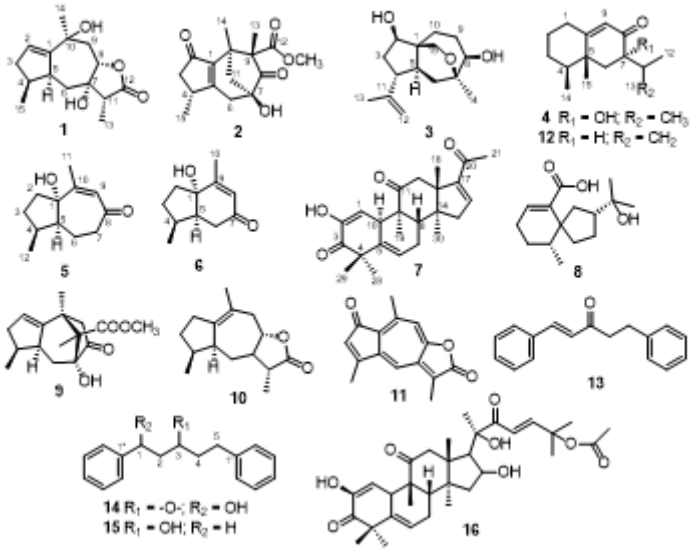

Fig. 1. Chemical structure of compounds **1-16.**

Keywords *Aquilaria malaccensis,* agarwood, hexanorcucurbitacin, isodaucane-type sesquiterpenoid, NLRP3 signaling

References

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S1.P176 Isolation of *Salvia apiana* Jeps. (Lamiaceae) essential oil—the influence of distillation technique and process parameters on the yield and composition of volatile fraction

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Salvia apiana (white sage) is an aromatic plant of mint family, endemic to California Floristic Province. It has long been known to native North American Chumash people who employed white sage as a medicinal and ritual plant. Modern studies confirmed antioxidant, antimicrobial, anti- inflammatory, analgesic and anti-cancer activities of *S. apiana*, which can be largely attributed to its high essential oil content (Krol et al., 2022). In the current work, the influence of distillation technique (conventional hydrodistillation [HD] vs simultaneous distillation-extraction [SDE]), as well as different parameters of the process (sample size, water volume, distillation time and condensate flow) on the yield and composition of essential oil, isolated from dried white sage leaves, have been examined. Of the techniques employed, SDE proved to be superior in terms of essential oil yield (83 ml/kg dry wt, ca. 1.5-2-times higher as compared to HD, depending on the apparatus type used). Regardless of the technique used, the composition of the volatile fraction remained largely the same, with 1,8-cineole, alpha-pinene, beta-pinene and bornanone being the major constituents, as revealed by GC-MS and GC-FID analysis. HD experiments demonstrated that sample to water ratio had negligible effect on essential oil yield and composition. However, the study showed that by extending HD time from 1.5 to 3 h, the yield of volatiles can be increased from ca. 40 to 46 ml/kg dry wt. Higher amounts of essential oil were also obtained at higher condensate flows which enables to shorten the HD process, while maintaining its effectiveness.

Keywords: *Salvia apiana,* essential oil, hydrodistillation, simultaneous distillation-extraction, process optimization

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S1.P177 Biomasses of *Phyllanthus* spp. (Euphorbiaceae) as a source of sulfonic derivatives of flavonoids

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Naturally occurring sulfonic derivatives of flavonoids are an extremely rare group of compounds. Due to their limited distribution, little is known about their biological activity, but also about their biosynthesis levels in plants. Moreover, there is no information on the accumulation of these compounds in *in vitro* conditions. In nature, some species of the genus *Phyllanthus* are unique sources of these secondary metabolites (Than et al., 2006; Thanh et al., 2014). The aim of the study was to evaluate *Phyllanthus* spp. biomasses obtained *in vitro* for the presence of sulfonic derivatives of flavonoids. The study included methanol extracts from biomasses (shoot, callus and adventitious root cultures) obtained from 5 species – *P. grandifolius*, *P. juglandifolius, P. glaucus, P. amarus* and *P. multiflorus*. Conditions for screening analysis using HPTLC and HPLC methods were developed and the presence of the tested compounds was demonstrated in *P. amarus* (shoot and adventitious root culture) and *P. multiflorus* (shoot culture and callus culture). Shoot cultures of both species were characterized by the presence of one compound, while the callus of *P. multiflorus* and adventitious roots of *P. amarus* were the source of a more complex set of compounds. Preliminary studies indicate that niruriflavone is the dominant compound in all tested biomasses (Fig. 1).

Fig. 1. Chemical structure of niruriflavone.

Keywords: *Phyllanthus,* sulfonic derivatives of flavonoids, niruriflavone, plan *in vitro* cultures

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S1.P178 Antibiotic potentiating effects of *Salix* twig extracts against *Bacillus cereus*

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Plant extracts and compounds are known to possess antibiotic potentiating effects (Abreau et al., 2017). The use of plant extracts and compounds as antibiotic adjuvants could be a way to repurpose antibiotics that have lost their effects against resistant bacteria. Moreover, in the combinations it is possible to use the antibiotics in extremely small concentrations, thus reducing their toxic effects. In addition, the risk of development of resistance is reduced for a combination. Only a few *Salix* species, such as the African *S. ledermannii*, have so far been evaluated for their combination effects with antibiotics (Demgne et al., 2022). Thus, we have chosen to study *Salix starkeana*, *S. myrsinifolia* and *S. aurita* that grow naturally in Finland. In our studies, methanol extracts of the winter twigs of these *Salix* species gave growth inhibitory effects against *B. cereus* with MIC values of 625, 1250 and 2500 µg/ml, respectively. In addition, two antibiotics, tetracycline with MIC of 0.488 µg/ml and ciprofloxacin hydrochloride with MIC of 0.06 µg/ml against *B. cereus* were chosen for the combination studies. A 96 well microplate checkerboard method was used to test the combination effects (Mordmuang et al., 2019). To evaluate the results of the combinations, we calculated the FIC indexes (SFIC or FICI) for each combination producing 90% or more of growth inhibition. The FICI was calculated as ΣFIC = FICA + FICB = (CA/MICA) + (CB/MICB), where MICA and MICB are the MICs of drugs A and B alone, respectively, and CA and CB are the concentrations of the drugs in combination, respectively. Several combinations of all tested *Salix* extracts with tetracycline hydrochloride resulted in synergistic effects. Moreover, our combination tests showed that even at concentrations of 0.03 ´ MIC (0.061 µg/ml), tetracycline produced synergistic effects when combined with a *Salix aurita* methanol twig extract at 625 µg/ml (**Fig. 1**).

			MIC	0,5 MIC	0,25 MIC	0,125 MIC	0,0625 MIC	0,0312 MIC	0,0156 MIC	0,0078 MIC	
Inh%		Extr in well	2500	1250	625	313	156,5	78,25	39,125	19,56	µg/ml
	Atb in well	Eppendorf	10000	5000	2500	1250	625	313	156,5	78,25	µg/ml
MIC	0,488	1,952	106,000497	100,80752	104,323339	99,105516	100,409972	99,7515322	100,086964	99,8757661	
0,5 MIC	0,244	0,976	132,077191	118,312076	109,690243	101,279609	98,8322014	100,03727	100,273315	100,472089	
0,25 MIC	0,122	0,488	92,2229584	92,272652	107,044062	101,006295	100,633593	100,03727	99,8384959	100,049694	
0,125 MIC	0,061	0,244	99,043399	94,4094749	104,459997	111,479212	98,6831208	91,1297002	84,4459168	93,105019	
0,0625 MIC	0,0305	0,122	124,958589	102,447408	100,496936	88,8686434	57,3629286	46,4676164	45,2128541	41,0510187	
0,0312 MIC	0,01525	0,061	126,113964	86,3591188	90,8936558	51,7351333	46,2688421	41,8958092	31,2862349	5,8555574	
	µg/ml	µg/ml									

Fig. 1. Checkerboard test results as FICI of combinations of a methanol twig extract of *S. aurita* (Extr) with tetracycline (Atb) against *Bacillus cereus*. The y-axis shows tetracycline and the x-axis the plant extract at their MIC related concentrations in Eppendorf tubes and in the microplate wells. The green colour indicates synergistic, the blue additive and the orange intermediate effects.

Keywords: *Salix myrsinifolia, S. aurita, S. starkeana,* antibiotic potentiating

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S1.P179 HPLC-MS and 2D-TLC analysis of secondary metabolites from the leaves of selected species and hybrids from the genus *Populus* with the estimation of their antioxidant properties

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Rheumatic diseases require long-term therapy, and usually steroid and non-steroidal anti- inflammatory drugs are used, which cause many side effects. Therefore, there is a growing interest among patients in medicines of plant origin, which effectively alleviate the symptoms associated with rheumatic diseases and are characterized by high safety of use (Uehleke et al., 2011). One of the herbal medicinal products used to treat rheumatism are poplar leaves (*Populi folium*). Currently, the pharmaceutical industry has problems with obtaining plant raw material that meets the requirements contained in the *Populi folium* monograph in the Polish Pharmacopoeia due to the increasingly rare occurrence of the *Populus nigra* species, which is the main source of plant raw material. Therefore, research is being conducted on other *Populus* species regarding compounds with high antioxidant and anti-inflammatory activity. This study presents the HPLC-MS analysis of leaves from three taxa from *Populus* genus - *P. alba*, *P.× candicans* and *P. nigra*, selected in our previous studies (Pobłocka- Olech et al., 2021). Moreover, the separation of secondary metabolites occurring in analyzed poplar leaves by 2D-TLC method were optimized and used for bioauthography of their antioxidant and anti- inflammatory properties. The antioxidant activity was estimated by DPPH, ABTS and FRAP assays. In the results, it was found that all analyzed leaves were characterized by the similar contents of phenolics (81.75-85.29 mg/g d.w.), flavonoids (6.23-8.69 mg/g d.w.) and differential content of salicylic derivatives (4.42-36.16 mg/g d.w.). The highest antioxidant activity was assessed for the leaves of *P. nigra,* and similar for *P.× candicans.*

Keywords: *Populus,* phytochemical analysis, antioxidant activity

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S1.P180 Antibacterial and phytochemical study of Bornean Myristicaceae and Clusiaceae species

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Antimicrobial resistance is a leading cause of morbidity and mortality worldwide. It is defined as the resistance of a microorganism to an antimicrobial agent, to which it was originally susceptible (de Sousa Oliveira, K. et al. 2016). In 2019, 1.3 million deaths were directly caused by antibiotic resistant infections, with estimations that by 2050, such infections could cause 10 million annual premature deaths worldwide, as well as a large economic burden (Murray et al. 2022; O'Neill, J. 2018). The island of Borneo has remarkable biodiversity, including a variety of unexplored plant species, some of which belong to families known for their pharmacological activities, such as the Myristicaceae and Clusiaceae. This project investigates the bioactivity and phytochemistry of the previously unexplored plant species *Horsfieldia splendida, H. polyspherula, Knema elmerii, K. latifolia, K. membranifolia, Gymnacranthera contracta, G. forbesii* (Myristicaceae), *Garcinia caudiculata, Calophyllum pulcherimum* and *Mesua calciphila* (Clusiaceae). Here, biological assays including broth microdilution and AlamarBlue, as well as chromatographic techniques (TLC, VLC and HPLC), spectroscopic techniques (LC-MS, NMR) and x-ray crystallography are used to isolate plant metabolites and assess their antibacterial and anticancer potentials. To date, 7 Myristicaceae and 3 Clusiaceae species have been extracted and assessed for antibacterial activities (MIC ≤ 64 µg/mL against *Staphylococcus aureus*). Following this, two known (example, Figure 1) and two novel anacardic acids, as well as one resorcinol and two acetophenones have been isolated from *K. membranifolia.* In addition, one known vitamin E derivative, alpha-tocopherol quinone has been isolated from *G. contracta*. Lastly, two lactone compounds, including one novel, have been isolated from *G. caudiculata*. Furthermore, potent antibacterial activities have been demonstrated by these compounds for the first time. Spectroscopic analysis of further isolated compounds is being undertaken.

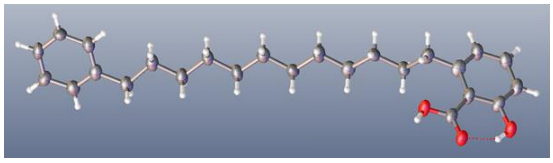

Fig. 1. 2-Hydroxy-6-(12-phenyl dodecyl)benzoic acid isolated for the first time from Knema membranifolia (Myristicaeae).

Keywords: ethnopharmacology, antimicrobial resistance, secondary metabolites, x-ray crystallography, NMR

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S1.P181 Screening for inhibition of β-haematin formation in *Rhaphiostylis beninensis* (Hook. f. ex Planch) Planch ex Benth. (Icacinaceae)

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Rhaphiostylis beninensis leaf has demonstrated antimalarial activity in a murine antimalarial model (Oluseje, 2016), but the mode of antimalarial action (important in rational antiplasmodial drug discovery) appears lacking in literature. Therefore, the screening of leaves and roots of *R. beninensis* for inhibition of β-haematin formation is hereby presented. Methanol and 70 % ethanol extracts (25 mg/mL) of each of air-dried, powdered leaves and roots as well as root acidified-water extract (25 mg/mL) of the plant were screened for inhibition of β-haematin formation by modified method of Vargas *et al.*, 2011. Quinine hemi-succinate (10 mg/mL) was used as reference antiplasmodial drug. Activity (Ianalysis, Absorbance Unit, AU) was detected by formation of pink colour and a positive net absorbance value; maximal inhibition was equivalent to absorbance of initial haematin in reaction mixture. The chemical profiles of major groups of secondary metabolites present were also obtained by chromatography (TLC, LC/DAD) followed by quantitative estimation. The hydroethanol extracts of both plant parts had higher Ianalysis than their methanol extracts: 0.173±0.127/ 0.115±0.036 AU and 0.121±0.051/0.091±0.051 AU for hydroethanol and methanol extracts of leaf/roots, respectively. The acidified water extract had Ianalysis of 0.113±0.070 AU. The reference drug had Ianalysis, 0.971±0.141 AU. Terpenoids, phenolics and alkaloids were detected in the parts especially in the hydroethanol extracts. Higher levels of terpenoids and phenolics occurred in the hydroethanol extracts of leaves.

R. beninensis showed inhibition of β-haematin formation in leaves and roots possibly due to the detected secondary metabolites suggesting potential for discovery of inhibitors.

Keywords: *Rhaphiostylis beninensis,* inhibition of β-haematin formation, antimalarial, LC/DAD

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S1.P182 New bryostatin derivatives from the North Atlantic Nudibranch *Janolus cristatus*

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Bryostatin-1 is a macrocyclic lactone first isolated from the bryozoan *Bugula neritina* in 1960 (Pettit et al., 1982). It was identified as a potent modulator of protein kinase C (PKC) and in the 1990's quickly progressed through phase I and II clinical trials for cancer treatment (Mohammad et al., 1993). Subsequently, bryostatin-1 has been under investigation for its potential in treating Alzheimer's disease and HIV. However, a significant challenge from the trials of Bryostatin-1 was its limited supply due to low yield within the bryozoan and complexity of its synthesis (Kollár et al., 2014). This underscores the importance of exploring alternative sources of Bryostatins. As part of our ongoing project to isolate bioactive compounds from Irish marine invertebrate, we conducted extensive screening against lymphoma cell lines and identified the nudibranch *Janolus cristatus* exhibiting potent and selective activity. The purification from 1g of *J. cristatus* led to the isolation of the known bryostatin-13 alongside a new C-18 demethylated bryostatin. Further analysis of unpure fractions also revealed the presence of the known bryostatin-11, and two other new C-20 deoxybryostatin derivatives. This study marks the first isolation of bryostatin derivatives from a mollusk. It is widely recognized that nudibranchs typically acquire chemical defence metabolites from their diet, and *J. cristatus* is reported to feed on *B. neritina* (Trindade-Silva et al., 2010). *J. cristatus* was found to exclusively contain C-20 deoxybryostatin derivatives potentially indicating a selective uptake of these specific analogues. Furthermore, *J. cristatus* was found to significantly bioaccumulate and concentrate the bryostatins, as evidenced by the quantity of material isolated.

Keywords: bryostatin, lymphoma, Nudibranch, Ireland

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S1.P183 Study of the metabolic diversity of Antarctic endophytic fungus *Aspergillus unguis* in relation to different macroalgal hosts and environmental stressors

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The extreme environmental conditions of the Antarctic continent formed organisms with unique adaptive abilities and intricated molecular relations. For that reason, the interaction between endophytic fungi and their macroalgal hosts are characterized by the production of unique metabolites (Araújo et al. 2019). To study the diversity of these commercial interesting compounds, the present study focused on the endophytic fungus *Aspergillus unguis.* In a multifactorial approach strain from two different macroalgal hosts, the Rhodophyte *Palmaria decipiens* and the Phaeophyceae *Ascoseira mirabilis,* were subjected to different environmental key factors (e.g., light regime and cultivation media) and subsequent metabolic characterizations were performed by UHLPC-UV-DAD-MS/M. This research sheds light on the diversity and dynamics of metabolites produced by *A. unguis* and might support further targeted metabolite extractions.

Keywords: marine natural products, *Aspergillus unguis*, macroalgal hosts, UVB

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S1.P184 Oxidation studies on selected betacyanins from the fruits of *Hylocereus polyrhizus* under influence of ABTS cation radicals

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Betalains, derived from tyrosine, include red-violet betacyanins and yellow-orange betaxanthins. In industry, these pigments can be valued for their potent coloring capabilities owing to their high extinction coefficients. Moreover, they exhibit antioxidant properties due to their ability to scavenge free radicals (Chander et al., 2008). In addition to their industrial utility, betalains demonstrate a spectrum of health benefits, including the induction of phase II enzymes, implicated in cancer protection (Lee et al., 2005), and the inhibition of Intercellular Adhesion Molecule-1 expression (Gentile et al., 2005). Our studies explored dehydrogenation process of selected betacyanins alongside with identifying newly formed derivatives through oxidation. The main objective was to decipher the mechanistic complexities underlying the interaction of cation radicals with betacyanins, thus increasing our understanding of the factors limiting betalain utilization in both food and pharmaceutical sectors. A dehydrogenation study was performed on three pigments present in *Hylocereus polyrhizus* fruits: betanin, as well as its acylated derivatives, phyllocactin and hylocerenin. The isolation of the pigments was conducted using preparative chromatography in a reversed-phase system. The composition of fractions obtained from preparative separations was examined using a mass spectrometer coupled with an HPLC system. The influence of the effect of the degree of the pigment extract purification on the stability of the pigments was performed. The results indicate that degradation of the pigments through reaction with the ABTS cation radicals not only catalyzes the dehydrogenation reactions but also leads to the formation of decarboxylated derivatives.

Keywords: *betalains, betacyanins, Hylocereus polyrhizus, dehydrogenation*

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S1.P185 Identification of novel betaxanthin pigments in jewel-like fruits of *Talinum paniculatum* (Jact.) Gaertn

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Betaxanthins belong to the unique pigments occurring in Caryophyllales order plants providing them yellowish color. Due to their health-promoting properties, natural sources of these pigments are continually sought (Kumorkiewicz-Jamro et al. 2021). Unlike betacyanins, betaxanthin pigments can be readily synthesized by coupling selected amines or amino acids to betalamic acid (Schliemann, et al., 1999). Previous studies have demonstrated that a plant of the *Talinum* genus – *Talinum triangulare* (Jacq.) Willd – produces dopamine-betaxanthin and tyrosine-betaxanthin (Swarna et al., 2013). However, *Talinum paniculatum* has not yet been studied for its betaxanthin profile. The plant belongs to a group of herbs used in folk medicine. It is distinguished by its succulent leaves, purplish flowers and yellowish fruits located on long stems (Tolouei et al., 2019). This study determined betaxanthin profile for fruits of *T. paniculatum* using liquid chromatography coupled with mass spectrometry. The results of the analyses indicated the presence of two novel, previously unknown betaxanthin derivatives with *m/z* ratio of 361. These compounds were identified as synephrine- betaxanthin and phenylephrine-betaxanthin. Their presence in the extract was confirmed based on the synthesized standards formed by coupling respective molecules with betalamic acid produced during betanin hydrolysis. These pigments were purified using flash chromatography and preparative high-performance liquid chromatography. The structures were confirmed by high-resolution mass spectrometry (LC-Q-Orbitrap-MS) and NMR spectroscopy. The finding of novel betaxanthin pigments in herbaceous plants suggests a promising direction for future research, including investigating their potential bioactive properties. Furthermore, this represents promising prospect for using betaxanthins for food fortification.

Keywords: *Talinum paniculatum,* betaxanthins, mass spectrometry, chromatography

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S1.P187 Evaluation of a standardized *Melissa officinalis* extract anti-obesity effects in high fat high sugar diet-induced obesity in rat

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Many studies suggest the use of *Melissa officinalis* extracts (MOE) to decrease anxiety. However, their anti-obesity effects remain insufficiently documented. In this context, the anti-obesity effect of a standardized MOE was evaluated in an induced obesity rat model, and compared to one of it's the main active compound: Rosmarinic acid (RA). Thirty-two 9-week-old Wistar rats were divided into 4 groups. A control group fed with a standard diet (ctSTD) and 3 experimental groups fed with High Fat High Sugar Diet (HFHSD) and respectively supplemented with placebo (ctHFHSD, 200 mg/kg), MOE (MOE200, 200 mg/kg) and RA (RA10.8, 10.8 mg/kg). The trial duration was 12 weeks and several measures linked to obesity such as body weight, adiposity index, abdominal circumference, glucose tolerance, and Total Antioxidant Status (TAS) from rats' livers were monitored at the end of the trial. Results showed that HFHSD significantly increased Body weight, adiposity Index, abdominal Circumference, and reduced glucose tolerance compared to ctSTD group ($p<0.05$ to $p<0.005$). These effects were attenuated by MOE and RA supplementations. As expected, the TAS of the ctHFHSD group was also significantly lower than the TAS of the ctSTD group. Nevertheless, surprisingly, TAS of rats supplemented with MOE and RA was also lower than the TAS of ctHFHSD group. These results highlight the advantage of MOE and RA supplementation to reduce visceral obesity. However, their anti-obesity effect was not correlated with TAS increase, suggesting that their metabolic benefit involves antioxidant-independent mechanisms. Further studies are underway to confirm this hypothesis.

Keywords: *Melissa officinalis,* rosmarinic acid, obesity, rats

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S1.P188 Biotransformation and antimicrobial studies of flavonoids with bromine and chlorine atoms

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Flavonoids are known for their anti-inflammatory properties, antioxidant, and antimicrobial ones. However, due to their low water solubility, orally administered flavonoids are poorly absorbed, and therefore, their therapeutic potential is limited (Karak et al., 2019; Lewin et al., 2013). It can be improved using glycosylation via biotransformation, which has been proven to be an effective way to increase water solubility and bioavailability of flavonoids (Yang et al., 2016; Hofer, 2016). In our study, we carried out the biotransformation of chalcone, flavanone, and flavone, which contained bromine and chlorine atoms, using the strains *Beauveria bassiana* KCH J1.5, *Isaria fumosorosea* KCH J2 and *Isaria farinose* KCH J2.6. Then, we checked the antimicrobial activities of aglycones and one glycoside 8-bromo-6-chloroflavone 4'-*O*-β-D-(4"-*O*-methyl)-glucopyranoside on four pathogenic and four probiotic bacteria. We measured the effect of flavonoids on bacteria growth over a 72-hour period at hourly intervals using a microplate reader. The chalcone exhibited highest antibacterial properties, followed by flavones and then flavanones. We also noticed that the glycoside derivative had enhanced antimicrobial effect against pathogenic bacteria, while being less harmful to probiotic bacteria than the corresponding aglycone. Our research suggests that bromine and chlorine atoms in flavonoids increase their antimicrobial properties, and that attachment of a glycoside unit to a flavone selectively enhances its bactericidal properties and bioavailability of the compound. This study adds to the knowledge about compounds not previously described in the literature with their antibacterial properties and further potential for their use in industry (Perz et al., 2023).

Keywords: flavonoids, biotransformations, entomopathogenic filamentous fungi, antimicrobial activity

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S1.P189 *In situ* approaches to marine and terrestrial metabolomics

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This poster will cover *in situ* chemical detection of secondary metabolites in marine environments. *In situ* metabolomics allows for more unbiased studies of complex microbial systems, natural metabolite expression and interactions between microbial communities than what is afforded by traditional laboratory culturing of microbes (Tomm et al., 2019). Historically *in situ* detection has been difficult, but with advances in both instruments, capture methods, and metabolomic approaches it is a promising avenue for answering basic research questions about the microbial world, as well as identifying novel and useful compounds for real world applications. Marine-based *in situ* systems have been studied utilizing custom designed devices for *in situ* transcriptomic and meta-genomic studies (Bech et al., 2024). These devices allow for the colonization of marine bacteria and formation of biofilms after placement in a natural harbor environment. Combining exo-metabolite capture via pumping of surrounding seawater through HP-20 resin, metabolomics of resulting biofilms and captured exo-metabolites, mass spectrometry imaging, transcriptomics and meta-genomics, key secondary metabolites can be identified and linked to the different life stages and organisms present during microbial colonization in marine environments. Additionally, these key metabolites are likely to be of great interest due to wide varieties of possible bioactivity associated with their ecological roles as marine microorganisms (Li et al., 2023).

Keywords: *in situ,* metabolomics, marine microbes, multi-omics, chemical ecology

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S1.P190 Correlation between saponin content and biological activities of *Beta vulgaris* L.

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Beta vulgaris L. represents functional food due to the presence of diverse bioactive compounds such as saponins, betalains, polyphenols, and minerals (Thiruvengadam et al., 2024). Saponins, which are natural glycosides, have exhibited varied pharmacological activities in clinical trials, effectively treating conditions such as type II diabetes, pancytopenia, and aplastic anemia (Galas et al., 2023). Triterpenoid saponins, notably prevalent in beetroot, are complex molecules composed of varied glycone and aglycone structures (Mikołajczyk-Bator et al., 2016). The diversity in structures and the proven pharmacological activity of certain saponins underscore significance of beetroot as a promising source of these compounds. The aim of the study is to investigate the correlation between saponin content in both the flesh and peel of five *B. vulgaris* varieties (Ceryl, Chrobry, Forono, Tytus, and Boldor) and their respective anticancer and antifungal activities. Using HPLC-ESI-MS/MS, 24 saponins with oleanolic acid, hederagenin, akebonoic acid, and gypsogenin as aglycones were identified. The Boldor cultivar (peel) exhibited the highest saponin content at 20.8 g/kg fresh weight. Notably, Tytus (peel) displayed the strongest antifungal activity against the majority of tested fungi (MIC = 0.125–0.5 mg/mL), and showed low cytotoxicity towards non-cancerous cells (CC50 = 405 µg/mL). Conversely, Tytus (flesh) exhibited high cytotoxicity against human cervical adenocarcinoma (HeLa) cells, with a CC50 of 282 µg/mL. Most saponins influenced the anticancer activity of beets against colon cancer cell line (RKO) and HeLa. Furthermore, betavulgarosides V and VIII, as well as compounds with m/z = 779 and 631, demonstrated effects on all tested fungi strains.

This research was funded in whole by National Science Centre, Poland for the years 2024–2027 (Project No. UMO-2023/49/N/NZ9/01706).

Keywords: *Beta vulgaris* L., saponins, anticancer, antifungal, LC-MS/MS

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S1.P191 A metabolomic approach in assessing the anti-biofilm potentials of *F. proliferatum*-a fungal endophyte isolated from an indigenous Nigerian medicinal plant

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Our research is aimed at isolating fungal endophytes from indigenous Nigerian medicinal plants and bio-prospecting their potential for the treatment of biofilm-forming methicillin-resistant *Staphylococcus aureus*. The fungal endophyte BLS1 was isolated from the stem of *Vernonia amygdalina*, an indigenous Nigerian medicinal plant used in the treatment of various ailments (Asante et al., 2016, Asante et al., 2019). DNA sequencing identified BLS1 as *Fusarium proliferatum* (Altschul, 1990).

Crude extract of BLS1, initially grown on malt agar, exhibited 96.3% antimicrobial activity, 99.8% biofilm prevention, and 67.3% biofilm eradication at concentration of 100 µg/ml. Culture optimization on rice, oat, malt extract and potato dextrose broth incubated at 7, 15, and 30 days determined the most suitable conditions to scale-up BLS1 on rice media for 15 days at highest extract yield with 89.5% antimicrobial activity while preventing biofilm formation at 100%. The scaled-up extract was fractionated by normal-phase flash chromatography using hexane and ethyl acetate. The polar fractions exhibited almost 100% antimicrobial and antibiofilm activity, while multivariate OPLS-DA of their NMR spectral data showed chemical shifts of 0-7 ppm representing fatty acids, acetylated compounds, hydroxylated aliphatics, and phenolics. Dereplication of the LC-HRMS data indicated that the antimicrobial- and antibiofilm-active fractions afforded known Fusarium chemotaxonomic markers, fusarin A and fusaric acid (Proctor et al., 2009).

Keywords: *Fusarium proliferatum,* antimicrobial, antibiofilm, fungal endophyte, MRSA

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S1.P192 Flavonoids content, anti-inflammatory effect and antioxidant activities methanol leaf extract of *Funtumia africana* (Benth)

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Funtumia africana (Apocynacea) a versatile plant in the Niger Delta region of Nigeria, have been used to treat various diseases, such as inflammation, diabetes and bacterial infections. Likewise flavonoids have received great attention due to the health benefits they offer varying from lowering blood pressure, inhibiting pro-inflammatory cytokines and protecting against oxidative stress (Al- Khayri et al., 2022).

This study was conducted to appraise the antioxidants activities and anti-inflammatory effect of aqueous methanol leaf extract of *F. africana*. The leaf extract was assessed for total phenol, flavonoids, total antioxidant capacity and free radical-scavenging activities. HPLC profiling of the plant sample was carried out for quantification of flavonoids. Acute anti-inflammatory activity was determined using the formalin induced paw edema method.

The antioxidant results show that *F. africana* has strong antioxidant activities (Table 1). HPLC fingerprinting gave kaempferol (154mg/100g), (+) – catechin (36.55 mg/100 g), naringin (35.90 mg/100 g), quercetin (35.67 mg/100 g), luteolin (31.26 mg/100 g) and apigenin (16.46 mg/100g) as the major flavonoids present in the sample. *F. africana* extract demonstrated significant anti- inflammatory effects in a dose-dependent manner. It showed significant reduction of the paw edema size in Wistar rats at 500 mg/kg dose compared to the standard drug (aspirin). The antioxidant and anti-inflammatory activities could be attributed to the high content of flavonoids and phenolics in *F. africana* leaves.

The presence of these phytocompounds might justify the use of the plant in the treatment of inflammation and other degenerate diseases by traditional practitioners in Niger Delta region of Nigeria.

Keywords: *Funtumia africana,* anti-inflammation, antioxidant, flavonoids

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S1.P193 Demystifying Bangladeshi ethnomedicines with modern approaches: *Congea tomentosa* as a source of potential anti-microbial and anti-cancer agents

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The traditional healers of indigenous communities of Bangladesh use several plant parts, with often unknown ingredients, for the treatment of cancers and microbial diseases. To investigate such treatments, we documented the traditional use of 276 ethnomedicinal plant species, utilising 292 informants of Bangladesh. Notably, 28 species were reported with new therapeutic uses and 13 species described have never been studied. Of these, *Congea tomentosa* was selected for *in-vitro* studies. Bioassay-guided screening led us to identify 10 compounds, and 5 compounds were isolated and identified. Among the isolated compounds, compound-2 (Stigmasterol) displayed promising antimicrobial activity against tested microorganisms, while compound-4 (5,6-Dimethoxy-2-methyl- 1H-benzoimidazole) significantly decreased the ratio of Bcl-2/Bax and increased the expression levels of cleaved caspases-9 and -3, suggested that compound-4 had the potential to induce apoptosis in U-251 cells, through activation of the intrinsic/mitochondrial pathway which might be triggered by the inhibition of Stat3 and Akt expression. Despite the ethnobotanical importance of *Congea tomentosa*, no chemical or biological studies have been published to date which support its traditional applications in Bangladesh. However, our study demonstrates the possible use of this plant in the treatment of cancers/microbial diseases, and support its traditional use.

Keywords: ethnobotany, *Congea tomentosa,* cytotoxicity, antimicrobial activity, apoptosis

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S1.P194 In silico testing of flavonoids of the genus *Mentha* (Lamiaceae) as potential inhibitors of NS3- protease domains of dengue viruses

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Dengue fever is a vector-borne disease with 4 serotypes. From 2000-2019, the WHO documented a ten-fold surge in reported cases worldwide, as cases skyrocketed from 500,000 to 5.2 million. In 2023, 80 countries/ territories cumulatively reported over 5 million dengue cases and over 5,000 dengue-related deaths. Clinical symptoms of the virus include severe headache, muscle and joint pain, nausea, vomiting, swollen glands, rash, and high fever. Currently, only one vaccine exists for the virus as a preventative measure, but according to the CDC, no antivirals have been identified to be used for treatment. In the present work, the flavonoids of medicinal plants (Eftekhari et al., 2021) known to block the virus infection in vitro were explored against the DENV NS3-protease of 4 serotypes. UCSF Chimera and AutodockVina were utilized for docking and optimal binding visualization to obtain the binding affinity scores for each ligand and DENV protein. Finally, the best ligands were evaluated by molecular dynamic simulations through GROMACS software.

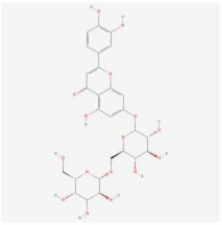

Fig 1. Structure of luteolin-7-*O*-rutinoside (LNR26)

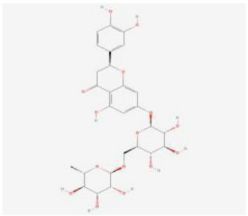

Fig 2. Structure of eriocitrin (ETN13)

Molecular docking, binding affinity scores suggested that the luteolin-7-*O*-rutinoside (**Fig 1.**) and eriocitrin (**Fig 2.**) compounds are able to tightly bind to NS3-pro. These molecules were then subjected to molecular dynamics to determine and visualize how they would bind to the DENV proteases.

Results indicate that LNR26 and ETN13 could block NS3-pro activity in DENV. For further research, follow-up in vitro experiments can be conducted to ensure the integrity of results, which can be later utilized for drug development.

Keywords: Dengue virus, NS3-protease, Molecular docking, *Mentha* sp., flavonoids

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S1.P195 Application of feature based molecular network analysis for the investigation of the Covid-19 remedy Hanshiyi

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In the nascent phase of the Covid-19 pandemic, the Traditional Chinese Medicine (TCM) formula known as Hanshiyi (HSYF) was developed with the aim of mitigating the risk of severe disease progression among patients (Tian et al., 2020). Comprising primarily botanical ingredients, HSYF consists of 20 components. Previous investigations have detailed the chemical composition through the application of analytical techniques such as High-Performance Thin-Layer Chromatography (HPTLC) and Liquid Chromatography-Mass Spectrometry (LC-MS) (Tiefenbacher et al., 2022), along with the exploration of its pharmacological properties (Tiefenbacher et al., 2023).

This study introduces the utilization of feature-based molecular network analysis to delve into the constituents of HSYF. Different decoctions, were prepared and subjected to LC-MS analysis using an Ultimate 3000 RS system coupled with a Q Exactive Orbitrap Mass Spectrometer. Subsequently, the acquired data underwent conversion; alignment and were then exported to the Global Natural Products Social Molecular Networking (GNPS) platform (Aron et al., 2020). Employing Cytoscape, the resulting visualizations revealed 1536 nodes and 2533 edges in the positive mode, and 2456 nodes and 3897 edges in the negative mode. For the validation of the proposed compounds, 108 reference substances were utilized, with approximately 60 confirmed to be constituents of the mixture. Consequently, feature-based molecular network analysis emerges as a contemporary and valuable approach, amalgamating traditional analytical methods with innovative database-driven methodologies.

Keywords: Hanshiyi, TCM, Covid-19, feature based molecular network, LC-MS

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S1.P196 Pathophysiological assessment of trypanocidal activitys of ethanolic leaf extract and fractions of A*zadiractha indica* on infected experimental model

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Trypanosomiasis is a vector borne parasitic infection caused by genus Trypanosome.This infection precipitates increased red blood cell destruction that predispose the host to anemia and tissue damage (Sirak, *et al.,* 2024;).The pathophysiological evaluation of the trypanocidal activity of ethanolic leaf extract and fractions of A*zadiractha indica* was studied. Phytochemical and toxicity studies were done using standard procedures (Mikail, 2009). A total number of 25 rats (5 groups (A - E) of 5 rats) were used for the study. All the groups were induced with 0.1ml of Trypanosome inoculum containing 10^6 of Trypanosome except the positive control. Groups A and B were treated with 400mg/kg and 600mg/kg (b.wt.) respectively for 5 days after the establishment of parasitemia. Groups C, D and E served as positive and negative controls. At the end of therapeutic study, the organs (liver and kidney) were harvested for histopathological studies.

The phytochemical screening revealed the presence of flavonoids, terpenoids, tannis, alkaloids, reducing sugar, phenols and saponins. The oral medial lethal dose(LD_{50}) of the extract was 3162mg/kg. Treatment with 600 mg/kg b.wt. cleared the parasite from circulating blood on day 9 post treatment(pt) similar to the positive control group. Out of the four fractions tested, Ethyl acetate fraction showed the highest activity compared to other test groups. This study established that the crude extract and fractions of *A. indica* were active against *T. brucei brucei* sub specie which has not been reported. Histopathological studies confirmed no infiltration of the intracellular cells of the organs examined. Therefore treatment with *A. indica m*ay not cause any serious toxic effects on the biological system of the host if utilized as a potential treatment for trypanosomiasis. Characterization of the most active fraction is ongoing.

Keywords: *Trypanosoma brucei brucei, Azadiractha indica*, phytotherapeutic, histopathology and albino rats

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S1.P197 Antioxidant activity and Structural-Activity Relationship (SAR) study of stilbenoids isolated from the stem bark of *Anisoptera laevis* (Dipterocarpaceae)

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Anisoptera laevis, a genus in the family of Dipterocarpaceae, is well-known to contain abundant sources of stilbenoids, exhibited a wide range of biological activities (Duta-Bratu et al., 2023). This study revealed antioxidant activities and structural-activity relationships of stilbenoids isolated from *A. laevis*. The stem bark of *A. laevis* (900g) was collected from Pahang, Malaysia and macerated in acetone for 24 hours, concentrated in vacuo to yield crude extract (65g). Fractionation using VLC, *n*- hexane:ethyl acetate as eluent gave eight fractions (AL1-8). Piceid (**1**) (4.0mg) and resveratrol-12-*C*- β-D-glucopyranoside (**2**) (1.9mg), were obtained by purifying AL5 (1.73g) via column chromatography (CC). Vaticanol B (**3**) (204mg) was obtained from AL4 (1g) using CC, solvent- system chloroform:methanol. The antioxidant activities of isolated stilbenoids were assessed using DPPH, ABTS and hydroxyl radicals scavenging.

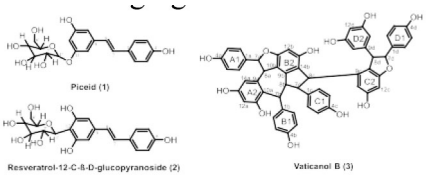

Fig. 1. Structures of compounds **1**-**3**

Compounds (**1**-**3**) (Fig.1) demonstrated significant efficacy in scavenging DPPH radicals, as evidenced by their respective IC_{50} values of 69.00, 50.58 and 66.34 µg/mL. Compounds **2** and **3** exhibited very strong activity against ABTS radicals (IC_{50}=16.44, 33.43µg/mL, respectively), while compound **1** demonstrated strong activity at 63.57 µg/mL. Compounds **1** and **2** exhibited potent antioxidant activity in scavenging hydroxyl radicals (IC_{50}=20.06, 17.08µg/mL, respectively), while compound **3** demonstrated weak antioxidant effect (IC_{50} =248.5µg/mL). The most potent antioxidant activity was observed in Compound **2**. This results indicated that the position of a sugar moiety (Xie et al., 2022) and the degree of polymerisation are the factors contributing the antioxidant activity. We would like to express our appreciation to UiTM, Malaysia for the financial support [600-UITMSEL (PI. 5/4) (127/2022)]

Keywords: *Anisoptera laevis,* stilbenoids, antioxidant activity, structural-activity relationship

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S1.P198 Resveratrol dimer and tetramers from the isolation of *Shorea pauciflora* King. (Dipterocarpaceae)

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Polyphenols are a broad chemical class of phytochemicals, with the stilbenoid (resveratrol) subclass being extensively studied. Both individual compounds and plant extracts enriched with stilbenoids have been widely examined (Kasiotis et al., 2013, Shen et al., 2009) due to their diverse biological effects (Duta-Bratu et al., 2023). Stilbenoids have garnered attention for their isolation and investigation as monomers, oligomers and glycosylated derivatives. In continuation to the previous research, the phytochemical study from the stem bark of *Shorea pauciflora* has successfully afforded three resveratrol oligomers. Fractionation of the crude acetone extract (148 g) was executed using vacuum liquid chromatography with the increasing polarity of *n*- hexane:ethyl acetate and ethyl acetate:methanol to give ten semi-purified fractions. Compounds **1** and **2** were obtained from fraction 9 (1 g) using column chromatography twice, with *n*-hexane: ethyl acetate followed by chloroform: methanol as a solvent system, respectively. Fraction 4 (1 g) was also subjected to column chromatography followed by purification using preparative thin layer chromatography with chloroform: methanol as eluent to obtain compound **3**. Based on the spectroscopic analyses and comparison with reported data, the compounds were elucidated as resveratrol tetramers, namely isohopeaphenol (**1**) (Kang et al., 2020) and hemsleyanol D (**2**) (Lim et al., 2023) as well as a new resveratrol dimer, compound Z (**3**) (Fig. 1). This research was supported by the Ministry of Higher Education, Malaysia through the Fundamental Research Grant Scheme (FRGS/1/2022/STG04/UITM/02/24).

Keywords: *Shorea pauciflora*, resveratrols, isohopeaphenol, hemsleyanol D, new compound Z

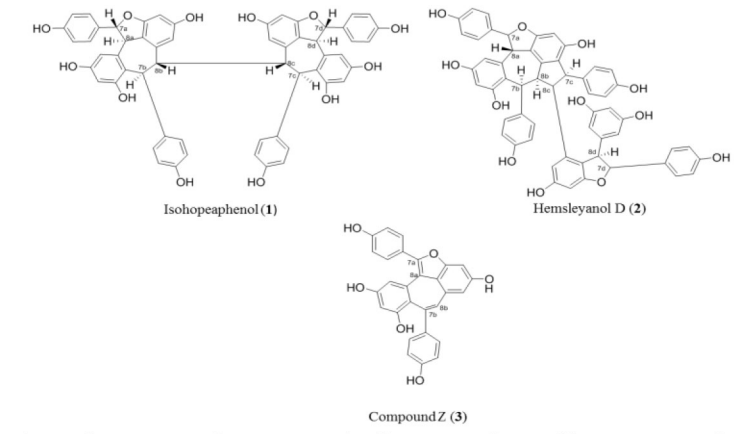

Fig 1. Structure of resveratrol oligomers from *Shorea pauciflora*

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S1.P199 In-silico and *in-vitro* evaluation of natural product scaffolds to overcome the scourge of drug resistant tuberculosis by virtual based drug design approach

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In pursuit of new anti-tubercular agents, we here report the anti-mycobacterial ($H_{37}Rv$) and DNA gyrase inhibitory potential of daidzein and khellin natural products (NPs). We procured a total of 16 NPs based on their pharmacophoric similarities with known antimycobacterial compounds (Coronado-Aceves et al., 2017, Krishna et al., 2019). The $H_{37}Rv$ strain of M. tuberculosis was found to be susceptible to only two out of the 16 NPs procured; specifically, daidzein and khellin each exhibited an MIC of 25 µg/mL. Moreover, daidzein and khellin inhibited the DNA gyrase enzyme with IC_{50} values of 0.042 and 0.822 µg/mL, respectively, compared to ciprofloxacin with an IC_{50} value of 0.018 µg/mL. Daidzein and khellin were found to have lower toxicity toward the vero cell line, with IC_{50} values of 160.81 and 300.23 µg/mL, respectively. Further, molecular docking study and MD simulation of daidzein **(Fig. 1)** indicated that it remained stable inside the cavity of DNA GyrB domain for 100 ns.

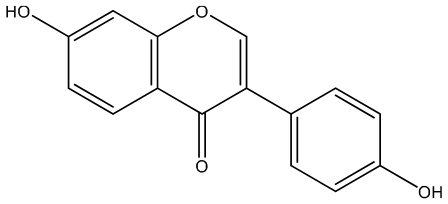

Fig. 1. Structure of daidzein natural product

Keywords: Tuberculosis, anti-mycobacterials, natural products, daidzein, DNA gyrase

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S1.P200 Exploring Malaysian biodiversity in the fight against antibiotic resistance

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Carbapenemases are enzymes produced as a resistance mechanism by various bacteria to hydrolyse carbapenem antibiotics. These enzymes propagate rapidly, contributing to the alarming ineffectiveness of these kind of large spectrum antibiotics all over the world (Taconelli *et al.*, 2018). A series of 824 Rutaceae and Annonaceae plant extracts from the extract library of the Institut de Chimie de Substances Naturelles (Gif-sur-Yvette, France) was screened for inhbition of three of the most extended varieties of carbapenemase enzymes: KPC-2, OXA-48 and NDM-1 (Bush *et al.*, 2020).

The bark extract of *Fissistigma litseaefolium* (King) Merr, a Malaysian endemic climber Annonaceae species, was found active and was thus selected for a bioassay-guided isolation. The fact that this species has not been explored before from a metabolomic standpoint added an extra dimension to its study. A comparison of the metabolome of this species with others of the genera by molecular networking using MetGem software (Olivon *et al.*, 2018) and annotation tools revealed differences and guided to the isolation of novel structures.

Two new molecules derived from an original double cyclisation of a prenylated-chalcone have been isolated. Their absolute configuration has been determined thanks to X-Ray spectroscopy. All these results **(1)**, as well as the proposed biosynthetic pathway conducting to these structures and hemisynthesis will be presented.

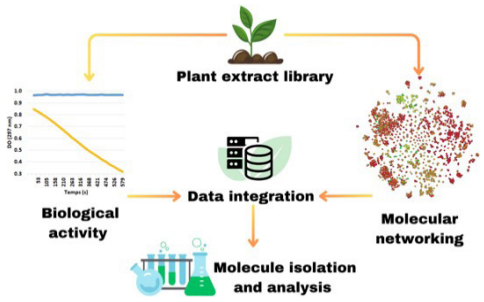

Fig. 1. Toward carbapenemase inhibitors isolation from ICSN's extract library

Keywords: *Fissistigma sp.*, carbapenemases, molecular networking, isolation, hemisynthesis

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S1.P201 Effect of seasonal and geographical location on the secondary metabolic contents of *Artemisia afra* and *Artemisia annua*. Anti-plasmodial properties

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Malaria is a disease of public health concern, and natural products have been investigated as potential treatments. Due to the emergence of resistance to Artemisinin, traditional medicinal preparation using *A. afra* and *A. annua* is practiced in Africa where malaria is endemic. This study explores the phytochemical diversity and anti-plasmodial potential of *A. annua* and *A. afra* sourced from distinct geographical locations within Cameroon aiming to discern potential variations in phytochemical profiles and correlated to observed activity. Crude extracts were prepared from plants collected from diverse regions in Cameroon during the rainy/dry seasons and metabolic contents analyzed by TLC, HPLC, and GC. Additionally, anti-plasmodial potential was assessed on 3D7 *Plasmodium falciparum* strain. The activity profiles of the samples were correlated with their environment, with distinct phytochemical compositions observed for each sample based on its geographical origin and season of collection. Traces of artemisinin were detected in some *A. afra* samples but present in all *A. annua* samples with a high concentration in the rainy season samples (better anti-plasmodial activity). The Adamawa region had the highest artemisinin contents (8.9% m/m artemisinin in dry extract) with better anti-plasmodial activity. These findings suggest that the selection of a suitable *Artemisia* sample for use as a potential antimalarial treatment should take into consideration its geographical origin and season. By understanding how location and seasonal changes influence the phytochemical composition of *Artemisia* samples, this study may provide an insight into how natural products can be effectively used as a preventative or curative measure against malaria.

Keywords: malaria, *Artemisia annua*, *Artemisia afra*, resistance, *Plasmodium*

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S1.P202 Ecology, metabolome and occurrence of *Pseudallescheria sp.* associated with termites

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Termites are eusocial insects that live in homeostatic environments, confined spaces and with high population density (Liu *et al.*, 2019). This life style leads to an intimate association with microscopic life forms. These microorganisms can be parasites, mutualists, opportunists or pathogens (Douglas *et al.*, 2016). Until now, studies on the termite holobiont have focused on trophobiosis-related symbioses that allows the degradation of lignocellulose in the termite gut. However, exploration of other symbiotic relationships may show their ecological role in termite survival whilst environmental conditions may promote the transmission of infectious agents. It can also lead to the discovery of new chemical entities with therapeutic interest (Beemelmanns *et al.*, 2016).

During previous investigations carried out by ICSN, fungal strains of the genus *Pseudallescheria boydii*, from different termite species of French Guiana were identified (frequently associated with *Nasutitermes sp.*). The fungi's metabolome was analyzed. When co-inoculated with the entomopathogen *Beauveria sp.*, antimicrobial compounds like Tyroscherin and *N*-methyltyroscherin (Fig. 1) where overproduced by the symbiont (Sorres *et al.*, 2017, 2022), indicating a probable host defense role under biotic stress when in association with the termite species. Current studies aim to determine if this termite-*Pseudallescheria sp.* relationship is widespread focusing on the occurrence of this interaction in anthropophized and non-anthropophized environments. Today, 14 new nests from French Guiana are being analyzed by LC-HRMS/MS, using Tyroscherin as an indicator of the presence of fungi in the various parts of the macro-holobiont „termite nest" (including termites, nest and tunnels).

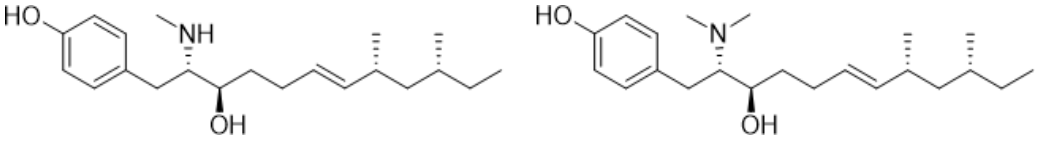

Fig. 1. Structure of Tyroscherin **1** and *N*-methyltyroscherin **2** from *Pseudallescheria sp.* strains.

Keywords: *Nasutitermes sp., Pseudallescheria sp., Tyroscherin, Symbiosis, Chemical ecology*

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S1.P203 *Tetracera alnifolia* (Wild) Drake popular in Guinean traditional medicines possesse some pharmacological activities

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Several parts of the woody plant *T. alnifolia* are traditionally used for treating infectious diseases, sexually transmitted infections, skin diseases, and malaria in Guinean traditional medicine (Baldé et al., 2020; Magassouba et al., 2007; Traore et al., 2013). In this context, based on the ethnomedical investigations conducted by IRDPMAG, *T. alnifolia* was selected to confirm some of its traditional uses in Guinea as well as to survey its biological properties We conducted an ethnomedical survey across several markets of the city of Conakry to identify and interview healers. Chloroform, methanol, dichloromethane, and aqueous extracts of *T. alnifolia* were tested for activities against protozoa, bacteria, fungi, HIV, and *SARS-CoV-2*.

39 traditional healers indicated that *T. alnifolia* is used in the treatment of more than 15 pathologies including *Fassa* (marasmus/malnutrition), *Soukhou kouyé* (white discharge in women), and *Tèmou bankhi* (sexual weakness in men). Leaves were the most used part. Modes of preparation included decoction and powder. Data from biological activities identicatied good activities of the methanolic extract against *Leishmania infantum* (MIC = 8.1 µg/ml) and a moderate activity against *Trypanosoma brucei* (MIC = 28.2 µg/ml) and *Staphylococcus aureus* (MIC = 29.9 µg/ml). Dichloromethane extracts inhibited up to 53.4% of *SARS-CoV-2* replication at 50 µg/mL.

These results explain at least in part the traditional use of *T. alnifolia*. Aiming to rationalise the use of *T. alnifolia,* bioassay-guided fractionations are in progress to determine useful fractions or molecules for clinical investigations.

Keywords: *Tetracera alnifolia,* ethnomedical investigations, biological activity, SARS-CoV-2, protozoa

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S1.P204 Specialized metabolites with antiviral properties from a Vietnamese plant: isolation and synthesis

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The plant metabolites group at the Institute of Chemistry of Natural Substances (ICSN) aims to study and valorize specialized metabolites isolated from plants. For that, the plant extract library named Extractothèque ICSN, containing more than 15,000 extracts, is regularly screened by biologist partners.

After the COVID-19 pandemic, as part of this project, a selection of 824 plant extracts from Extractothèque ICSN was evaluated on an inhibition cell-based assay on human Coronavirus (HCoV- 229E and SARS-CoV-2). The EtOAc and MeOH extracts of the leaves of the Vietnamese species *Melodorum fruticosum* were selected for their good activity on HCoV-229E. Bio-guided fractionation led to the isolation of 11 pure products, including 3 novel compounds. Their antiviral evaluation confirmed the interest of some metabolites with IC_{50} around 1 M for toussaintine C. To validate their activity and improve our knowledge of the structure-activity relationships, we first performed the total synthesis of racemic toussaintine C (Fig. 1) and developed a divergent synthesis of analogs. As one of the enantiomers of toussaintine C (Fig. 1) has been found to exhibit greater activity against HCoV-229E, we developed an asymmetric total synthesis leading to both enantiomers.

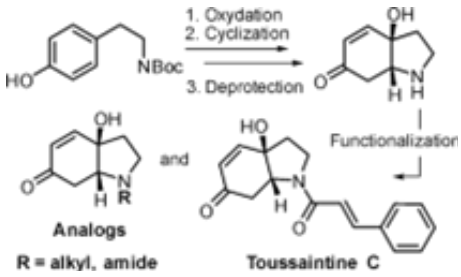

Fig. 1. Total synthesis pathway of toussaintine C and synthesis analogs *Keywords: Melodorum fruticosum,* HCoV-229E, SARS-CoV-2, antiviral, toussaintine C

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S1.P205 From signaling molecules to secondary metabolites: harnessing c-di-GMP for enhanced natural product biosynthesis in *Streptomyces*

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The regulatory mechanisms governing natural product biosynthesis in Streptomyces species have long intrigued researchers due to their potential in drug discovery and biotechnology. Among these mechanisms, cyclic di-GMP (c-di-GMP) signaling has emerged as a key player, orchestrating various cellular processes (1). In this study, we delve into the intricate world of c-di-GMP signaling in Streptomyces and its implications for natural products research. In addition to unraveling the interplay between c-di-GMP signaling and natural product biosynthesis, our study highlights Streptomyces' crucial role in antibiotic discovery, especially amidst rising resistance, tapping into their potential to produce new antibiotics.

Through capture compound experiments, utilizing a modified c-di-GMP molecule with a UV-reactive group for crosslinking and biotin-residue for affinity purification, we elucidated novel c-di-GMP effectors (2). This molecule was synthesized in-house. Investigating the strain Streptomyces sp. Tu6071, we are searching for potential targets involved in transcriptional regulation, that are crucial for activating new biosynthetic gene clusters.

These findings not only deepen our understanding of c-di-GMP signaling dynamics, but also offer promising avenues for manipulating natural product biosynthesis. In the future, these potential targets will be confirmed, and their direct connection to the biosynthesis of natural products will be elucidated.

Moreover, our study opens doors to the activation of silent gene clusters, harboring untapped reservoirs of bioactive compounds. Unraveling the intricate interplay between c-di-GMP signaling and natural product biosynthesis holds tremendous potential for accelerating drug discovery efforts and harnessing the vast chemical diversity of Streptomyces.

Keywords: c-di-GMP signaling, *Streptomyces*, natural products, capture compound experiments, gene regulation

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S1.P206 A novel natural product from the leaves of a *Solanum spec.* (Solanaceae) shows antimicrobial activity against multiple multi-resistant pathogens

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Due to the extensive use of antibiotics in both human medicine and factory farming, there is an alarming increase of infections with antibiotic-resistant bacteria that do not respond to standard therapy. These strains develop resistance to reserve antibiotics, posing a threat to public health (Frieri et al., 2017). Between 1981 and 2019 approximately 55 % of new antibiotics are natural products or their derivatives (Newman et al. 2020), and consequently, substances from natural sources are promising in drug development.

A new natural product was isolated and identified from the leaves of a wild potato species (Solanaceae) and was shown to be bioactive against the Colorado potato beetle (*Leptinotarsa decemlineata*) and plant pathogenic fungi (not published). Here we investigated this product for its activity against human pathogens.

Hence, seven different bacteria from the WHO-Pathogen-Priority-List (*Acinetobacter baumannii*, *Campylobacter jejuni*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and *Candida albicans* were tested against the natural product and the MIC-values were determined using the broth microdilution method of EUCAST. The substance showed activity against every pathogen (MIC between 1024 µg/mL and 32 µg/mL).

Atomic force microscope imaging indicated a short-term membrane toxic effect on the bacteria.

To assess the cytotoxicity, an MTT assay using the immortalized epithelial kidney cell line Vero E6 (*Cercopithecus aethiops*) and human primary fibroblasts was performed. These data indicated high toxicity (IC_{50} = 2.2 µg/mL and 1.2 µg/mL) making the natural product most likely to be unsuitable for use in human medicine to treat infections.

Keywords: *Solanum,* Solanaceae, anti-infective, cytotoxicity, bioactive

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S1.P207 Prenylated phenolics from Fabaceae as inhibitors of the multidrug efflux pump NorA

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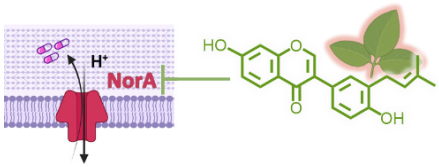
Considering their key role in the emerge of antimicrobial resistance (AMR), efflux pumps are an interesting target for the development of novel resistance-modifying agents, such as efflux pump inhibitors (EPIs). Among different efflux pumps, members of the Major Facilitator Superfamily (MFS) are the most prevalent efflux pumps in gram-positive bacteria, including *Staphylococcus aureus*, for which the overexpression of the MFS efflux ppump NorA is observed in 43% of strains, particularly in MRSA strains (Patel et al., 2010). EPIs are a promising solution as they can re-sensitize resistant bacteria to common antibiotics and reduce the emerge of AMR.

Plants are an excellent source of bioactive compounds, including EPIs. Some specific plant EPIs identified contain prenyl groups (isoprene moieties) in their backbone (Prasch & Bucar, 2015, Ika Irianti et al., 2023). These prenyl groups increase hydrophobicity of the molecule and their affinity towards biological targets. The Fabaceae plant family produces prenylated phenolics as main defence mechanism against microbial stress.



To understand the structure-function relationship as potential NorA EPIs, an extended collection of prenylated phenolics was investigated, belonging to different flavonoid and isoflavonoid subclasses, substituted with different number and configuration of prenyl groups. Characterization was done in terms of NorA inhibition using ethidium accumulation and antibiotic potentiation assays, using both *norA* overexpressing and knockout *S. aureus* strains. Cytotoxicity of promising candidates was assessed using Caco-2 cell lines. Given the chemical diversity of the collection investigated, an in- silico analysis was performed to understand key molecular properties for NorA inhibition.

Keywords: Prenylated isoflavonoids, Fabaceae, *Staphylococcus aureus*, NorA, efflux pump

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S1.P208 Improving the activity of pleuromutilin antibiotics

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With rising levels of antimicrobial resistance and the slow development of new antibiotics, there is increasing interest in re-vitalizing existing agents. Pleuromutilin is a diterpenoid natural product antibiotic that was first isolated in the 1950s from *Pleurotus mutilis* (now *Clitopilus scyphoides*) (Kavanagh, et al., 1952). The ease of modification of the C-14 glycolate side chain has led to the discovery of more active variants, however, they remain predominantly active against Gram-positive and mycoplasma bacteria (Egger et al., 1976, Goeth e et al., 2019).

In our ongoing efforts to develop antibiotics against Gram-negative bacteria, we synthesised a series of pleuromutilins with modified sidechains that explored several strategies to turn Gram-positive- only antibiotics into Gram-negative agents. The first is increasing the presence of ionizable amines which has been suggested to increase the uptake and accumulation of small molecule antibiotics in Gram-negative bacteria (Richter et al., 2017). We have also extended the sidechain with a variety of end groups through peptide coupling to facilitate targeted delivery (Fig. 1). The synthesis and antimicrobial activities of these derivatives, along with their cytotoxic and hemolytic activity, will be presented.

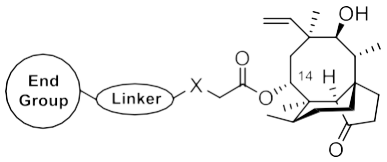

Fig. 1. General structure of pleuromutilin derivatives for targeted delivery. X is S or NH.

Keywords: pleuromutilin, antibiotics, antifungal activity, targeted delivery

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S1.P209 Bioactivity-guided investigations on biofilm inhibition with *Rubus chamaemorus* leaf extracts

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Cloudberry (*Rubus chamaemorus* L., Rosaceae) is known for its health-promoting fruits rich in antioxidants and historical use against scurvy (Luca et al., 2011; Kähkönen et al., 2001). While the fruits are well studied, cloudberry leaves remain less investigated (Fig.1). Traditional use as antidiarrheal and for wound treatment are reported. (Rocabado et al., 2008). Recent investigations show biofilm inhibition against multidrug-resistant Gram-negative (MRGN) *Escherichia coli* (Neumann et al., 2022). Further investigation of this plant is highly interesting, considering its potential for dual utilization of leaves and fruits, cultivation in CO_2-storing bogs, and therapeutic application against multidrug-resistant pathogens. This study examines cloudberry leaf extracts, fractions, and sub-fractions using a bioactivity-guided approach.

Fig. 1. Cloudberry leaves.

Five different methods were used subsequent to methanol extraction: solid phase extraction utilizing a reversed-phase column for extract fractionation, High-Performance Liquid Chromatography for a fingerprint of the extracts, size exclusion chromatography for further fractionation, and mass spectrometry to identify active compounds. In parallel to all extraction and separation steps, bioactivity-guided tests were performed using a macrocolony biofilm assay with *E. coli*.

One fraction exhibited biofilm inhibition against MRGN *E. coli* at 10 µg/mL, outperforming the crude extract at 30 µg/mL. Mass spectrometric analysis suggested sanguiin-H6 and lambertianin C, ellagitannins, as potential biofilm inhibition contributors. The fraction containing ellagic acid, quercetin-glucuronide, quercetin-galloylglucuronide, and kaempferol-glucuronide showed no biofilm inhibition. Ongoing assays aim to elucidate the potential involvement of quorum sensing in this biological activity. Understanding cloudberry's biofilm inhibition holds promise for phytopharmaceutical development and peatland preservation through cultivation.

Keywords: *Rubus chamaemorus*, anti-biofilm, MRGN *E. coli*, bioactivity-guided, tannins

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S1.P210 Insights into the nature of the microalgal toxins from the *Chrysochromulina leadbeateri* blooms in Northern Norwegian fjords

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This presentation will cover the discovery and analysis of two novel polyhydroxylated polyketides, leadbeaterin-1 (**1**) and leadbeaterin-2 (**2**), from *Chrysochromulina leadbeateri*, identified as potential fish-killing toxins. In May–June 2019, the microalga *C. leadbeateri* caused a massive fish-killing event in several fjords in Northern Norway, resulting in the largest direct impact ever on aquaculture in northern Europe due to toxic algae (Samdal and Edvardsen, 2020; John et al., 2022). Motivated by the fact that no algal toxins have previously been described from *C. leadbeateri*, we set out to investigate the chemical nature and toxicity of secondary metabolites in extracts of two strains (UIO 393, UIO 394) isolated from the 2019 bloom, as well as one older strain (UIO 035) isolated during a bloom in Northern Norway in 1991. Bioassay-guided fractionation using the RTgill-W1 cell line (Bols et al., 1994; Dorantes-Aranda et al., 2011) and metabolomics analysis pointed to a major compound affording $[M+H]^+$ ions at m/z 1399.8333 as a possible toxin. Moreover, our study unveiled a series of minor analogues exhibiting distinct patterns of chlorination and sulfation, together defining a new family of compounds, which we propose to name leadbeaterins. Leadbeaterin-1 (**1**) and leadbeaterin-2 (**2**) were isolated and purified from UIO 394 cultures. Their structures were determined by 1D and 2D NMR experiments and tandem mass spectrometry. Remarkably, these suspected toxins were detected *in situ* in samples collected during the 2019 bloom close to Tromsø, thereby substantiating their likely role in fish kills.

Keywords: *Chrysochromulina leadbeateri,* algal bloom, ichthyotoxins, bioassay-guided fractionation, RTgill-W1

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S1.P211 Revisiting F.P. Porcher's Resources of Southern Fields and Forests (pub. 1863): Insights into the Forgotten Medicinal Botany of the Southeastern United States

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Following the American Civil War, the Southeast United States was under blockade with limited the access to medical supplies. Dr. Francis Pyre Porcher, a physician and medicinal botanist at the Medical University of South Carolina was contracted to create a "repertory of scientific and popular knowledge as regards the medicinal, economical, and useful properties of the trees, plants, and shrubs found within the limits of the Confederate States." In this seminal work, Porcher reported on the uses of nearly 800 species of plants with a focus on those native to his home region. Discussed here, with a focus on infectious disease, is an overview of Porcher's reported medicinal plant uses. Additionally, a bioassay guided isolation of anti-mycobacterial compounds from *Baccharis halimifolia* is presented. Porcher describes *B. halimifolia* thus, "This plant is of undoubted value and of very general use in popular practice in South Carolina as a palliative demulcent in consumption and cough. I have frequently seen it used with advantage and have often heard those employing it confess the benefit derived from it… Like many other of our indigenous plants possessed of unequivocal utility, is unnoticed in the dispensatories and other works."

Keywords: *Mycobacterium, Baccharis*, antimicrobial, ethnobotany, medicinal plants

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S1.P212 Bioassay-guided interpretation of antimicrobial compounds from *Limonia acidissima*

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Medicinal plants are a primary source of natural products which are used for the treatment of various infections throughout the world (Abdallah et al., 2023). Among, many medicinal plants, *Limonia acidissima* L. (Wood apple), Rutaceae, emerged out to be one of the most valuable plants, owing multiple medicinal properties (Gopinath et al., 2023). The present study was conducted to investigate the antimicrobial components of *L. acidissima* through bioassay-guided purification against *Staphylococcus aureus, Micrococcus luteus*, *Escherichia coli, Pseudomonas aeruginosa*, *Candida albicans* and *Saccharomyces cerevisiae*. The active peaks were collected and identified via LC- MS/MS. For identification, the COCONUT Database and a manually built LA database were used, in combination with prediction and calculation of mass fragmentation and retention time. A total of 10 active compounds were identified, including 3 alkaloids, 4 flavonoids, 1 anthraquinone, and 1 oxidoreductase against *S. aureus*; 1 alkaloid, 1 flavonoid, and 1 anthraquinone against *E. coli;* and only 1 alkaloid was active against *C. albicans*. All of them exhibited strong antimicrobial activities with good potential to be developed as antibiotics.

Keywords: plants, microbes, infections, *Limonia acidissima*, chromatography

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S1.P213 Targeted isolation of potent antiviral cyclopeptides from tropical plant extracts

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Viral diseases represent a significant public health concern. The emergence of novel viruses, such as coronaviruses, and the resurgence of known pathogens such as dengue and Zika viruses, underscores the necessity for effective treatment options. The rich chemical diversity of plant metabolites is of great interest in the search for antiviral agents and requires innovative approaches to be explored.

In this context, biological assays were conducted on 824 plant extracts from the ICSN's extract library. Among them, eight extracts from phylogenetically related species exhibited significant inhibitory activities against Zika virus replication. The 824 samples were analyzed by LC- HRMS/MS, and the resulting data were processed using MZmine3 (Schmid et al., 2023). The metabolite content of the extracts was then organized and visualized as molecular networks using MetGem (Wang et al., 2016; Olivon et al., 2018). Overlaying the biological activity results onto the networks subsequently highlighted a cluster of ions highly specific to the eight active extracts (Fig. 1) (Olivon et al., 2017). Targeted isolation of the corresponding analogues afforded 8 undescribed cyclopeptides, which were further evaluated against a broader panel of viruses including dengue, chikungunya, Ross River and SARS-CoV-2 viruses. The most active compounds demonstrated antiviral activity in the nanomolar range, with low cytotoxicity. A medicinal chemistry project is underway for the development of an antiviral lead compound. Two patent applications are currently pending.

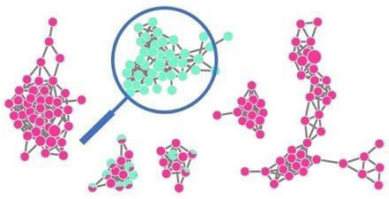

Fig. 1. Bioactivity-based molecular networking revealing a cluster of antiviral cyclopeptides

Keywords: Tropical plants, emerging viruses, antiviral agents, molecular networking

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S1.P214 Anti-*Helicobacter pylori* compounds isolated from *Sambucus williamsii*

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Sambucus williamsii Hance (Viburnaceae) has been widely used in herbal medicine and food industry. It is known to have various pharmacological effects including antitumor, antioxidant, anti- inflammatory, and antimicrobial activities (Xiao et al., 2016). During our search for anti-*Helicobacter pylori* compounds from natural resources, the methanol extract of the *S. williamsii* branch significantly inhibited the growth of *H. pylori*. Four lignans and two phenolic compounds were isolated from the methylene chloride fraction that showed the most potent anti-*H. pylori* activity among the five fractions of hexane, methylene chloride, ethyl acetate, butanol, and water fractions. The chemical structures were identified to be boehmenan (**1**), pinnatifidanin B VII (**2**), guaiacylglycerol β-coniferyl ether (**3**), guaiacylglycerol-β-*O*-6'-(2-methoxy) cinnamyl alcohol ether (**4**), guaiacylglycerol (**5**) and 3-hydroxy-5-methoxy benzenemethanol (**6**) by UV, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry (Fig. 1) (Huang et al., 2013). Among the isolates, compounds **1** and **5** exhibited significant anti-*H. pylori* activity against strains 26695 and 51. Compound **5** displayed more potent anti-bacterial activity with MIC values of 3.13 and 6.25 μM, and MIC_{50} values of 28.5 and 56.8 μM against the two strains, respectively. Their inhibitory activities were higher than those of a positive control, quercetin. Furthermore, these two compounds showed moderate urease inhibitory activity. A molecular docking study revealed the high binding ability of **1** and **5** to the active site of *H. pylori* urease. These results will provide further insights into the design of more potent natural products for eradicating *H. pylori*.

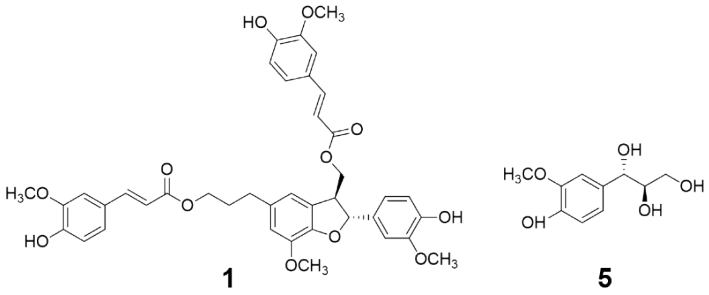

Fig. 1. Two anti-*H. pylori* compounds isolated from *S. williamsii*

Keywords: *Sambucus williamsii*, Viburnaceae, anti-*Helicobacter pylori*, boehmenan, guaiacylglycerol

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S1.P215 Lignans from autochthonous Argentinians *Larrea* species: potential use in the control of microorganisms in agriculture

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For decades, the growing global food demand has driven agricultural intensification, necessitating increased output in both crop and livestock production (Tudi *et al.*). Despite advancements in global food safety, crops and livestock remain susceptible to infectious diseases. Ensuring this safety requires the constant control of such illnesses. Hence, the ongoing search for new molecules is crucial to combat pathogens that are increasingly resistant to chemical treatments (Qiu *et al.*). This study aims to discover new antifungal and antibacterial compounds through bioguided isolation from two active plant extracts native to Argentina, *Larrea cuneifolia* and *Larrea divaricata (Vogt et al.)*. Twenty molecules were isolated, especially lignans, flavonoïds and original esters. Seven of them were tested against two *Salmonella* serovars affecting chicken farms and a phytopathogenic fungus, *Fusarium graminearum*. Nordihydroguaiaretic acid (NDGA) demonstrated significant activity against the tested *Salmonella* strains, with a minimum inhibitory dose (MIC) of 1.6 µg, and the (*R*- *R*) ent of Parakmerin A showed notable activity against the *Fusarium* strain with a MID of 3.1 µg. Isobolographic analysis revealed the synergistic effect of these two molecules with commonly used agricultural controls such as chloramphenicol and tebuconazole. Finally, the content of Ent (*R*-*R*) of Parakmerin A in *Larrea divaricata* was measured by HPTLC representing 0.90% of the total ethanolic extract.

Keywords: *Larrea*, lignans, antimicrobial activity, isobologram, agricultural control

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S1.P216 Traditional Middle Eastern spice blends (Baharat): Antimicrobial activity, metabolomic profile, and trace element analysis

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Spices and aromatic herbs have traditionally served culinary, preservative, and health purposes (Liu et al., 2017). This study assessed Middle Eastern spice blends (Baharat) for antimicrobial activity, comprising black and red pepper, cinnamon, clove, allspice, garlic, mint, black dry lime, turmeric, cumin, and ginger. EtOH:H_2O (3:7) was used to extract the spices individually and in four mixtures (blends 1-4). Blends 2 and 4 exhibited good antibacterial activity through the plate microdilution method (Wiegand et al., 2008) against Gram-positive bacteria (MIC = 0.390 and 3.125 mg/mL against *Staphylococcus aureus* and *Listeria monocytogenes*, respectively) and inhibition against *Enterococcus faecalis* (MIC = 12.5 and 6.25 mg/mL for blends 2 and 4, respectively). A reduced inhibitory activity was found against Gram-negative bacteria, except blend 4 against *Salmonella typhimurium* (MIC = 6.25 mg/mL). A mild bactericidal activity was shown against Gram-positive bacteria (50-200 mg/mL). The MIC values of positive control (gentamycin) ranged 7-28 µL/mL. Blends 2 and 4 underwent metabolomic analysis using UHPLC-HR-Orbitrap/ESI-MS. Altogether, 123 compounds were identified, including phenolic and hydroxycinnamic acids, flavonoids, piperamides, and organosulfur compounds (Ali et al., 2021). Blend 4 also contained nine limonoids exclusive to *Citrus* L. fruits due to black dry lime inclusion. Finally, ICP- QQQ-MS analyzed 12 trace elements in the spices, comparing their concentrations to FAO/WHO limits (FAO/WHO, 2023): Al ≤ 49, Cu ≤ 9.9, Fe ≤ 588, Mn ≤ 484, Zn ≤ 17.4 mg/L; As ≤ 38, Cd ≤ 207, Cr ≤ 636, Hg ≤ 10, Ni ≤ 1266, Pb ≤ 1548, Sn ≤ 60 µL/L.

Keywords: spices, metabolomics, antimicrobial, UHPLC-HR-Orbitrap/ESI-MS, ICP-QQQ-MS

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S1.P217 Ethnopharmacological survey of medicinal plants traditionally used in Tanzania for the management of sickle cell disease

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Introduction: Previous ethnopharmacological and bioactivity studies reported the use of medicinal plants in the management of sickle cell disease (SCD)[1,2]. *In vitro* and *in vivo* experiments showed anti-sickling, anti-inflammatory, antioxidant, analgesic, anti-anemic and antibacterial activities for these plants[3–5]. The use of medicinal plants against infectious and non-infectious diseases was reported, but little is known about medicinal plants used to manage SCD in Tanzania[6–8].

Methods: In-person interviews were conducted with traditional health practitioners (THPs) using semi-structured, open-ended questions and medicinal plants traditionally used to manage SCD were documented. The interview was followed by a field visit to collect plant specimens. A comprehensive literature review was performed for each of the medicinal plants mentioned by THPs and little studied plants were selected for further investigation.

Results: In total, 37 participants were interviewed. Most participants had primary education (81%) and mainly gained their knowledge on medicinal plants via revelations (54%). A total of 95 medicinal plants were collected belonging to 35 families. Roots (38%) and leaves (33%) were most frequently used, and decoction was the most common preparation method (56%). The vast majority of herbal treatments was recommended to be taken orally (89%).

Conclusion: This is the first ethnopharmacological survey on medicinal plants used in Tanzania for the management of SCD. The survey reported 95 medicinal plants claimed to have anti-sickling potential. The results provide a starting point in the search for new bioactive phytochemicals for the management of SCD.

Keywords: ethnopharmacology, sickle cell disease, Tanzania

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S1.P218 Bio-elicitation of isoquinoline alkaloids in *Chelidonium majus in vivo* & *in vitro*

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Cell culture technology and hydroponic systems were used for the examination of *Chelidonium majus* metabolic response to biotic stimuli. In *iv vitro* experiments, *C. majus* cells were cultured on *Komagataeibacter xylinus* derived non-purified nanocellulose matrices (BNC) (Zielińska et al., 2022). Three microbial strains (*Candida albicans*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) were used as bio-elicitors and were inoculated at the top or the bottom of cellulose discs. In turn, in the hydroponic plant cultivation *in vivo* system, *P. aeruginosa* at three different concentrations (1, 10, 50 ml/L) as well as methyl jasmonate (50 ml/L) were used. After the biotic elicitors treatment the isoquinoline alkaloid profile in plant material was examined using LC-MS/MS and MALDI-MS imaging. MALDI MSI chemical maps showed higher content of coptisine, sanguinarine, berberine, chelerythrine, chelidonine and allocryptopine in BNC containing *S. aureus* compared to *C. albicans* and *P. aeruginosa* or elicitor-free cellulose carriers. In hydroponic experiments, the concentration of 10 ml/L of lyophilized *P. aeruginosa* was the most effective in terms of protoberberine and benzophenanthridine derivatives' production in the *C. majus* plants. Further investigation on the individual microbial strains effect including the concentration and the exposure time, both for plants grown *in vitro* and *in vivo* is required to understand their possible mechanism of action and specialized metabolites production control.

Keywords: isoquinoline alkaloids, *Chelidonium majus*, biotic elicitors

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S1.P219 *In vitro* anti-*Helicobacter pylori* activity of extracts and essential oil of *Thymus pannonicus* All. (Lamiaceae)

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Thymus pannonicus All. is an aromatic herbaceous plant distributed in Central and Eastern Europe (Jalas, 1972). The aerial parts of the lemon-scented chemotype from Serbia are considered a traditional remedy for various gastrointestinal complaints (Maksimović et al., 2008). In this work, we tested its antibacterial activity against *Helicobacter pylori*, a bacterium associated with the risk of developing complicated chronic diseases of the gastrointestinal tract.

T. pannonicus hexane (HE), dichloromethane (DE), and methanol extracts (ME), the fraction of diethyl ether extract soluble in 80% aqueous methanol (FDM), and essential oil (EO), were tested by broth microdilution method against *H. pylori* ATCC 43504 (reference strain) and 10 clinical isolates, including strains resistant to metronidazole and/or clarithromycin.

ME showed the best activity, with MIC_{50} (minimum concentration that inhibits the growth of 50% of the tested strains) and MIC_{90} of 4 and 16 µg/mL, respectively. FDM exhibited MIC_{50} 16 µg/mL and MIC_{90} 32 µg/mL, while EO showed MIC_{50} 2 µg/mL and MIC_{90} 64 µg/mL. HE and DE were less active (MIC_{50} and MIC_{90} were 32 and 128 µg/mL, respectively).

Liquid chromatography with ultraviolet and mass spectrometric detection revealed that ME was characterized by a high content of rosmarinic acid (64 mg/g) and salvianolic acid H, while FDM was rich in methylated flavonoid aglycons. The dominant compounds in EO, analyzed by gas chromatography with mass spectrometry and flame-ionization detectors, were geranial and neral (36.7% and 27.2%, respectively).

The results indicate that preparations of *T. pannonicus* may be potential therapeutic agents against *H. pylori* infections.

Keywords: *Thymus*, *Helicobacter pylori*, rosmarinic acid, geranial, neral

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S1.P220 Assessing the structural interaction profile differences between natural products and synthetics, a data-driven approach

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The potential of natural products (NPs) is a largely untapped source when comparing its prevalence in the drug market with that of its synthetic counterparts. By leveraging structure-based drug design (SBDD), we explore the coupling of conventional computational tools and the impact of state-of-the- art artificial intelligence (AI)-based tools. Moreover, NPs have in the past years, demonstrated increased efficacy and potency. In our work, we aimed to elucidate the target promiscuity profiles of synthetic and NP compounds to shed light on the rationale behind NPs' selection as primary candidates for drug discovery. To achieve this, we conducted a comprehensive comparative structure analysis of the two compound groups (i.e. NPs and synthetics) targeting similar biological targets. By systematically analyzing the interactions between synthetic and NP compounds and their respective targets, we sought to uncover insights into the unique properties and mechanisms underlying NP efficacy and potency. Our findings contribute to a deeper understanding of the molecular interactions and functional characteristics of synthetic and NP compounds, providing valuable insights for optimizing drug discovery strategies and harnessing the therapeutic potential of NPs. Further research in this area holds promise for the development of novel therapeutics and the advancement of drug discovery efforts.

Keywords: structure-based drug design (SBDD), in-silico computation, natural products, small molecules, protein-ligand interaction (PLI)

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S1.P221 Guttiferones-targeted dereplication integrating LC-HRMS/MS and ^{13}C-NMR data: A comparative metabolomic study of *Symphonia globulifera* latex

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Symphonia globulifera is a Clusiaceae species, mostly distributed in South America and Africa. This medicinal plant has been the focus of phytochemical studies leading to the identification of several PPAPs. Guttiferones are the main PPAPs isolated from *S. globulifera* latex, exhibiting various pharmacological properties such as antiparasitic, antimicrobial, antiviral, and cytotoxic activities (Conceição et al., 2023). To gain access to this natural resource, our team has established a collaboration with French Guiana (FG), Ivory Coast (IC) and Brazil (BR), to conduct a comparative metabolomics study. Latex was collected from these three regions, and guttiferones were extracted by maceration (48h), using methanol. Extracts were then analyzed by LC-HRMS/MS and a molecular network (MN) was constructed using GNPS and Cytoscape® software. The MN revealed a main cluster for guttiferones with *m/z* 602 or 670, corresponding to guttiferones A and C and their isomers, respectively. Due to the presence of numerous stereoisomers, a very similar MS/MS fragmentation pathway is often observed, limiting their putative identification. We then proceed with ^{13}C-NMR dereplication experiments using MixONat® software (Bruguière et al., 2020). Latex spectral data were compared with predicted NMR data from a Clusiaceae database (DB) including 1992 natural compounds described on LOTUS. This MS/MS-NMR integrated approach allows the annotation of guttiferones C and D (*m/z* 670) in all samples, suggesting these are important chemical markers for *S. globulifera*. Guttiferone A (*m/z* 602) was found in FG latex, while guttiferone E/xanthochymol were identified in BR latex. Phytochemical investigations are ongoing in order to isolate the unknown guttiferones.

Keywords: Clusiaceae, dereplication, guttiferones, PPAPs (PolycyclicPolyprenylated Acylphloroglucinols)

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S1.P222 QSAR-derived opportunities of prenylated phenolic compounds from Fabaceae as antimicrobial phytochemicals

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Prenylated phenolic compounds (Fabaceae), bearing C5-isoprene moieties, are antimicrobial phytochemicals known for their wide structural diversity (Al-Maharik, 2019). Evidence shows that even minor structural variations can greatly influence their antimicrobial properties (Araya-Cloutier et al., 2018).

This presentation aims to provide an overview of opportunities identified in recent structure-activity relationships (SARs) studies of prenylated phenolics as antimicrobial agents. Using a diverse collection of prenylated phenolics, tested against various microorganisms, including healthcare- associated pathogen methicillin-resistant *Staphylococcus aureus* (MRSA) and oral pathogen *Streptococcus mutans*, SARs were defined and in-silico quantitative SARs (QSAR) models developed and validated. The models were compared to unravel the most important molecular properties for antimicrobial activity of prenylated phenolics.

SARs showed that the number of prenyl groups attached to the backbone influences the spectrum of activity of these phytochemicals, i.e., diprenylated phenolics were among the most active antibacterials against Gram positive bacteria, whereas monoprenylated phenolics showed superior activity against yeast or Gram negative bacteria *Escherichia coli* (for the latter one in the presence of an efflux pump inhibitor). The backbone of the compounds was also shown to play a major role in the SARs; e.g. C-ring opening of prenylated flavanones, resulting in prenylated chalcones, significantly restored antimicrobial activity.

Based on the QSAR models, extent of hydrophobicity, shape, flexibility, charge and hydrogen- bonding were identified as critical contributors of antimicrobial activity of prenylated phenolics. Overall, prenylated phenolics are potent natural antimicrobials and the developed QSAR models are valuable tools for gaining insights into the complex SARs governing their activity.

Keywords: Prenylated phenolic compounds, Fabaceae, Structure-Activity Relationships, QSAR modelling

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S1.P223 Phytochemistry and biological activities of *Ostericum palustre* (*Angelica palustris*) (Apiaceae)

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The genus *Angelica* has a Euroasiatic-continental range that includes several Central European countries (Dittbrenner et al. 2005). *Ostericum palustre* Besser (= *Angelica palustris* (Besser) Hoffm.) is a representative of genus Angelica. In Poland, this species has been subject to strict and active species protection since 2001 (Journal of Laws 2014, item 1409). The species is a rhizomatous perennial (hemicryptophyte) herb 140-160 cm high that prefers humid and eutrophic habitats on mineral substrates. The genus *Angelica* is indicated as a pharmaceutical raw material due to the richness of chemical compounds with anti-allergic, anti-cancer, and anti-inflammatory properties and is used in the treatment of diabetes, rheumatism, injuries, burns, and blood deficiencies (Patents 2012, 2014, 2015, 2016). This presentation will cover the phytochemical analysis and biological evaluation of the extracts prepared from aerial and underground parts of this species.

The antimalarial activity was determined against the chloroquine-sensitive (D6) strain of *Plasmodium falciparum* by measuring plasmodial LDH activity according to the procedure of Makler and Hinrichs (1993). Laboratory tests showed high biological activity of the methanol extract from the aerial part (stems, leaves, and flowers) of this species, which inhibited the growth of the malaria- causing strain D6 of *Plasmodium falciparum* in 41%, In comparison, chloroquine (positive control) showed 94–98% inhibition. Evaluation of cytotoxicity was conducted using selected normal and cancer cell lines.

Among the secondary metabolites of *O. palustre*, coumarins, organic acids (angelic, valeric, succinic, malonic, oxalic, citric, fumaric), phenolics (coumaric, feruloylquinic, caffeoylquinic and di- caffeoylquinic acids, luteolin rutinoside) and terpenes (cymene, camphene, limonene) were identified.

Keywords: *Ostericum palustre*, Apiaceae, antimalarial activity, *Plasmodium falciparum*, cytotoxicity

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S1.P224 Discovery of two new bicyclic macrolides from *Kitasatospora acidiphila*

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Forests are an under-explored habitat for chemically prolific actinomycetes (Srivastava et al., 2019). Because rare actinomycete phylogenetic groups, besides commonly studied *Streptomyces*, are recognized as important sources of novel bioactive, we prioritized rare actinomycetes strains from forest soil for deeper chemical analysis (Tiwari et al., 2012).

The MMS16-CNU292 strain was isolated from a soil sample collected from the surface of a pine forest in Daejeon City, Republic of Korea. It has been identified as a new species of the genus *Kitasatospora*, and named *Kitasatospora acidiphila* based on its phylogenetic, chemotaxonomic, and phenotypic properties (Kim et al., 2020).

Cultivation and LC/MS-based chemical analysis of *K. acidiphila* MMS16-CNU292 enabled the detection of two previously unreported compounds. Chromatographic purification and spectroscopic analysis based on 1D/2D NMR, HR-ESI-MS, and UV data elucidated the planar structures of the new natural products as unique bicyclic macrolides bearing 14- and 6-membered rings. Further analysis of $^{3}J_{H,H}$ and ROESY correlations determined the geometry of two *trans-trans* dienes in the new macrolides.

Based on *J*-based configuration analysis, ROESY correlations, and acetonide derivatization of an 1,3-diol (Rychnovsky et al., 1997), the relative stereochemistry of the new polyketides was established. The determination of the absolute configuration of the compounds is in progress based on multi-step chemical derivatizations, including Mosher's esterification.

Keywords: *Kitasatospora acidiphila*, rare actinomycetes, macrolide, structure elucidation

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S1.P225 The potential of curry leaf extract in controlling SARS-CoV-2 infectious syndromes

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Coronavirus disease 2019 (COVID-19) is caused by infection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a worldwide pandemic with asymptomatic, mild or severe symptoms. The severe symptoms linked to a massive viral load may induce a severe immune response that causes tissue damage (Purbey et al., 2023). Evidence supports that several eatable spices, including curry leaves, could potentially control the cytokine storm of lung infectious diseases (Kunnumakkara et al., 2021). This study aims to identify the active components of a spice curry leaf (*Murraya koenigii*) that can prevent SARS-CoV-2 infection and its related allergy response. The assays of cell-free spike RBD-ACE2 binding and calcium ionophore A23187-induced mast cell degranulation guided the plant extraction. Curry leaves were initially extracted using 95% ethanol and partitioned by ethyl acetate and water with 1% HCl. The n-hexane and methanol subjects partitioned the ethyl acetate (non-alkaloids) fraction, which showed potent inhibition of spike-ACE2 binding. The most bioactive active hexane extract was fractionated using MPLC column chromatography. The acid water fraction was partitioned by dichloromethane (DCM) and water with ammonium hydroxide, and the DCM fraction with anti-allergy activity was further fractionated. The current evidence supports that leaf extract fractions of non-alkaloid have potential activities to inhibit spike-ACE2 protein binding by a concentration range of ng/mL, and alkaloid fractions have the potential to inhibit cellular allergy response by a concentration range of μg/mL.

Keywords: Curry leaf (*Murraya koenigii*), SARS-CoV-2

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S1.P226 Antiviral effect of *Hoveniae Semen Seu Fructus* against Influenza A viral infection

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Hoveniae Semen Seu Fructus is the fruit and seed of Hovenia dulcis Thunb. Here, we first demonstrate that *Hoveniae Semen Seu Fructus* water extract contains a potent anti-influenza A virus effect via inhibition of neuraminidase and hemagglutinin and a direct virucidal effect. We investigated the effect of *Hoveniae Semen Seu Fructus* on Influenza A virus infection using the GFP- tagged Influenza A virus. FACS analysis and fluorescent microscopy showed a dose-dependent inhibitory effect of *Hoveniae Semen Seu Fructus* against Influenza A virus infection. *Hoveniae Semen Seu Fructus* also suppressed the cytopathic effects of H1N1 and H3N2 Influenza A virus. Immunofluorescence and qPCR analyses confirmed that *Hoveniae Semen Seu Fructus* significantly represses Influenza A virus protein and RNA expressions. Time of addition and plaque assays showed that *Hoveniae Semen Seu Fructus* changes hemagglutinin and causes virucidal effects early on in the viral replication process. Furthermore, *Hoveniae Semen Seu Fructus* inhibited the neuraminidase activity of H1N1 and H3N2 Influenza A virus. Quercetin, not taxifolin in *Hoveniae Semen Seu Fructus*, inhibited Influenza A virus infection. Our results suggest that *Hoveniae Semen Seu Fructus* could be developed as a natural antiviral agent to prevent Influenza A virus infection.

Keywords: Hoveniae Semen Seu Fructus, Influenza A virus, hemagglutinin, neuraminidase, quercetin

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S1.P227 *Thuja orientalis Folium contains antiviral effect against* Influenza A viral infection via modulating hemagglutinin and neuraminidase

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Thuja orientalis Folium (TOF) has been traditionally used as an expectorant for inflammatory airway disease. In the present study, we examined the antiviral activity of TOF using GFP-tagged influenza A virus (IAV). The fluorescence microscopy and fluorescence-activated cell sorting analysis showed that TOF potently inhibits IAV infection, dose-dependently. Consistent with that, immunofluorescence and Q-PCR analysis results confirmed TOF significantly reduces IAV protein and RNA expression. TOF blocked IAV infection at the binding and entry step upon viral infection and interfered with HA protein. Further, TOF exhibited a direct virus-killing effect and inhibited the neuraminidase activity of IAV. Additionally, TOF prevented the cytopathic effect caused by H1N1 and H3N2 IAV infection. Amentoflavone among the constituents in TOF exerted the strongest anti- IAV effect. Myricetin, quercetin, and quercitrin in TOF also inhibited IAV infection. However, the potent anti-IAV effect of TOF may be related to the synergistic effect of constituents, not by a single specific compound. Our results suggest TOF exhibits a significant inhibitory effect against IAV infection at multi-stages via the blockage of viral attachment and entry, inhibition of neuraminidase, and induction of virucidal effects.

Keywords: *Thuja orientalis Folium*, influenza A virus, virucidal effect, hemagglutinin, neuraminidase

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S1.P228 Exploring the phytochemistry, antibacterial and anti-inflammatory activities of three Ericaceous species native to Ireland

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Plant derived natural products have promising therapeutic candidates with multifaceted bioactivities. As part of the 'Unlocking Nature's Pharmacy from Bogland Species' (UNPBS) project, we have investigated the chemistry and biology of a range of bogland species. The present study investigates three Ericaceous species (Heathers) native to Ireland which grow abundantly across raised bogs and mountain heath. We report the antibacterial and anti-inflammatory activities of *Erica cinerea* L., *Erica tetralix* L., and *Calluna vulgaris* (L.) Hull. toward the highly virulent and antibiotic resistant bacterial ESKAPE pathogens *E. faecalis, S. aureus, A. baumannii* and *E. cloacae. C. vulgaris* demonstrated the most potent antibiofilm activity reducing *A. baumannii* biofilm formation by 38%, at a concentration of 0.05 mg/ml. The effects of extracts on Interleukin 6 (IL6), RANTES (CCL5), TNFα and IFNβ were evaluated in the THP-1 cell line, after stimulation with LPS. All three extracts significantly reduced the LPS induced cytokine release, most notably of IL6 at a subtoxic concentration range of 10-100 ug/mL.

Chemical fingerprints of the extracts were generated by LCMS, GCMS, HPTLC (normal and reverse phase) and NMR. Bioactivity guided fractionation of *E. cinerea* was carried out and the most bioactive fraction 'S5' was shown to contain high concentrations of scopoletin, protocatechuic acid, p-coumaric acid along with isofraxidin, ferulic acid, caffeic acid and ursolic acid.

This research highlights the significance of three Irish heathers. From the findings it can be concluded that the three plants have antibacterial, antibiofilm and anti-inflammatory potential and serve as promising candidates for further development of novel therapeutic or cosmetic agents.

Keywords: Ericaceous species, antibacterial, anti-inflammatory, IL6, phytochemical profile

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S1.P229 Funicone-like compounds: potential antiviral agents towards canine coronavirus

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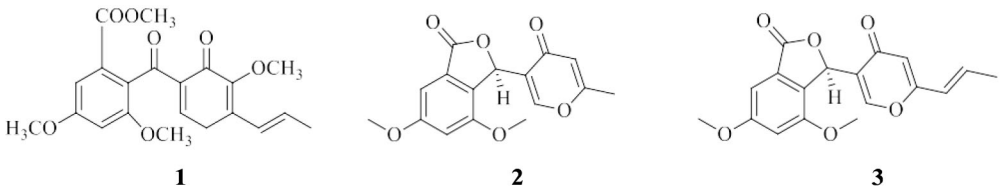
The exploitation of bioactive properties of microbial products is of great pharmaceutical interest. Fungi are a promising source of novel drugs with a broad spectrum of activities and functions, including antiviral properties. Funicone-like compounds, as well as related com- pounds, constitute a homogeneous group of fungal polyketides possessing remarkable biological activities which have promoted their consideration as drug possibilities (Salvatore et al. 2022). This group of secondary metabolites is essentially produced by species in the genus *Talaromyces* (Eurotiales: *Trichocomaceae*). This communication provides interesting findings concerning the antiviral properties of three well-known members of this group (i.e., 3-*O*-methylfunicone (**1**), penisimplicissin **2**) and vermistatin (**3**) (Fig.1) obtained from an isolate of *Talaromyces pinophilus*, previously recovered from the rhizosphere of tobacco (*Nicotiana tabacum*) (Salvatore et al. 2018). In particular, the antiviral effects of **1-3** were investigated on canine coronavirus (CCoV) infection. The virus, an alphacoronavirus, causes self-limiting enteric disease in dogs, especially in puppies but, due to noteworthy plasticity of CoVs, mutation and recombination processes occur generating new dangerous variants. In this context, developing antiviral compounds certainly may have potential applications in the treatment of CoVs infection. Thus, during CCoV infection in canine fibrosarcoma cells (A72), a fibrosarcoma cell line suitable for investigating CCoV, a non-toxic dose of these funicone-like compounds markedly increased features of cell viability and reduced virus yield. In addition, we observed that these compounds caused a strong inhibition in the expression of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor which is activated during CCoV infection.



1 **2** **3**

Fig 1. Structures of 3-*O*-methylfunicone, penisimplicissin and vermistatin (**1**-**3**).

Keywords: coronavirus, *Talaromyces*, fungal secondary metabolites, metabolomics

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S1.P230 Efficacy of food waste as a potential source of antibacterial agents and antibiotic adjuvants against MRSA and MSSA isolates

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Staphylococcus aureus is a leading cause of nosocomial and community acquired infections, with up to 50% of isolates exhibiting methicillin resistance (European Centre for Disease Prevention and Control, 2014). Methicillin-resistant *Staphylococcus aureus* (MRSA) infection is associated with increased healthcare costs and higher mortality rates, with over fifty thousand MRSA associated deaths reported in the WHO European region for 2019 (O'Neill, 2014). In this study, the antibacterial properties of extracts, derived from thirteen fruit and vegetable waste streams, were investigated against clinical MRSA and MSSA isolates,

using a broth microdilution assay. Of the 65 crude extracts tested, 11 were shown to have activity against both strains at minimum inhibitory concentrations (MICs) ≤ 1 mg/mL. Crude extracts from onion skins (0.25 mg/mL), kiwi skins (0.8 mg/mL) and broccoli stalks (0.8 mg/mL) demonstrated the greatest antibacterial activity. Extracts were also evaluated for their efficacy as synergistic agents with several beta-lactam antibiotics against clinical MRSA isolates. The strongest antibacterial effect was shown from the combination of the onion skin extract with amoxicillin, resulting in a twenty-five-fold reduction of its MIC (0.31 µg/mL) and a Fractional Inhibitory Concentration Index of

0.29. Total phenolic content analysis of the onion skin extract (262.39 ±

0.05 mg GAE/g DW suggested that these compounds may play a key role in its outlined bioactivity. Overall, the results of this study demonstrate the potential of food waste revalorisation as a viable strategy for combatting the rise of AMR. Studies regarding the antibacterial mechanism of action and identification of bioactive compound(s) are currently ongoing.

Keywords: antimicrobial resistance, methicillin-resistant *Staphylococcus aureus*, food waste, antibiotic modulation

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S1.P231 Coumarins of *Angelica archangelica* roots inhibit efflux pumps in *Escherichia coli*

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Angelica archangelica L. (Apiaceae), native and widespread in Europe, has been cultivated as a medical plant with antibacterial, antispasmodic and antianxiety properties. *A. archangelica* has a complex chemical composition of essential oils and coumarins, contributing to the plant's biological activities (Lunz et al., 2021).

Former studies unveiled, that essential oils obtained from the roots show antibacterial activity against Gram-positive bacteria (Fraternale et al., 2014). Additionally, *A. archangelica* arrested attention as coumarins were identified as being responsible for antibacterial activity against *Staphylococcus aureus, Escherichia coli* (*E.coli*) and *Pseudomonas aeruginosa* (Nemeth et al., 2015; Alloush et al., 2022).

So far, with increasing prevalence of untreatable infections induced by antibiotic resistance of bacteria, coumarins were identified as inhibitors of bacterial efflux pump systems (Roy et al., 2013). While many former studies focused on efflux pumps in Gram-positive bacteria, the effects of coumarins on Gram-negative bacteria like *E. coli* are still poorly investigated. Hence, combined analytical systems like thin-layer-chromatography (TLC) and high-pressure-liquid-chromatography coupled with mass spectrometry (HPLC-MS) were used, to get a deeper insight into coumarin pattern of *A. archangelica* and to identify coumarins with efflux pump modifying effects.

To this, the antimicrobial and resistance-modifying profile of plant-derived coumarins were evaluated, and *E. coli* AG100 and the acrB gene knockout mutant *E. coli* AG100A were treated with isolated fractions. It could be shown, that fractions containing archangelicin and 2´-angeloyl-3´- isovaleryl vaginate (Wszelaki et al., 2011) induced the most potent modulatory effects on ciprofloxacin (CIP) against *E. coli* AG100 in comparison to *E. coli* AG100A.

Keywords: *Escherichia coli, Angelica archangelica*, coumarins, antibacterial activity, efflux

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S1.P232 Thymoquinone potentiate activity of antibiotics against clinical isolates of *Mycobacterium tuberculosis*

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A numerous plant species are being screened in order to find molecules inhibiting the growth of Mycobacterium tuberculosis (Mtb). These include in vitro determination of minimal inhibitory concentration against sensitive and multidrug resistant (MDR)-Mtb strains, inhibition of intracellular bacilli in vitro and ex vivo, and killing of replicating and dormant bacteria (Gupta et al., 2017). However, because molecules active in nano- or micromolar concentrations are found rarely, the plant secondary metabolites are evaluated for their ability to enhance activity of antimycobacterial antibiotics. The synergistic action of plant extracts or isolated compounds against Mtb was already described (Gupta et al., 2017; Polak and Kapka-Skrzypczak, 2017). Interestingly, the synergy was more frequently found when natural compounds were combined with rifampicin rather than with isoniazid or ethambutol (Sieniawska et al., 2018). These combinations showed variable activity against sensitive and drug resistant Mtb clinical strains (Sieniawska et al., 2018, 2020) suggesting the possible differences in their mode of action.

To better understand the differences in the sensitivity of Mtb clinical strains we evaluated the possible synergistic action of first line antibiotics in a combination with thymoquinone, a natural antimicrobial and anti-inflammatory agent. The studies of tuberculostatic activity of antibiotics in the presence of subinhibitory concentrations of thymoquinone showed enhanced activity of rifampicin against all tested strains. This activity was further evaluated in order to investigate the antimycobacterial mechanism of action of thymoquinone.

Keywords: antimicrobials, *Nigella sativa,* tuberculosis, MIC, FICI

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S1.P233 Exploring olikomycin a: a novel lipopeptide antibiotic - insights into its mechanism of action

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In the world of antibiotics, Streptomyces stand out for their large number of biosynthetic gene clusters (BGCs), which enable them to produce a diverse range of natural products. Typically, only a limited number of these BGCs are abundantly expressed under laboratory conditions, while the rest are either poorly expressed or completely silent for unknown reasons.

By specifically deleting the regulatory gene *wblA* in a *Streptomyces* strain, we successfully awakened a silent BGC, leading to the discovery of the natural product olikomycin A with a strong antibiotic activity. Olikomycin A is a cyclic lipopeptide synthesized via NRPS.

Olikomycin A was extracted by liquid–liquid extraction and purified by reversed-phase HPLC. In order to investigate its antibiotic efficacy, a comprehensive analysis was carried out to assess its effects on both peptidoglycan biosynthesis and the structural integrity of the cell membrane. In particular, isothermal titration calorimetry (ITC) was used to analyse interactions with a model membrane.

Olikomycin A has been found to show a similar mechanism of action to daptomycin, which is based on dependence on calcium and phosphoglycerol. Interestingly, however, it exhibits unique pattern in binding behaviour.

This study underlines the potential of *Streptomyces* in producing diverse natural compounds through its wealth of BGCs. The discovery of Olikomycin A, coupled with insights into its purification and antibiotic mechanisms, provides valuable contributions to the field of antibiotics and opens doors for further exploration.

Keywords: Streptomyces, novel antibiotics, cyclic lipopeptide, calcium-dependent, non-ribosomal peptide synthetases (NRPS)

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S1.P234 GC-MS analysis of essential sandalwood oils with in vitro activity against *Madurella mycetomatis*

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Mycetoma is a chronic subcutaneous infection occurring in tropical and subtropical regions. Since 2016, Mycetoma is included in the WHO´s list of neglected tropical diseases. Depending of the causative organism, mycetoma is classified as actinomycetoma, caused by bacteria, and eumycetoma, caused by fungi (e.g. *Madurella mycetomatis*). With limited treatment options due to high costs, low availability and poor efficacy of antimycotic drugs, the urge to find new active compounds against eumycetoma is high (Zijlstra et al., 2016).

In an in vitro screening of essential oils against *M. mycetomatis* (Abd Algaffar et al., 2021), Indian sandalwood oil (SWO) the essential oil of *Santalum album* (Santalaceae), with MICs ≤ 0.0039% (v/v) was the most active among 27 tested oils. Therefore, 15 SWOs from 5 different *Santalum* species were tested and thoroughly analyzed by GC-QTOF-MS on two columns (DB-5 and DB-HeavyWax). A biotechnological SWO-surrogate and two oils from other plants marketed as "SWOs" were also tested and analyzed. Over 270 constituents were detected, and

>100 of them identified, many for the first time in the investigated oils. All tested oils showed considerable activity with MICs ranging from 0.0078-0.0019% (v/v). Column-chromatographic separation of SWO on silica yielded fractions of sesquiterpene-hydrocarbons and -alcohols. Only the latter showed activity against *M. mycetomatis*. The main compounds of SWO (Fig. 1), (*Z*)-a-santalol (**1**) and (*Z*)-b-santalol (**2**) were obtained by CC on $AgNO_3$-impregnated silica (Daramwar et al., 2012). The former was twice as active as the latter (MIC = 27.5 vs. 55 µg/mL).

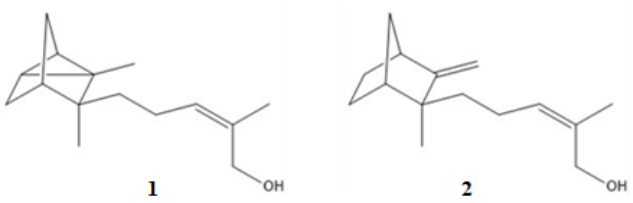

Fig. 1. Structures of (*Z*)-a-santalol (**1**) and (*Z*)-b-santalol (**2**)

Keywords: *Santalum*, Santalaceae, essential oil, GC-MS, Mycetoma, *Madurella mycetomatis*

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S1.P235 Bio-guided approach as a valuable tool in the phytochemical investigation of three underexplored Myrtaceae plants

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Myrtaceae Juiss is a large family of flowering plants with privileged chemical and biological profiles (Celaj et al., 2020). In particular, it is a very rich source of phloroglucinol derivatives, which have become attractive targets for organic chemists thanks to their structural features and biological activities, including antimicrobial activity (Nicoletti et al., 2018). Nevertheless, some species in this family, have been scarcely investigated. Thus, this work explores the chemical complexity of the underexplored species, *Myrcianthes cisplatensis*, *Psidium friedrichsthalianum* and *Psidium oligospermum* as a potential source of antimicrobial phytochemicals, through the combination of antimicrobial assay and NMR analysis. Three extracts at increasing polarity, hexane, chloroform and methanol, obtained from leaves of each plant, grown and collected in Arizona, were evaluated for their antimicrobial potential against two strains of *Staphylococcus aureus*: ATCC 29213 and 43300 (a methicillin-resistant *Staphylococcus aureus* strain, MRSA). Hexane and methanol of *M. cisplatensis* (MIC of 16 μg/mL and 64 μg/mL, respectively) and polar extracts of *P. friedrichsthalianum* (MIC of 64 μg/mL and 128 μg/mL, respectively) were the most promising against both the strains. Starting from the most polar ones, a liquid-liquid separation was useful to concentrate the flavonoidic components in ethyl acetate fraction, giving back a more active dichloromethane fraction that reported an increasing antimicrobial potential against both the strains (MIC of 16 μg/mL for *M. cisplatensis;* MIC of 128 μg/mL for *P. friedrichsthalianum*). The fractionation, using different chromatographic techniques, has let to identify the component responsible of the highlighted antimicrobial properties. Four new cinnamoylated alkylphloroglucinol glucosides (Guzzo et al., 2023), along with tricyclic sesquiterpenes, acylated pentacyclic triterpenes, were isolated from bioactive fractions and characterized by extensive 2D NMR analysis (HSQC, H2BC CIGAR-HMBC, COSY, HSQCTOCSY) and HR-MS analyses. The antimicrobial potential of the isolated compounds was evaluated, showing how cinnamoylated alkylphloroglucinol glucosides derivatives were the most active respect to the others and how the bioguided approach is an excellent tool for the characterization of new natural bioactive compounds.

Keywords: bio-guided approach, *Myrcianthes cisplatensis*, *Psidium friedrichsthalianum*, *Psidium oligospermum*, *Staphylococcus aureus*, NMR analysis

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S1.P236 From poison to potential: Nb-methyl usambarensine reveals antiviral activity against SARS- CoV-2

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Nb-methyl usambarensine is a quaternary alkaloid originally isolated from the roots of *Strychnos usambarensis* (Loganiaceae) (Angelot. L *et al.*, 1975). This plant was historically recognized for its curarizing properties, traditionally employed as an arrow poison in Africa. In response to the SARS- CoV-2 pandemic in 2020, an investigation for potential antiviral agents from the enriched pharmacognosy library was undertaken. Although Nb-methyl usambarensine exhibited no significant activity against *Plasmodium falciparum* in previous studies (Wright *et al.*, 1991), it emerged as a promising candidate during screening against SARS-CoV-2. Additionally, a structurally similar compound, 10-hydroxyusambarensine, was identified as a hit-compound through an in-silico approach, suggesting its potential as an inhibitor of the coronavirus 3-chymotrypsin-like protease (Gyebi *et al.*, 2021).

The antiviral activity of Nb-methyl usambarensine was evaluated in BSL-3 facilities using VERO- E6 cells. The virus was introduced with a multiplicity of infection ratio or MOI of 0.01, alongside varying concentrations of the molecule (1.56 µg/ml- 50 µg/ml). Sampling occurred at 24-hour intervals over 3 days to capture the viral kinetics. The RT-qPCR results at 72h demonstrated a reduction of more than 4 $\log_{10}$ in RNA copies/ml at the concentration of 50 µg/ml of the Nb-methyl usambarensine, while no signs of cytotoxicity were observed. This study highlights the previously unrecognized antiviral potential of Nb-methyl usambarensine against SARS-CoV-2. Further research is needed to elucidate the molecular mechanisms underlying this antiviral activity and to explore its potential as a therapeutic agent against COVID-19.

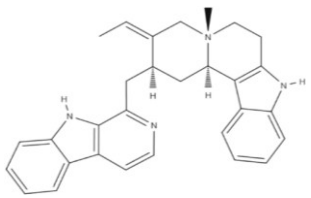

Fig. 1. Structure of Nb-methyl usambarensine

Keywords: Nb-methyl usambarensine, SARS-CoV-2, antiviral-activity, RT-qPCR

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S1.P237 Semi-synthetic ecdysteroids as promising new antichagasic agents

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The parasite *Trypanosoma cruzi* is the causative agent of a severe infectious disease, the so-called Chagas' disease, which is one of the neglected tropical diseases, according to WHO. As therapy is limited to two available drugs, (WHO; Rassi et al 2010) seeking new effective agents is crucial.

In this study, the screening of fifty-two ecdysteroids for their antichagasic effects revealed that cinnamic ester and *tert*-butyl oxime ether moieties might have a key role in the antiparasitic effect. Based on the screening results, new compounds were designed and prepared, incorporating the two potential pharmacophores. 20-Hydroxyecdysone (**20E**) was used as starting material, which is the most widespread natural ecdysteroid. Six compounds were semi-synthesised, among which two cinnamic mono-esters of 20E 6-*tert*-butyl oxime ether derivatives exerted the most potent activity. (Háznagy et al., 2022) IC_{50} measurements (MTT and XTT assays) (Salm A, et al, 2021) were carried out with compound (**1**) (Fig. 1). The results demonstrated that compound (**1**) has six times selective toxicity on *T. cruzi* epimastigotes as compared to cytotoxicity on the native cell line. The FACS- based quantitation of released parasites from infected cells indicated that (**1**) exerted an IC_{50} value of 2.7 µm.

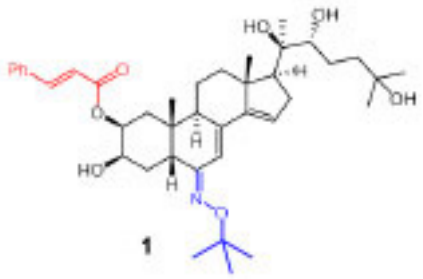

Fig. 1. Structure of the most active ecdysteroid derivative **1**

Semi-synthetic preparation of new derivatives is currently ongoing. A further aim is to investigate the role of the substitution pattern of the cinnamic acid moiety.

Keywords: *Trypanosoma cruzi,* ecdysteroids, semi-synthesis, cinnamic esters

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S1.P238 Exploring the potential biopesticide application of nemertide alpha-1 on parasitic and disease vector mosquitoes

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This poster presents the initial exploration of nemertide alpha-1 as a potential biopesticide targeting pest insects. Nemertide alpha-1, a neurotoxic peptide consisting of 31 amino acids, has shown potent biopesticidal activity, with an EC_{50} of 8.6 nM on BgNa$_v$1 (*Blatella germanica*) (Jacobsson et al., 2018). Additionally, it demonstrated complete inhibition of inactivation in Na$_v$ channels of *Drosophila melanogaster* and *Varroa destructor* at 1 µM (Jacobsson et al., 2018). Bell et al. (2021) also reported the insecticidal efficacy of a recombinant nemertide alpha-1 propeptide on cabbage moth larvae (*Mamestra brassicae*) and proboscis feeding insects such as adult aphids (*Acyrthosiphon pisum* and *Myzus persicae*) and adult bees (*Apis mellifera*). Building upon these promising findings, our study aims to explore the insecticidal potency of alpha-1 while considering its implications for human health and the emergence or re-emergence of infectious diseases. Here, we present a preliminary investigation into the acute toxicity effects of nemertide alpha-1 on *Anopheles* mosquitoes (vectors of malaria: *Plasmodium falciparum*) and *Aedes* mosquitoes (vectors of viral diseases: dengue fever, Zika fever, yellow fever, and Chikungunya).

Keywords: nemertide, marine, peptide toxin, biopesticide, mosquito

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S1.P239 Chemical constituents of *Sambucus nigra* fruit (European Elderberry)

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Sambucus spp. are broadly dispersed throughout the tropical/subtropical and temperate areas. *Sambucus nigra* L. (syn. *S. nigra* subsp. *nigra*) is one of the five best-known among 30 species in the genus *Sambucus* L. (elder) (Viburnaceae). *S. nigra*, a shrub commonly known as elderberry, elder, European elderberry, European elder, black elder, and European black elderberry, is prevalent in Europe, North Africa, and West and Central Asia. Various biological activities such as anti- inflammatory, diuretic, and antioxidative have been associated with *S. nigra* (Ahmadiani et al., 1998; Ebrahimzadeh et al., 2006). *S. nigra* fruit is a cultivated as well as a wild-harvested species and is one of the richest sources of flavonoids and anthocyanins (Duymuş et al., 2014).

Elderberry products famed over the past couple of years due to their use in the COVID-19 pandemic, though, there is no robust evidence to support the prevention or treatment of this disease. Two iridoids including one previously undescribed, an indole alkaloid, and various flavonoid glycosides (Fig.1) were isolated and characterized from the dried fruit of *S. nigra.* The compounds were purified from the hydroalcoholic extract of the dried berries after various chromatographic procedures. Structure elucidation was accomplished from NMR and mass data analysis.

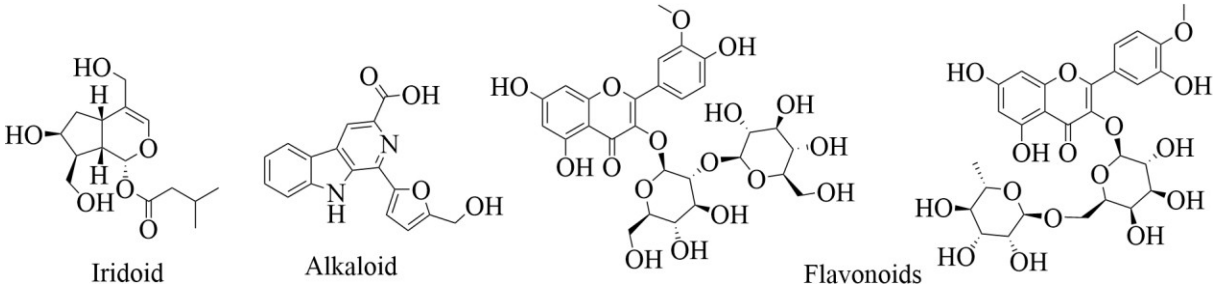

Fig. 1. Structure of iridoid, alkaloid, and flavonoids

Keywords: *Sambucus nigra,* Viburnaceae, iridoid, alkaloid, flavonoid

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S1.P240 Invasive plants as promising solutions against biofilms

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Biofilm is a widespread way of life among human pathogenic microorganisms and conventional drugs are poorly effective against biofilm-associated infections. Invasive plants, due to their often higher chemical diversity than native species, constitute interesting sources of active molecules.

Among 73 extracts obtained from 10 invasive plants collected in France, the essential oil (EO) from the leaves of *Baccharis halimifolia* was the most active against *Candida albicans* (Ca) biofilms, acting against the maturation phase and the 24h biofilm (IC_{50}=4 and 74 µg/mL respectively). The GC-MS analysis of this EO showed the presence of 48 compounds mainly identified as oxygenated sesquiterpenes (62%). The most abundant compound was β-caryophyllene oxide (37%) which showed, as well as aromadendrene oxide-(2) and (±)-β-pinene also present, significant anti- maturation and antibiofilm effects of Ca (IC_{50}=9-630 µmol/L) (Desrini et al., 2023). Also, the 2- methyltetrahydrofuran stem extract of *Ludwigia grandiflora* was the most active against the bi- species biofilm *Staphylococcus aureus* (Sa)-Ca, inhibiting the mature biofilm by more than 50% at 50 µg/mL. An approach using bioguided molecular networks correlating data from UHPLC-MS/MS analyzes of extracts and fractions with the results of antibiofilm tests revealed seven compounds of interest in *L. grandiflora*, belonging to pentacyclic triterpenes and lipids including acylglycerols and hydroxylated and epoxidized derivatives of fatty acids. The most correlated compound, betulinic acid, significantly reduced Sa-Ca preformed biofilms by several pairs of strains (inhibition>40% at 25 µg/mL) (Hamion et al., 2022).

This study clearly confirms the potential of invasive plants as promising solutions against biofilms.

Keywords: invasive plants, biofilm, *Baccharis halimifolia, Ludwigia grandiflora,* antibiofilm compounds

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S1.P241 Isolation and characterization of antimicrobial compounds from *Picralima nitida* (Apocynaceae)

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Our current work is focused on re-isolating akuammidine (**1**), and akuammine (**2**), two major monoterpene indole alkaloids from *Picralima nitida* Stapf. Th & H. Durand (Apocynaceae) and their biological evaluation. The plant is used traditionally for treating infectious diseases (Menzies et al., 1998). The global quest to curb antimicrobial resistance elicits the need to harness antimicrobial and resistant modifying compounds from nature. Seeds were collected from Kpetoe in the Volta region of Ghana. The alkaloidal extract showed significant resistance-modifying properties in efflux pump inhibition and biofilm formation assays against resistant microbial strains.

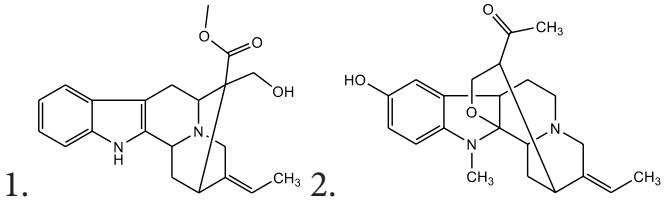

Fig 1. Chemical structures of isolated compounds, akuammidine (**1**) and akuammine (**2**)

Structure elucidation of the compounds was achieved using spectroscopic techniques. Biofilm inhibition assay revealed the compounds to be ineffective against *E. coli* and *C. albicans*. However, they demonstrated significant inhibition of biofilm formation in *P. aeruginosa*, *S. aureus*, and *B. subtilis* for akuammidine by 60%, 67%, 53%, respectively and akuammine by 61%, 27%, and 61% respectively. Additionally, they exhibited superior efflux pump inhibition activity on the organisms except *S. aureus*. These findings suggest the potential application of the compounds as promising leads in the development of antimicrobial agents to combat antimicrobial resistance.

Keywords: *Picralima nitida*, resistant strains, isolation, biofilm formation, efflux pump inhibition

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S1.P242 Identification of a Potent Antibiotic Compound from *Geodermatophilus* Bacteria

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The majority of antibiotics in clinical use today are natural products derived from microorganisms in the environment. One such example is gentamicin, which Dr. Marvin Weinstein discovered in soil bacteria and produced through fermentation of *Micromonospora echinospora*. One of Dr. Weinstein's gentamicin project members, Dr. Luedemann (Weinstein et al., 1963) continued to collect microbial samples from arid desert environments over twenty years and left a large collection of source organisms when he passed away. Herein, we screened over 300 *Geodermatophilus* bacteria from the over 2000 isolate Luedemann collection to discover potent antibiotics candidates from uncharacterized microbial sources. We identified five species with potent antibacterial activity. Among them, lyophilized 9005BA culture supernatant exhibited a relative strong MIC of 100 µg/mL against *Acinetobacter baumannii* and weaker activity against *S. aureus*. The 9005BA was selected for further isolation and structural elucidation studies on the active compound. Bioactivity-guided isolation was performed following a microfractionation technique using HPLC-DAD to target the active compound. In parallel to HPLC-based fractionation, HPLC-ESI-Q-TOF MS in positive ion mode was used for the early detection and prediction of the active compound presented in the extract and fraction. Compound isolation was achieved by solid phase extraction and further semi-preparative HPLC and its structure was established by carrying out 1D and 2D NMR experiments along with HRMS analyses. Here we present our findings on a novel potent antibiotic alkaloid for the gram- negative pathogen, *A. baumannii*.

Keywords: *Geodermatophilus* bacteria, antibiotic, alkaloid, microfractionation, *Acinetobacter baumannii*

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S1.P243 Ethnobotany as a tool for targeted discovery of bioactive natural products for emerging infectious diseases

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Out of the estimated 374,000 species of plants on Earth, 9% have been documented as being used in medicine. Billions of people across the globe currently rely on plants to meet their primary healthcare needs, including for the treatment of infection. Many of our life-saving therapies for treating cancer, pain, heart disease, and infection were developed based on chemical scaffolds first discovered in plants. Yet, most medicinal plants have never been examined through the lens of modern science. This presentation will cover the historical importance of plants in the evolution of current therapeutics and explore emerging technologies, such as microelectron diffraction (Delgadillo et al., 2023), enabling scientists to look deeper into the pharmacological properties of plants. Major classes of antimicrobial natural products will be discussed, and insights on research bottlenecks and challenges in scientific rigor in the field will be addressed (Porras et al., 2021). Examples of a novel antibiotic potentiator **1** (Dettweiler, et al. 2020) from *Callicarpa americana* L., Lamiaceae and antivirulence **2** (Tang et al., 2020) and antifungal **3** (Marquez et al., 2023) agents from *Schinus terebinthifolia* Raddi, Anacardiaceae will be used to illustrate the ethnobotanical approach to drug discovery (Fig. 1).

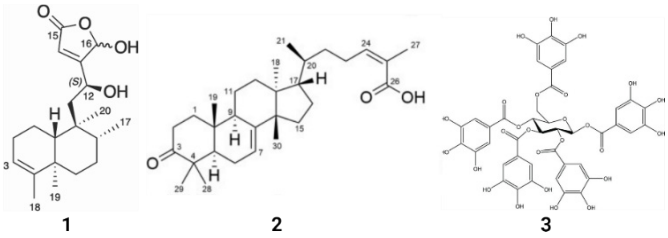

Fig. 1. Structure of a clerodane diterpene **1**, triterpenoid acid **2**, and gallotannin **3**.

Keywords: ethnobotany, antimicrobial, drug discovery, antimicrobial resistance

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S1.P244 Amaryllidaceae alkaloids as promising anti-dengue phytochemicals

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Amaryllidaceae alkaloids (AAs) are compounds belonging to the large group of plant-derived isoquinoline alkaloids, which exhibit various pharmacological activities such as anticancer, antiplasmodial, anti-inflammatory, antiviral, etc (Ding et al, 2017). This presentation will cover the anti-dengue serotype-2 (DENV-2) evaluation of AAs isolated from *Hymenocallis littoralis* (Le et al., 2022; 2023a), *Scadoxus multiflorus* (Le et al., 2023b) and *Pancratium maritimum* (Le et al., 2023c). In total, a library of 37 Amaryllidaceae alkaloids was built from the three plant species, belonging to seven skeletal subclasses: 14 lycorine-type, 3 lycorenine-type, 3 narciclasine-type, 8 crinine/haemanthamine-type, 6 galanthamine-type, 1 montanine-type and 2 norbelladine-type compounds. The library was screened *in vitro* against the DENV2 virus and cytotoxicity was assessed on Vero- E6 cells. As a result, 12 compounds displayed potent inhibition of DENV2 replication (EC_{50} = 0.023 – 3.22 µM); 4 compounds exhibited weak inhibition (EC_{50} = 12.9 – 29.4 µM); and 21 compounds were devoid of activity and cytotoxicity. All potent anti-DENV-2 AAs described in this study showed a certain level of cytotoxicity, but did possess selective antiviral activity (SI = 15 - 127). Preliminary evaluation of structure-activity relationships revealed four skeleton-types (lycorine, homolycorine, narciclasine, and haemanthamine) as promising scaffolds for further research. Synergy was assessed for dual combinations of the four most potent AAs and moderate synergistic effects were also observed.

Keywords: Amaryllidaceae alkaloids, anti-DENV2, cytotoxicity, synergy

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S1.P245 Multi-target action of volatile compounds from essential oils in the treatment of periodontitis

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Periodontal disease has a multifactorial etiology including genetic predisposition, environmental factors and microbial dysbiosis, where Gram-negative anaerobic bacteria play a crucial role. Essential oils have long been used to treat a variety of disorders affecting the oral cavity, and they are mostly known for their antibacterial effect. However, emerging evidence suggests that the predominant bioactive compounds within these oils confer distinct specificity in targeting various bacterial strains. The main mechanism of action seems to be the disruption of the bacterial cell membrane and the potency is higher for the alcohols and phenols (e.g. thymol and carvacrol). Other volatile compounds prevent bacterial replication and transcription by inhibiting the expression of some genes linked to bacterial energy metabolism, and DNA metabolism (Álvarez-Martínez et al, 2021).Evidence from the clinical studies suggests that the antibacterial and anti-inflammatory effects of essential oils are maintained *in vivo,* by inhibiting the growth and biofilm formation of these pathogenic microorganisms (Azad et al, 2016; Takallu et al, 2024). Recent studies suggest that some volatile compounds target pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), as well as inflammatory enzymes, including cyclooxygenase-2 (COX- 2) and matrix metalloproteinases (MMPs) (Tazehjani, 2021). Future research should focus on elucidating the molecular mechanisms underlying compound-specific antibacterial and anti- inflammatory effects in order to develop targeted strategies that can enhance the treatment of periodontal disease.

Keywords: periodontal disease, essential oils, inflammation modulators, antibacterial activity

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Acknowledgments

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S1.P246 Metabolomic-guided phytochemical investigation and antimicrobial activity of *Morus alba* L. twigs extract

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In the realm of public health, a pressing concern is antimicrobial resistance, a significant global threat (De Oliveira et al., 2020). Plant natural products offer promise in combating multi-drug resistance by acting as antibacterial agents through various mechanisms (Woo et al., 2023). Widespread natural product classes, such as terpenes, flavonoids, alkaloids, and phenolics have demonstrated antimicrobial activity (Drasar, 2020).

Morus alba L. (Moraceae) twigs have historical use in traditional medicine for muscle-related symptoms, yet their chemical constituents and clinical potential remain underexplored (Kwak et al., 2023). Here, we conducted a metabolomic-guided phytochemical investigation, based on a LC- HRMS/MS and Molecular Networking combined approach, of an industrial extract of *M. alba* L. twigs, obtained in the field of our scientific partnership with Indena Spa (Milan, Italy). Our analysis allowed us to swiftly profile and isolate 17 secondary metabolites including stilbenoids, flavonoids, flavanones, and chalcones. Molecular networking uncovered a cluster of prenylated polyphenols, with several nodes having a similar parent mass. Manual annotation based on LC-HRMS combined with isolation and NMR-based structure elucidation allowed the identification of a new chromenochalcone derivative.

Isolated metabolites were tested for their antimicrobial activity against *Staphylococcus spp*. The most active compound resulted to be kuwanon C, exhibiting a MIC of 8 *m*g/mL against *S. aureus* ATCC 43300 (methicillin resistant, MRSA) and *S. epidermidis* ATCC 3598 (a biofilm producers' strain). The same concentration resulted bactericidal. We also observed an additive interaction between 4 *m*g/mL kuwanon C in combination with low oxacillin dosage against the MRSA.

Keywords: *Morus alba,* metabolomics, phytochemical investigation, antimicrobial, MRSA

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S1.P247 A new approach to study the mechanisms of action of antimicrobial plant-derived molecules in prokaryotic cells

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The identification of molecular targets of small-molecule drugs is a major challenge of pharmaceutical research (Lomenick et al., 2009). In this context, proteomic-based approaches appear to be very promising. In particular, Drug Affinity Responsive Target Stability (DARTS) coupled with mass spectrometry emerged as an efficient, quick and easy method (Dal Piaz et al., 2016). Although the DARTS approach is widely used to investigate protein interactions in eukaryotic cells and protein extracts, it is still not exploited in bacteria. Therefore, the use of this approach to study antibacterial compounds is limited. The main problem with bacteria is their very fast duplication times and stress- response. To overcome such a limitation, we developed an experimental protocol based on the use of minimal medium, thus slowing down bacterial proliferation and metabolism. We used this approach to identify the molecular target of a plant diterpene with antimicrobial activity. As a bacterium, we chose *Streptococcus mutans*, which recently emerged as a novel Gram-positive model organism (Lemos et al., 2013). The experiment was performed by incubating *S. mutans* with the diterpene while cultured in minimal media, and then performing the typical DARTS protocol followed by LC-MS/MS analysis. The DARTS approach was also carried out using *S. mutans* protein lysate, and the results obtained in the two experiments were compared. Remarkably, most of the proteins identified in the two procedures were the same and their inhibition by the diterpene could actually underlie its antimicrobial activity. These results confirmed that our DARTS experimental model can also be applied to prokaryotic cells.

Keywords: prokaryotic cells, drug affinity responsive target stability assay, plant-derived molecules, bacteria, molecular target(s)

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S1.P248 Harnessing the constrained macrocyclic peptide frameworks for the development of antimicrobial peptides to treat infections

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The need for new innovative treatments for common infections like urinary tract infections (UTI) and skin infections is critical, and among the top 10 reasons to visit outpatient clinics. This is also of particular concern because of increasing antibiotic resistance. Despite widespread acknowledgment of this huge and demanding problem, progress in developing alternative therapeutics has been slow, and current efforts are insufficient. Where do we find novel chemical entities with therapeutic potential against pathogens? Nature has been a tremendous inspiration for novel chemistry throughout history and continues to be a rich reservoir of new chemical entities. One such source of inspiration is antimicrobial peptides (AMPs) produced by all living organisms as important and indispensable components of their innate immune system (Moretta et al., 2021).

Several AMPs have reached clinical trials. However, their development is hampered by a lack of stability because of enzymatic degradation, and a lack of activity when tested in biological contexts. The constrained macrocyclic peptides have experienced a renaissance in medicinal chemistry in recent years (Torres et al., 2019).

Over the years, we have investigated several macrocyclization methodologies, which involve dimerization, backbone cyclization, and the creation of cross-links via a disulfide bond. Our research has utilized KR-12, a truncated peptide derived from the human host defense peptide LL-37, for peptide engineering purposes (Muhammad et al., 2023). Furthermore, we have refined a lead compound for its application in treating bacterial infections (White et al., 2022).

Keywords: antimicrobial peptides, infections, LL-37, KR-12, macrocyclic peptides

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S1.P249 A steroid-rich fraction from parasitic climber *Cocculus hirsutus* (L.) W.Theob inhibits coronavirus disease and neutrophilic inflammation

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Infections from coronavirus diseases and their complications pose significant threats to human health, economies, and societies. Excessive inflammatory responses in human neutrophils are linked to high mortality rates (Chiang et al., 2020). There is a pressing need for affordable and safe treatments, prompting interest in herbal remedies.

Cocullus hirsutus (L.) W.Theob (CH, Menispermaceae) is a perennial climbing plant found widely across Asia and Africa. We have obtained aerial parts of CH from farm in India where it grows as weed and parasite, damaging crops. Notably, various parts of CH are used in folk medicine to treat fever, skin issues, stomach ailments, and urinary disorders. Alkaloid compounds are among major constituents identified in this plant (Logesh et al., 2020). However, other CH secondary metabolites are unexplored.

This study evaluated crude extracts, partitioned layers, and subfractions using SARS-CoV-2 spike/ACE2 pseudovirus neutralization assay (crude CH extract 27.16% and CHEA6 subfraction 54.85% inhibition at 10 μg/mL). Moreover, the anti-inflammatory assays including superoxide anion generation and elastase release in fMLF/CB-induced human neutrophils showed notable activity (crude CH extract 53.95% and 80.39%, respectively; CHEA layer and CHEA6 subfraction >90% inhibition for both assays at 10 μg/mL). Importantly, the bioactivity-guided fractionation of non- alkaloid subfraction CHEA6 (ethyl acetate fraction 6) yielded major steroid triterpenes, including ecdysone type, including 20-hydroecdysone (**1**), makisterone A (**2**), and phytosterols, such as β-sitosterol glucoside (**3**). Additionally, three compounds, including (+)-*proto*-quercitol (**4**), lutein (**5**), and formononentin (**6**) were isolated. The structure of the compounds was confirmed by 1D, 2D NMR, and mass spectroscopic analysis.

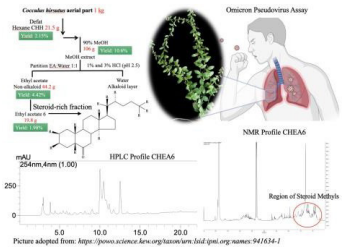

Fig. 1. Steroid-rich fraction isolation scheme, HPLC, and NMR chemical profile

Keywords: *Cocculus,* SARS-CoV-2, human neutrophils, triterpene, phytochemistry

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S1.P250 *Sargassum fusiforme* and its components inhibits Respiratory Syncytial Virus replication *in vitro* and *in vivo*

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Here, we demonstrate that *S. fusiforme* extract (SFE) has antiviral effects against the respiratory syncytial virus (RSV) *in vitro* and *in vivo* mouse models. *Sargassum fusiforme* is an edible brown alga widely distributed around the coastlines of China, Korea, and Japan, and this nutritious marine vegetable has been applied as a therapeutic in traditional Chinese medicine for thousands of years. It is a popular functional seaweed that can prolong life expectancy. (Chen et al., 2018) Treatment of HEp2 cells with non-cytotoxic concentrations (30, 50, or 100 μg/mL) of SFE significantly reduced RSV replication, RSV-induced cell death, RSV gene transcription, RSV protein synthesis, and syncytium formation. Moreover, oral inoculation of SFE significantly improved RSV clearance from the lungs of BALB/c mice.

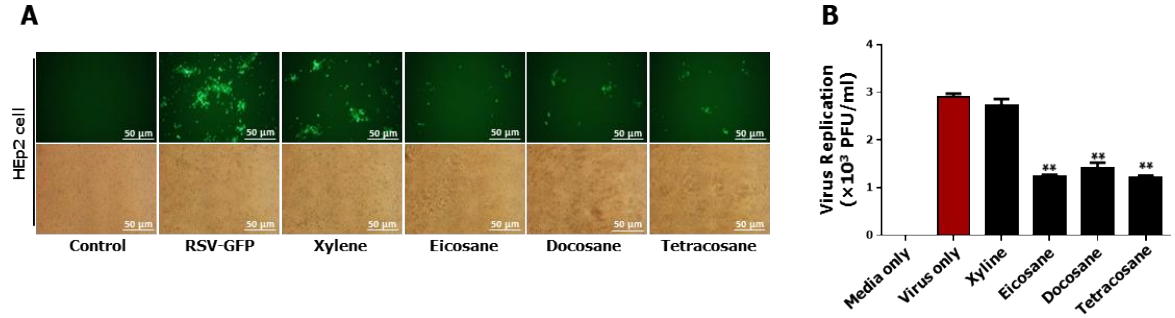

Fig. 1. Anti-RSV effect of eicosane, docosane and tetracosane in Hep2 cells.

Previous reports identified several active components of SFE. Among these, eicosane, dotriacontane, tritetracontane, docosane, heptacosane, and tetracosane are major components of SFE and have been proposed to have antimicrobial activity (El Shafay et al., 2016). Interestingly, the phenolic compounds eicosane, docosane, and tetracosane were identified as active components of SFE. Treatment with a non-cytotoxic concentration of these three components elicited similar antiviral effects against RSV infection as SFE in vitro. Collectively, these results suggest that SFE and its potential components are a promising natural antiviral agent candidate against RSV infection, and consumption of SFE would have beneficial effects in the prevention or therapy of RSV infection.

Keywords: *Sargassum fusiforme,* therapeutic effects, RSV, eicosane, docosane, tetracosane

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S1.P251 Inhibition of Respiratory Syncytial Virus *in vitro* and *in vivo* by *Apostichopus japonicus* (Selenka) extract

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Apostichopus japonicus (Selenka) a sea cucumber, is an invertebrate animal inhabiting the coastal sea around Korea, Japan, China, and Russia. It is one of the highest commercially valuable species of seafood, and has been commonly used for centuries in indigenous and folk medicine. *A. japonicus* is used in Chinese medicine to replenish the kidney, and supplement the essence, nourish the blood, and moisten dryness (Lu et al., 2023, Wang et al., 2021).

Although it has many therapeutic effects, its antiviral activity against Respiratory Syncytial Virus (RSV) has not been reported in detail. In this study, we show that extracts from *A. japonicus* (AJSE) have antiviral effects against RSV *in vitro* cell cultures and an *in vivo* mouse model. Treatment of a human respiratory tract cell line (HEp2) with a non-cytotoxic concentration of AJSE significantly reduced RSV replication, RSV-induced cell death, RSV gene transcription, and RSV protein synthesis.

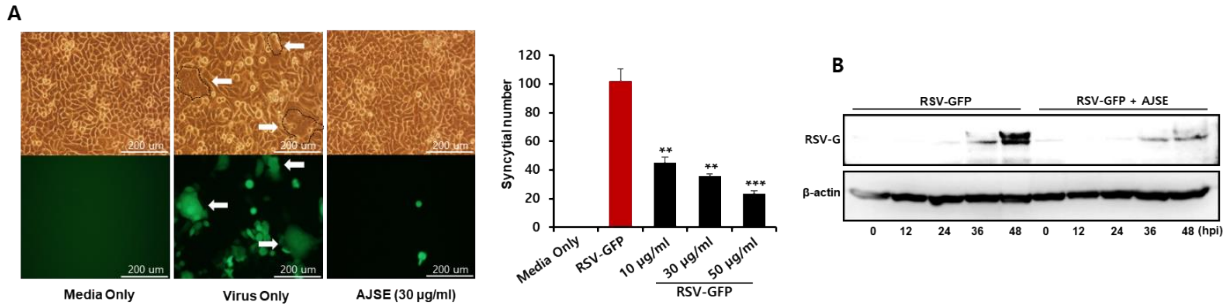

Fig.1. AJSE abrogates RSV-mediated syncytial formation (A), and protein translation (B)

Additionally, the treatments significantly diminished syncytial formation after RSV infection in HEp2 cells. Time-dependent treatment of AJSE after RSV infection in HEp2 cells showed that treatment with two-hour post-infection of virus infections can provided a better result by demolishing further replication of the RSV virus in the Hep2 cell line. Interestingly, oral inoculation with AJSE significantly improved viral clearance in the lungs of BALB/c mice. Together, our results suggested that extracts of *A. japonicus* could be used as a potent natural anti-RSV candidate.

Keywords: *Apostichopus japonicus* (Selenka), RSV, therapeutic effects, virus replication

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S1.P252 *Aster tataricus* and its components display a broad spectrum of antiviral effects *in vitro* and *in vivo*

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This presentation will cover the antiviral activities and mechanisms of action associated with *Aster tataricus* both *in vitro* and *in vivo*. *Aster tataricus*, a member of the family Asteraceae, is a perennial terrestrial herb with a rich history in traditional medicine, native to Siberia, Korea, Japan, and eastern Asia (Shen et al., 2018). It is characterized by violet-blue flowers with a conspicuous yellow center (Chen et al., 2022). The investigation comprised cytotoxicity assessment, antiviral tests, mRNA analysis, and assessment of protein phosphorylation. In the context of *in vivo*, mRNA induction, viral challenge in mice, histopathological examination, and cytokine analysis followed by HPLC chemical characterization and compound antiviral testing.

Preliminary assessments indicate that *Aster tataricus* Extract (ATE) exhibits low toxicity with a CC_{50} of 134.0 ± 1.08 µg/ml, while an effective concentration of 10 µg/ml is demonstrated marked inhibition of Influenza A virus (PR8), Newcastle disease virus (NDV), and Herpes simplex virus (HSV) replication in immune cells (RAW264.7). This inhibition was attributed to the induction of an antiviral state through the upregulation of interferon (IFN)-related genes, coupled with the secretion of IFNs and pro-inflammatory cytokines. *In vivo*, experiments with ATE-treated BALB/c mice revealed enhanced survivability and reduced lung viral titers when challenged with lethal doses of the highly pathogenic influenza A subtypes (H1N1, H5N2, and H9N2). The observed prophylactic effects correlated with increased secretion of IL-6, IFN-λ, and IFN-β in bronchoalveolar lavage fluid (BALF) of treated mice.

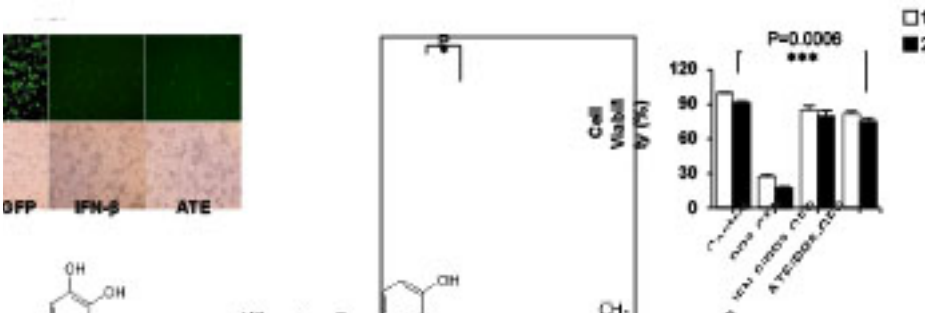

Fig. 1. Antiviral effect of *Astar tataricus* in RAW264.7 cells and structures of HPLC analysis identified active compounds in the aqueous fraction

High-performance liquid Chromatography (HPLC) analysis identified three main active compounds in the aqueous fraction. Subsequent evaluation highlighted the antiviral properties of Quercetin, Kaempferol, and Ferulic acid. This study confirms *Aster tataricus* and its components as potent broad-spectrum anti-viral and anti-influenza agents.

Keywords: *Aster tataricus,* antiviral effect, anti-influenza effect, Active compounds, Herbal medicine

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S1.P253 Extracts of *Costus speciosus* suppresses influenza A H1N1 activity *in vitro* and *in vivo*

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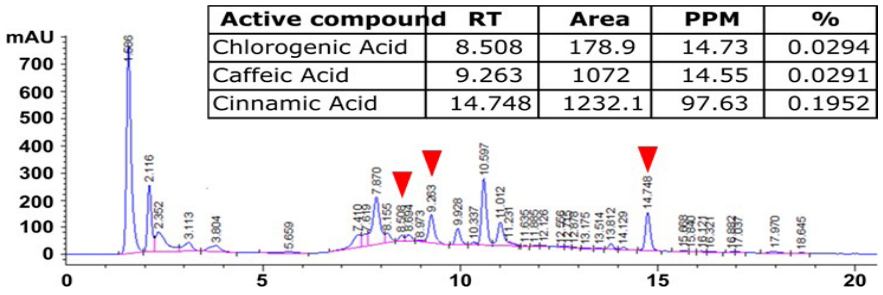
This study demonstrates the antiviral activity of aqueous leaf extract of *Costus speciosus* (J. Koenig) Sm, (TB100) against influenza viruses. *Costus speciosus* (J. Koenig) Sm is a well-known medicinal herb used in traditional medicine in South Asian countries such as Sri Lanka, India, and other tropical regions (Waisundara et al., 2015). TB100, a perennial herb, contains many medicinal phytochemicals with potent antimicrobial, antioxidant, insecticidal, anticancer, and antidiabetic properties (Wang et al., 2015).

Initially, antiviral activity against green fluorescence-expressing influenza A (H1N1) PR8-GFP virus was assessed. EC_{50} and CC_{50} values for RAW264.7 cells were determined as 15.19 ± 0.61 and 117.12 ± 18.31 µg/mL, respectively. TB100 reduced GFP expression and viral copy number, confirming its inhibition of viral replication in murine RAW264.7 and human A549 and HEp2 cells. In vitro pre- treatment with TB100 induced phosphorylation of transcriptional activators TBK1, IRF3, STAT1, IKB-α, and p65, associated with interferon pathways, indicating activation of antiviral defences. The antiviral effects of TB100 against multiple influenza A strains (H1N1, H3N2, and H9N2) were investigated in BALB/c mice. TB100 demonstrated the highest protective response against H1N1, followed by H3N2 and H9N2.



Active compound	RT	Area	PPM	%
Chlorogenic Acid	8.508	178.9	14.73	0.0294
Caffeic Acid	9.263	1072	14.55	0.0291
Cinnamic Acid	14.748	1232.1	97.63	0.1952

Fig. 1. Chemical compounds in TB100 were analysed by the reversed-phase HPLC

High-performance liquid chromatography (HPLC) analysis of aqueous extracts led to the identification of cinnamic, caffeic, and chlorogenic acids as potential chemicals for antiviral responses. Further confirmatory studies using these acids revealed that each of them confers significant antiviral effects against influenza when used as pre-treatment and enhances the antiviral response in a time-dependent manner. These findings suggest that TB100 has the potential to be developed into an antiviral agent that is effective against seasonal influenza.

Keywords: *Costus speciosus,* leaf extract, TB100, influenza A, antiviral effect, transcriptional activator

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S1.P254 Exploring faba bean pods as a potential nutritional supplement for piglet gut health

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One of the significant health concerns in the farm industry is post-weaning diarrhea (PWD) in piglets (Fairbrother et al., 2005). Due to a reduction in the use of antibiotics, it is necessary to find a source to uphold the gut health of piglets (Cheng et al., 2014). A potential source could derive from a by- product, such as faba bean pods (FBP, *Vicia faba* L.), which are rich in bioactive compounds (Valente et al., 2018). Our research focuses on nutritional profile, phytochemical analysis and biological activity of FBP.

Nutritional analysis revealed notable concentrations of crude protein (144 g/kg), potassium (27.8 g/kg), and iron (126 mg/kg) in dry FBP. Phytochemical analysis of methanolic extract (ME) showed significant levels of total polyphenols (38.43±0.59 mg gallic acid equivalents (GAE)/ g dry FBP), tannins (30.67±1.03 mg GAE/ g dry FBP), and procyanidins (0.66±0.13 mg procyanidin B1/g dry FBP). Utilizing a UHPLC-DAD-MSn method, 34 compounds were found in the ME; 16 were identified and classified as derivatives of quercetin and kaempferol, alongside levodopa and piscidic acid. The extract was also tested for inhibiting two common gastrointestinal pathogens. A dose- dependent growth inhibition of *Escherichia coli* ATTC25922 and a total elimination of visible growth of *Salmonella enterica* when exposed to the ME concentrations of 1–8 mg/mL was obtained. Additionally, the ME showed insignificant activity against porcine digestive enzymes (α-amylase, lipase, and trypsin) (IC_{50}>4mg/mL).

Our research demonstrates that FBP is a rich reservoir of varied polyphenols with antimicrobial properties, including the influence of FBP on porcine digestive enzymes. It is a promising feed additive for mitigating PWD in piglets.

Keywords: faba bean pods, food by-product, antimicrobials, post-weaning diarrhea, phytochemical analyses

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S1.P255 Anti-inflammatory properties of twelve Norwegian medicinal plants and their protective properties against impairments in the intestinal epithelial barrier

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Chronic inflammation is linked to various diseases like cardiovascular diseases, cancer, and diabetes. There is a lack of satisfying treatments for these diseases and medicinal plants may represent sustainable and effective sources for new drugs or drug leads. Polyphenols are known to act on several immune signaling pathways and can modulate the production and release of cytokines and affect proliferation and differentiation of leukocytes. The aim of this study was to investigate the *in vitro* anti-inflammatory properties and the ability to counteract impairments in the intestinal epithelial barrier of polyphenol rich extracts from Norwegian medicinal plants. Based on the traditional use of plants towards various inflammatory diseases, twelve Norwegian plants were identified. The plant material was extracted with 80% ethanol and dichloromethane, and the extracts were analyzed by ^{1}H NMR spectroscopy. By stimulating a dendritic cell line to produce NO, the extracts' ability to inhibit NO secretion was determined. Furthermore, inhibition of TNF-α secretion was performed using ELISA with peripheral blood mononuclear cells. The ability to counteract impairments in the intestinal epithelial barrier was investigated by indomethacin treated Caco-2 cells. The results show that the most promising ethanol extracts were *Alnus incana* (59% reduction in NO, 100 µg/mL; 79% reduction in TNF-α, 10 µg/mL), *Antennaria dioica* (38% reduction in NO, 100 µg/mL; 42% reduction in TNF-α, 10 µg/mL), and *Geranium sylvaticum* (36% reduction in NO, 100 µg/mL; 59% reduction in TNF-α, 10 µg/mL). Among the dichloromethane extracts, *Lepidotheca suaveolens* (100% reduction in NO, 100 µg/mL; 81% reduction in TNF-α, 10 µg/mL), *Malva moschata* (96% reduction in NO, 100 µg/mL; 77% reduction in TNF-α, 10 µg/mL), and *Tanacetum vulgare* (100% reduction in NO, 100 µg/mL; 99% reduction in TNF-α, 10 µg/mL) showed strongest effects. Results from Caco- 2 barrier protection experiments will be presented. The NMR spectra indicate a high phenolic content in the active ethanolic extracts with a diverse content in structure type.

Keywords: Medicinal plants, polyphenols, anti-inflammatory, TNF-α, nitric oxide

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S1.P256 Could Bistort rhizome serve as a potential alternative to antibiotics in preventing diarrhea in weaned pigs? *In vitro* study

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Post-weaning diarrhea is a major threat in the swine industry, which results in the high use of antibiotics for its treatment. There is a global agenda to decrease the use of antibiotics in order to reduce the risk of antimicrobial resistance. Therefore, alternative methods to prevent the development of post-weaning diarrhea and maintain piglets' gut health are needed.[1]

Bistort (*Bistorta officinalis*) rhizome (BR) has long been esteemed worldwide for its therapeutic properties, particularly in alleviating diarrhea. Its efficacy is attributed to its high tannin content, making it a popular choice in traditional medicine. However, in piglet nutrition, tannins are widely regarded as anti-nutritional factors.[2]

This study investigated the phytochemical and nutritional content of BR. Considering the attributed anti-nutritional properties and the potential application of BR as feed additives, the in vitro biological activity was evaluated, including the influence of BR on porcine digestive enzymes and antimicrobial activity against porcine gastrointestinal pathogens.

The UHPLC analysis of the aqueous extract of BR revealed 28 major compounds, mainly procyanidins and their galloylated derivatives. Extract from BR inhibited porcine amylase (IC_{50}= 0.05 mg/ml) and lipase (IC_{50}= 0.55 mg/ml) and had no impact on porcine trypsin (IC_{50}= 17.04 mg/ml). Antibacterial activity of the extract against major porcine intestinal pathogens *Salmonella enterica* and *Escherichia coli* in concentrations 0.25 – 8 mg/ml was also tested. Additionally, BR extract exhibited low cytotoxicity (IC_{50}= 330 µg/ml) on the intestinal porcine epithelial cell line (IPEC-J2). The obtained results provide basic evidence of the usage of BR as a novel feed additive in the prevention of post-weaning diarrhea in piglets.

Keywords: *Bistort rhizome, Bistorta officinalis,* antibacterials, cytotoxicity, gut health

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S1.P257 Detection and insight into structural requirements of SARS_CoV2 antibodies by peptide- oligonucleotides derived from sunflower trypsin inhibitor 1 (SFTI-1) peptide

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SARS_CoV2 virus represents the most devastating pandemic of modern times. As part of epidemiology studies, it has been crucial to understand the prevalence of antibodies following COVID-19 infection and vaccination, providing insight into protection against the virus. Recently Antibody proximity extension assay (AbPEA) was successfully used in SARS_CoV2 antibody detection (Zhao et al. 2022), offering high sensitivity with minimum samples volumes. However, the AbPEA typically utilizes complete viral antigenic protein domains (eg. spike protein subunit S and nucleocapsid protein), examining total antibody reactivity.

To enhance antibody detection specificity, we designed peptide-oligonucleotides containing short epitopes (10-15 residues), originating from immunodominant regions of the spike protein. These constructs were then employed in AbPEA to detect antibodies in convalescent sera and dry blood spots of vaccinated individuals. Two different counterpart epitopes were compared, in their linear form and incorporated within a cyclic scaffold derived from sunflower trypsin inhibitor 1 (SFTI-1) (Korsinczky et al. 2001; Eriksson et al. 2023). Peptides were synthesized by Fmoc-SPPS and their secondary structural characteristics were determined by ^{1}H NMR.

Several epitopes from S1and spike protein subunit S2 regions were able to detect antibodies in seropositive samples. Notably, peptide conformation appeared to influence antibody binding for some epitopes derived from the S1 and S2 regions, but not for several other epitopes. Certain epitopes reacted with antibodies regardless of whether they were linear or cyclic, indicating that the conformation does not affect binding. Overall, we gained insight into heterogeneity in antibody profiles across patient samples as well as structural requirements of antibodies for antigen binding. This study highlights the potential for improving current SARS-CoV2 antibody detection methods to achieve greater specificity in antibody profiling. If these fine specificity profiles in patient samples can be correlated with clinical symptoms and disease severity, AbPEA will become an invaluable tool for better understanding of seroprevalence.

Keywords: SARS_CoV2, antibody proximity extension, seroprevalence, peptide, SFTI-1

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S1.P258 Chemical analyses and *in vitro* biological properties of *Lythrum salicaria* L. for potential use in piglet nutrition

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Post-weaning diarrhoea (PWD) is a significant gastrointestinal disease affecting piglet production. Traditionally, PWD was treated by antibiotics, however this has contributed to the spread of antimicrobial resistance, making new therapeutic strategies urgently needed (Fairbrother et al., 2005). One possible approach to reduce the risk of PWD is the application of hydrolysable tannins, which were known for anti-diarrhoeal properties in the pre-antibiotic era. (Girard et al., 2020).

The present work aims to establish the phytochemical composition and biological activity of Purple loosestrife (*Lythrum salicaria* L.) aqueous extract (LSH) to assess the potential beneficial effects on gastrointestinal health of post-weaning piglets (Piwowarski et al., 2013). Firstly, we found a high content of tannins (TTC=268.06 µg/mg) as dominating compounds among polyphenols (TPC=274.75 µg/mg) in LSH. Then, we verified the inhibitory activity of LSH against *Salmonella typhimiurium* LT2, determining the minimal inhibitory concentration at 0.5 mg/mL and the dose-dependent effect on *Escherichia coli* ATTC 25922 in concentrations 0.5-2 mg/mL. We also determined cytotoxicity of LSH for the intestinal porcine epithelial cell line J2 (IC_{50}>100µg/mL). Additionally, we studied LSH interaction with porcine digestive enzymes. LSH had no effect on porcine trypsin with IC_{50}=27.45 mg/mL, and minimal effect on amylase with IC_{50}=2.65 mg/mL. However, we found significant activity against lipase with IC_{50}=0.33 mg/mL. To further investigate the bioactivity of the compounds found in LSH, we fractionated the LSH using column chromatography and spectroscopic methods. We isolated several hydrolysable tannins including vescalagin, castalagin, and salicarinin A, B, and C. Our results support the usage of LSH as novel feed additive in reducing the incidence of PWD in piglets.

Keywords: anti-microbial properties, hydrolysable tannins, *Lythrum salicaria* L., phytochemical composition, post-weaning diarrhoea

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S1.P259 Therapeutic potential of stilbene/β-diketone/gingerol allied naturally occurring non- flavonoids in triple-positive breast cancer: tracing the path from extraction to efficacy

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Triple-positive breast cancer (TPBC) is a less aggressive type of breast cancer often treated with long-term oral medications, but resistance can occur (Wang et al., 2019). To alleviate this issue, researchers are exploring natural and therapeutic interventions, including polyphenols, which have excellent anticancer properties (Schmidt et al., 2020). However, only a few studies have explored the role of non-flavonoid polyphenols in TPBC, while the major focus was on ER+ or triple-negative BC.

The present investigation aimed to evaluate the antiproliferative activity of three distinct non- flavonoids derived from different plant sources – resveratrol with a stilbene moiety from *Vitis vinifera*, curcumin with a β-diketone moiety from *Curcuma longa*, and 6-gingerol with a gingerol moiety from *Zingiber officinale* against two breast cancer cells: TPBC-BT-474 and TNBC-MDA- MB-231 via MTT assay (Mosmann 1983). Plant extracts of dried+sieved grapes, turmeric, and ginger powder were prepared via reflux extraction using ethanol and water mixture. Isolation and purification of respective polyphenols were carried out by Flash chromatography and HPLC/MS. Structures were elucidated by ^{1}H-NMR, ^{13}C-NMR, FT-IR, and HPLC/MS.

Among the three natural polyphenols, resveratrol showed superior antiproliferative activity against TPBC-BT-474 compared to TNBC-MDA-MB-231 cells. The other two polyphenols isolated from the rhizome family – curcumin and 6-gingerol showed better inhibiting properties against MDA-MB-231 cells. The chemopreventive effect of polyphenols is attributed to their excellent anti- inflammatory properties, especially through their antioxidant activity. The study highlights the potential of non-flavonoid polyphenols, particularly resveratrol (comprising stilbene moiety) as a scaffold for developing novel therapeutic derivatives for TPBC suggesting that their synergistic effect with anticancer drugs could enhance future treatments.

Keywords: non-flavonoids, reflux extraction, MTT, BT-474 cells, anti-breast cancer

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S1.P260 Drug discovery based on essential oils concurrently active against eumycetoma and actinomycetoma with *Pimpinella anisum* emerged as a forerunner

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Mycetoma is a neglected infection of the subcutaneous tissues. It is caused by fungal (eumycetoma) and bacterial (actinomycetoma) pathogens (Relhan et al., 2017). There is an urgent need to develop novel, effective, broad-spectrum antimycetomal agents. Treatment differs for both entities, and a diagnostic tool differentiating eumycetoma from actinomycetoma is limited and unaffordable. Therefore, discovering drugs concurrently acting on both types would circumvent the diagnosis to initiate treatment promptly. Eight essential oils (EOs) from diverse taxonomical origins belonging to five botanical families were screened for antimicrobial activity against *Madurella mycetomatis* and *Actinomadura madurae* strains. EOs were prepared by steam distillation, tested *in vitro* against mycetoma strains (Abd Algaffar et al., 2021, 2022), and subsequently subjected to gas chromatography, mass spectrometry, and radical scavenging activity (RSA). The most promising EOs of *Myristica fragrans* L. (Myristicaceae) and *Pimpinella anisum* L. (Apiaceae) demonstrated dual *in vitro* activity against all tested strains with MICs of 0.004 to 0.125% v/v, and DPPH inhibition of 90.30±0.008 to 98.00±0.003 %RSA±SD, respectively. Both EOs were further tested for *in vivo* toxicity and efficacy in *Galleria mellonella* larvae models (Konings et al., 2021). *P. anisum* EO enhanced the survival of *M. mycetomatis*-infected *Galleria* larvae, with no signs of toxicity to uninfected larvae. A tendency towards enhancing survival was noted for *A. madurae*-infected larvae. The use of these EOs as broad-spectrum dual antimycetomal agents, could cutting diagnosis cost and time and subsequently resulting in an early treatment. This approach could possibly pave the way for the next generation of antimycetomal agents.

Keywords: *Madurella mycetomatis, Actinomadura madurae,* essential oils*, Galleria mellonella*, metabolomics

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S1.P261 Synthesis of autoinducer-2 prodrugs and analogues as a new antimicrobial strategy

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Many species of bacteria control gene expression on a community-wide scale by producing, secreting, detecting and responding to extracellular signaling molecules that accumulate in the environment in proportion to cell density. This process is called quorum-sensing and enables these organisms to coordinate their behaviours which are more beneficial when cells are working in unison.[1,2] Autoinducer-2 (AI-2, Fig.1) is one of the extracellular signal molecules and is unique as it is produced and detected by many phylogenetically distinct bacteria.[1,2]

In past years we have developed the synthesis of (*S*)-4,5-dihydroxypentane-2,3-dione (DPD **1**, Fig.1),[3] which is the AI-2 precursor, and analogues in optically pure form.[2,4-6] Based on previous results, we hypothesise that AI-2 plays an important role in controlling colonisation and homeostasis of mammalian gut microbiota contributing to the protective properties of these poly-species against pathogens.[1] This community of bacteria is crucial for production of nutrients, maturation of the immune system and host protection against pathogens. Therefore, the ability to drive this community from disease to healthy states by manipulating the native signals and interactions occurring between its members offers great potential for therapeutic benefit. AI-2 analogues have high potential as a complementary therapy that can avoid the use of antibiotics, contributing to the fight against the development of antibiotic resistance, a worldwide problem.

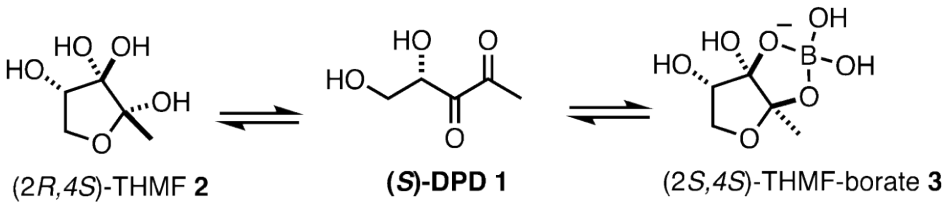

Fig. 1. Structures of linear and two cyclic forms of Autoinducer-2 (AI-2)

Here we present the synthetic strategies to obtain new analogues and prodrugs of AI-2 and the results of their biological evaluation.

Keywords: autoinducer 2, organic synthesis, quorum sensing, antibiotic resistance

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S1.P262 Bioactive compounds, antibacterial and antioxidant activities of methanol extract of *Axonopus compressus* (Sw.) P. Beauv

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This presentation will cover the various phytochemical constituents, antibacterial, antioxidant activities present in the methanol extract of *Axonopus compressus* responsible for its pharmacological activities. *Axonopus compressus* is a perennial, terrestrial stem compressed grass with a bearded or hairy nodes belonging to the family Poaceae [Cabi, 2019] commonly known as carpet grass. *Axonopus compressus* is widely used in the treatment of a wide range of infectious diseases by a large number of locals [Tow et al., 2018].

It has become very crucial to explore natural compounds due to their therapeutic potential in the treatment of infections [Vaou et al., 2021]. The extract was obtained through standardized maceration methods and subjected to various phytochemical screening tests, gas chromatography-mass spectrometry analysis to determine the chemical compounds present and the antibacterial activity was tested against some pathogens invitro.

The phytochemical screening tests revealed the presence of tannins, saponins, flavonoids, phenols in varying concentrations. The result of GC-MS analysis revealed the presence of 61 bioactive compounds in the extract with peak area such1,2, 3 – Benzentriol (20.69%), gamma. -Sitosterol (10.89%), 9,12-Octadecadienoic acid (Z, Z) (6.27%), 5-Hydroxymethylfurfural (6.62%) with different activities such as antibacterial, anti-inflammatory, anticancer. Reports on antioxidant activity indicated a notable rise with increasing plant concentration. The extracts demonstrated inhibitory potential against all tested pathogen.

GC-MS analysis revealed the presence of the bioactive constituents of the plants with various biological activities and this justifies the folkloric use of this plant as a therapeutic remedy by a large number of locals.

Keywords: *Axonopus compressus*, phytochemical constituents, antioxidant activity

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S1.P263 The anti-inflammatory and pro-resolving role of selected South African medicinal plants used traditionally as immunomodulatory remedies

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There is a growing interest in the use of natural products as alternative and/or combination therapy against inflammatory conditions. The use of medicinal plants to treat auto-immune disorders is a common practice in southern Africa and a primary source of healthcare for many individuals. However, the pharmacodynamic and pharmacokinetic properties related to the traditional use of herbal remedies as immunomodulatory agents remain relatively unknown. This study investigates the anti-inflammatory properties of extracts prepared from the leaves of *Warburgia salutaris* (G. Bertol.) Chiov., and the bark of *Pterocelastrus rostratus* (Thunb.) Walp., widely used traditionally to treat inflammation and pain. The inhibitory effect of aqueous and ethanol extracts as well as fractions on the release of pro-inflammatory cytokines was determined in lipopolysaccharide (LPS) stimulated and unstimulated RAW 264.7 murine macrophage cells. The levels of interleukin (IL)-1β, IL-6, tumour necrosis factor-α (TNF-α), and monocyte chemoattractant protein 1 (MCP-1) release were determined using cytokine multiplex-bead assays. The inhibitory properties of extracts and fractions were also evaluated on the release of prostaglandin E2 (PGE2), leukotriene-B4 (LTB4), cyclooxygenase-2 (COX-2), and nuclear factor-kappa B (NF-κB) in LPS-stimuted RAW 264.7 cells. The ethanol extracts and fractions of *W. salutaris* and *P. rostratus* demonstrated the greatest inhibitory activity, with over 50-fold inhibition of IL-1β, IL-6 and TNF-α levels in LPS-stimulated RAW 264.7 macrophages without toxicities in cell viability assays. These findings give an insight into understanding the mechanism of action of widely used southern African medicinal plants against chronic pain and rheumatological diseases.

Keywords: medicinal plants, inflammation, cytokines, cyclooxygenases, southern Africa

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S1.P264 Phytochemical analysis and antiplasmodial activity of *Pseudolachnostylis maprouneifolia* fruits extracts

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Pseudolachnostylis maprouneifolia, a member of the Phyllanthaceae family, is recognized for its distinctive botanical features and potential medicinal properties. In this study, we conducted a comprehensive phytochemical screening and evaluation of the antiplasmodial activity of a methanolic extracts derived from the fruits of *Pseudolachnostylis maprouneifolia*, sourced from Katanga in the Democratic Republic of the Congo. The extracts exhibited significant antiplasmodial activity against the chloroquine-sensitive *Plasmodium falciparum* strain (3D7), with an IC50 value of 4.56 ± 0.19 µg/mg, underscoring their potential as a source of antimalarial compounds (Jansen et al., 2010).

Using a multidisciplinary analytical approach, we employed thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) for component identification, followed by fractionation using column chromatography (CC). While gallic acid and methyl gallate were identified as key bioactive compounds through nuclear magnetic resonance (NMR) spectroscopy (Arsianti et al., 2018), ongoing fractionation efforts acknowledge the potential contribution of additional constituents to the observed antiplasmodial activity (Degotte et al., 2021, Arsianti et al., 2018). The goal of this study is to fully understand the chemical makeup of fruit extracts from *Pseudolachnostylis maprouneifolia* and find new antimalarial drugs that show in different mechanisms of action.

Our findings highlight the therapeutic potential of *Pseudolachnostylis maprouneifolia* as a valuable botanical resource for the development of antimalarial agents and underscore the importance of continued exploration to harness its full pharmacological potential.

Keywords: *Pseudolachnostylis maprouneifolia,* Gallic acid, Methyl gallate, Malaria, NMR

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S1.P265 Reconstruction of an ancient treatment of infections by incense

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The increasing number of antimicrobial resistances (AMRs) is one of the major health challenges nowadays. Effective strategies for management of AMRs include revitalizing already existing antimicrobials and combination therapies. In ancient drug books written in the 17th century in Austria herbal combinations have been used as incense to treat infectious diseases. Within of that treatment, incense was burned and smoked through sheets. Afterwards, the sheets were put on wounds or wombs in case of urinary infections. The aim of this work was to reconstruct and proof the described treatment. Therefore, an apparatus containing Erlenmeyer flasks, heater and hoses was created to fumarate herbal mixtures and draw the smoke through a solvent by vacuum. In short, herbs were burned in an Erlenmeyer flask on a heater and the fume was suck via vacuum through a hose in a second flask containing a solvent (10% DMSO in water). The hose transporting the smoke ended in the solvent. A second hose reaching into the flask was connected to the vacuum and drew the smoke through the solvent. To contain 1 ml of extract 2.56 mg dried material were burned. The resulting extracts were tested against MRSA by microdilution assays using a time-kill methodology (Fladerer et al., 2023). One formulation containing eggshell, coriander, silver birch and myrrh significantly reduced the growth of MRSA ($p < 0.05$) pointing towards the effectivity of this 300-year-old treatment against bacterial infections. As a result, we were able to develop a new method to obtain extracts from incense formulations and to prove their antimicrobial activity against MRSA.

Keywords: Antimicrobial resistance, MRSA, incense, method development

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S1.P266 Chelation of metal ions by tannins: from protolytic equilibrium to antibacterial activity

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Ellagitannins are a subgroup of tannins, ubiquitous secondary polyphenolic plant metabolites. They are technologically important compounds with several health-enhancing properties like antibacterial activity. Essential metal ions depletion is one of the major mechanisms of antibacterial action (Serrano et al., 2009). We studied the interactions of six ellagitannins (vescalin, castalin, vescalagin, castalagin, roburin A and D), isolated from chestnut wood extract, with Fe^{2+} and Al^{3+} ions. The main goal of the present study was to determine, how structural motifs of ellagitannins (NHTP and HHDP groups; green and orange in Fig. 1) affect the stoichiometry of complexes formed. This was performed using UV-Vis spectroscopy in conjunction with Job's method. Moreover, the effect of pH on tannins affinity for metal ions was examined. Since the tannin-metal ions interactions strongly depend on pH (Frešer et al., 2021; Frešer et al., 2024), the detailed study of ellagitannins microscopic protolytic equilibriums was performed using a combination of ^{13}C NMR and DFT methods. The results provided us with information on exact deprotonation positions, as well as pK_a constants. This enabled us to develop a model that explains the observed ellagitannin-metal ions interactions and can predict the stoichiometry and metal affinity of new ellagitannins, consisting of the NHTP and HHDP groups. The significance of the study does not end here because insight into ellagitannins' microscopic protolytic equilibrium is crucial for understanding their other properties, such as their bioavailability, reactivity, interactions with proteins and pH-dependent oxidative stability.

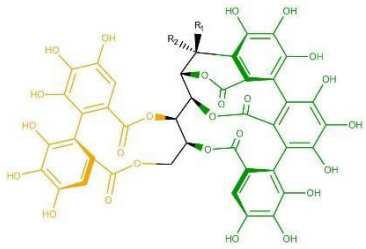

Fig. 1. Chemical structure of vescalagin (R_1=OH, R_2=H) and castalagin (R_1=H, R_2=OH)

Keywords: ellagitannins, metal ions chelation, proteolytic equilibrium, Job's method, NMR

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S1.P267 Effect of pH on UV/Vis and CD spectrum of cohumulone and colupulone

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This presentation will cover the isolation and characterization of cohumulone and colupulone from hop (*Humulus lupulus* L.) cones. The isolated compounds are of utmost technological importance because especially cohumulone is the source of the bitter beer taste, while they are researched also for other health-enhancing effects, such as antimicrobial (Kolenc et al., 2023), diuretic, and progestogenic properties. (Arruda et al. 2021; Zanoli et al., 2008)

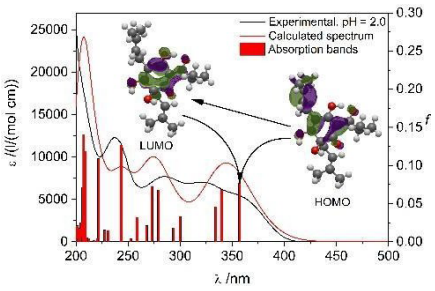

Fig. 1. Comparison of experimental and calculated spectrum of cohumulone and visualisation of orbitals taking place in HOMO→LUMO transition.

The compounds were dissolved in aqueous buffers of various pH values. The corresponding dependence of cohumulone and colupulone UV/Vis spectrum was measured. Additionally, the CD spectrum of cohumulone various species was examined. The UV/Vis spectrum of all possible species of cohumulone and colupulone species was calculated using TD-DFT on B3LYP level of theory and 6—311++G(d,p) basis set in the implicit CPCM water model. Additionally, CD spectra of all possible rotamers of cohumulone were calculated and compared to the experimental spectra. Next to observing which spectral transitions are responsible for the experimental absorption spectrum, the used method also enabled us to examine, which species are most likely present in the solution. What is more, the stereoconfiguration of cohumulone was examined and confirmed to be in good agreement with the stereoconfiguration already determined (Urban et al. 2013) – demonstrating that combination of CD spectroscopy and TD-DFT can relatively easily provide information on absolute stereoconfiguration.

Keywords: cohumulone, colupulone, stereoconfiguration, CD-spectroscopy, TD-DFT

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S1.P268 Inhibitory activity of *Vitex negundo (Lagundi)* leaf extracts against biofilm formation and quorum sensing of *Vibrio cholerae*

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Cholera, endemic in 69 countries including the Philippines, affects 1.3 billion individuals, with antibiotic use threatening antibiotic-resistant strains due to the biofilm formation of *Vibrio cholerae (*Almagro-Moreno et al., 2015; Yoon et al., 2019). The bacterium's virulence is regulated by quorum sensing (QS), controlled by LuxO, which is regulated by *hapR* and *qrr4*, quantified in this study (Watve et al., 2020; Hema et al., 2017). Lagundi (*Vitex negundo*), a traditional Philippine cholera treatment, is investigated for anti-QS and anti-biofilm properties (Basri et al., 2014; WIPO, 2015; Haq et al., 2012; Dwivedi et al., 2021).

This study determined the efficacy of *V. negundo leaf* methanolic extracts against *V. cholerae* by measuring the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), assessing antibiofilm activity against both *V. cholerae* and *Chromobacterium violaceum*, and evaluating anti-QS activities against *C. violaceum*. Quantifying gene expression of QS-regulating *qrr4* and *hapR* was also conducted. Positive control used is gentamicin.

Lagundi leaf extracts showed promise against cholera-causing *V. cholerae*. The minimum concentration needed to inhibit (MIC) and kill (MBC) *V. cholerae* was 7.81 and 125 mg/mL, respectively. Lagundi extracts, especially at 500 mg/mL, significantly reduced biofilm formation in both *V. cholerae* and a standard bacterium (C. violaceum). Interestingly, this concentration even seemed more effective than gentamicin against biofilms in *C. violaceum*. Lagundi extracts also inhibited *C. violaceum*'s cell-to-cell communication (quorum sensing) in a dose-dependent manner, with an inhibition zone similar to gentamicin (0.8 cm vs 1.5 cm). However, Lagundi appears to work through a different mechanism than directly suppressing a key QS gene (*qrr4*).

These findings underscore the therapeutic potential of *V. negundo* against *V. cholerae*. Mechanistic studies are necessary to identify bioactive compounds, optimize dosing regimens, and assess pharmacokinetics. Exploring synergistic combinations with existing antimicrobials are essential.

Keywords: *Vitex negundo*, *Vibrio cholerae*, quorum sensing, biofilm formation, gene expression

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S1.P269 Exploring the role of tannin-metal ion complex formation in antimicrobial activity

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The focus of the study was the antibacterial effect of ellagitannins, which are polyphenolic compounds known for their wide range of biological activities [1]. We specifically examined the Minimum Inhibitory Concentration (MIC) values of chestnut extract components, including a) gallic acid, b) vescalin and castalin as well as c) vescalagin and castalagin, against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli* using the broth microdilution method [2]. Our findings reveal that all tested samples displayed significant antibacterial activity. It was also observed that the concentration of the growth medium impacts MIC values by affecting the availability of nutrients to the bacteria. In the case of *E. coli*, MIC values increased proportionally to the medium concentration, whereas for tannin concentrations below the MIC, the duration of the lag phase extended exponentially with rising tannin concentration [3]. This effect was less pronounced for *Staphylococcus aureus* [4]. Additionally, when essential metal ions were added to the Mueller-Hinton Broth (MHB), an elevation in MIC values was observed for all tannins, suggesting that tannins' chelation of metal ions inhibits *E. coli* growth. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was employed to track metal ion concentrations in MHB, revealing that *E. coli* accumulates specific essential metal ions while others are unaffected. Notably, gallic acid was found to reduce the accumulation of these ions significantly.

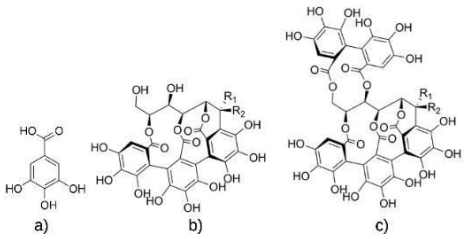

Fig. 1. Structural formula of a) gallic acid, b) vescalin (R1=H, R2=OH) and castalin (R1=OH, R2=H) and c) vescalagin (R1=H, R2=OH) and castalagin (R1=OH, R2=H).

Keywords: metal ions, tannins, *Escherichia coli,* antimicrobial activity, ICP-MS, MIC

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S1.P270 Isolation of flavonoids from the stem bark of *Cynometra malaccensis*

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Cynometra malaccensis, belongs to the Fabaceae family (Leguminosae). Even though there are 122 species of *cynometra* (GBIF, 2024), less than 10% of the species has been studied (Sabiha, 2022). *Cynometra* species were reported to contain flavonoids and phenolic compounds which are secondary metabolites known for their potent antioxidant and antibacterial activities (Ado, 2019 & Yuan, 2021). In this study, *C. malaccensis* was selected to be phytochemically investigated. The powder from the stem bark of *C. malaccensis* (750 g) was macerated in acetone at room temperature for 24 hours and repeated three times. The initial crude acetone extract (15 g) was subjected to treatment with diethyl ether in order to reduce tannin, resulting in a tannin-reduced extract (7.9 g). The process of fractionation was carried out using vacuum liquid chromatography (VLC) with the eluent *n*- hexane:ethyl acetate, which was gradually increased in polarity. This resulted in the separation of the mixture into eight semi-purified fractions (CMS 1-8). Fraction CMS 5 (425 mg) underwent purification using column chromatography (CC) with the solvent system of chloroform:ethyl acetate. This process resulted in the isolation of a pure compound **1** (0.9 mg). Compound **2** (28.4 mg) was obtained from CMS 6 (195 mg) using CC (chloroform:ethyl acetate). Isolation of compound **3** (0.9 mg) from CMS 7 (438 mg) was conducted via CC (chloroform:ethyl acetate) and further purified using preparative thin layer chromatography (pTLC). Based on spectroscopic techniques and comparison with existing literature data, the isolates were identified as apigenin (**1**), luteolin (**2**), and 7,4'-dihydroxyflavone (**3**).

Keywords: *Cynometra malaccensis,* apigenin, luteolin, 7,4'-dihydroxyflavone

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S1.P271 Chemical constituents from the leaves of *Dipterocarpus costulatus* (Dipterocarpaceae)

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Dipterocarpus is the third largest genus in the group of Dipterocarpaceae family and is particularly prominent in the tropical rainforests of Southeast Asia (Appanah & Turnbull, 1998). This genus is reported to possess a broad spectrum of potential biological activities due to the presence of their resveratrol oligomers, flavonoids and triterpenoids (Xue et al., 2014) showing anti-bacterial, anti- fungal, anti-cancer, anti-diabetic and hepatoprotective effects (Fernandes & Maharani, 2019). In this study, *D. costulatus* was selected to be phytochemically studied. The granules from the leaves of *D. costulatus* (1040 g) were macerated in methanol to give crude methanol extract (765 g). Six fractions (DC 1-6) were obtained from the fractionation of the crude via vacuum liquid chromatography. Purification of fraction DC 4 (1 g) by column chromatography (CC) with the solvent system chloroform:methanol yielded two pure compounds **1** and **2**. Meanwhile, compound **3** was obtained from the purification of DC 3 (500 mg) with the same chromatographic method with the eluent chloroform:ethyl acetate. The structural elucidation of the pure compounds was accomplished based on the spectroscopic analyses and comparison with the literature data. The pure compounds were determined as suffruticosol A **(1)** (7 mg), vaticanol B **(2)** (14 mg) and scopoletin **(3)** (4 mg) **(Fig. 1).** We would like to express our greatest appreciation to the Ministry of Higher Education, Malaysia through Fundamental Research Grant Scheme (FRGS/1/2022/STG04/UITM/02/24).

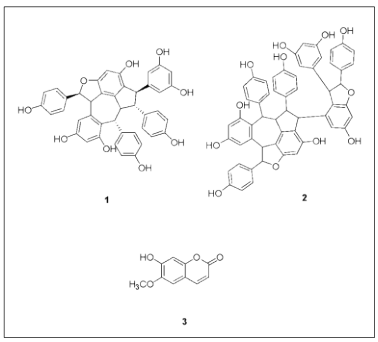

Fig. 1. Structures of compounds **1** – **3**

Keywords: *Dipterocarpus costulatus,* resveratrol oligomers, vaticanol B, suffruticosol A, scopoletin

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S1.P272 16-Angeloyloxy tigliane diterpenoid esters from *Euphorbia cooperi* with anti-HIV activity

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Tigliane diterpenoids constitute a significant class of anti-HIV diterpenoids in the *Euphorbia* genus (Wang et al., 2015, Otsuki et al., 2023). In an investigation to identify anti-HIV diterpenoids from South African *Euphorbia* species, an extract of *Euphorbia cooperi* N.E.Br. ex A.Berger, with 100% viral inhibition and IC_{50} = 0.14 mg/mL, was investigated by a luciferase-based antiviral assay. The extract was analysed using LC-TOF-MS-MS for the presence of diterpenoids. The purification of the extract afforded four 16-*O*-tigliane diterpenoids, including two new compounds (**1**) (Fig. 1) and a 7- oxo derivative of **1**. Two known 12-deoxy phorbol esters were also identified. Tigliane phorbol esters fragment well in the ESI positive mode of the MS due to the ester bonds that are prone to MS fragmentation. The MS fragmentation patterns of the minor peaks in the LC chromatogram enabled us to propose structures for these compounds.

Other isolated compounds included a new norsesquiterpenoid and its known glycoside, arachiside A. Three phorbol esters demonstrated anti-HIV activity, with compounds **1** and one of the known phorbol esters exhibiting potent viral inhibition concentrations when compared with AZT (zidovudine). The enhanced activities of the two active diterpenoids may be attributed to structural features on the seven-membered ring of the tigliane diterpenoids.

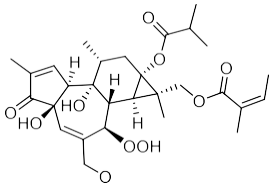

Fig. 1. Structure of 12-deoxyphorbol ester **1**.

Keywords: *Euphorbia cooperi,* 16-*O*-tigliane diterpenoids, anti-HIV, LC-TOF-MS-MS

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S1.P273 Anti-inflammatory and anti-oxidative effects of STW 42 and root extract of *Althaea officinalis* L. on endothelial cells, fibroblasts and macrophages *in vitro*

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Introduction: The root extract of marshmallow (*Althaea officinalis* L., REAo), known to cure mild irritation of pharygeal mucosa as well as stomach/gut mucosa[1], has a physical action and also induces regeneration of irritated mucosa[2]. Damage is associated with mucosal infiltration of activated leukocytes, such as MΦ, which produce excessive ROS and pro-inflammatory cytokines.

Objectives: We aimed to investigate whether REAo has anti-inflammatory/-oxidative properties in human dermal fibroblasts (NHDF), human umbilical vein endothelial cells (HUVEC), and human acute monocytic leukemia (THP-1) differentiated MΦ, critical cellular components of the gastrointestinal and oral mucosa.

Materials and methods: The cells were pre-treated with REAo or its formulation STW 42, containing REAo, and selected excipients, followed by incubation with H_2O_2 (pro-oxidative) or lipopolysaccharide (LPS, pro-inflammatory). Intracellular ROS were quantified with DCFDA. The releases of IL-6 and TNF-α were determined by ELISA. THP-1 cells were differentiated into MΦ with 0.1 µg/ml phorbol-12-myristate-13-acetatefor 3–5 days.

Results: Pre-treatment (24 h) of HUVEC, NHDF or THP-1-MΦ with STW 42 or REAo (100-1000 µg/ml) significantly inhibited the H_2O_2 -induced intracellular ROS production by 30.0% to 58.7% and the LPS-activated IL6 release by 25.0-67.0%. The observed effects of STW 42 or REAo were similar to 25-100 µM diclofenac, which was used as an anti-inflammatory control.

Conclusion: These properties may support the benefit of STW 42 and its constituent REAo in patients with mucosal irritation with subsequent irritative cough or gastric complaints.

Keywords: *Althaea officinalis* L, STW 42, anti-inflammatory, anti-oxidative, mucosa

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S1.P274 Ancient remedies for modern challenges: novel anti-infectives from Egyptian mummy embalming

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The escalating global threat of antibiotic resistance and the resurgence of neglected tropical diseases (NTDs) underscore the urgent need for new anti-infective therapies.[3] Of all anti-infective drugs approved from 1941-2014, 73% are natural products (NPs) or derivatives, underscoring the importance of NPs in drug discovery and development for this therapeutic area.[1,2] Leveraging the rich pharmacopoeia of Traditional Egyptian Medicine from the Pharaonic Era (ca. 3000-323 B.C.), we embarked on a collaboration to unearth potential anti-infective agents from ancient remedies.

Partnering with Egyptologists, we decoded and analyzed ancient medical recipes, focusing on identifying compounds with anti-infective properties. Concurrently, we formed alliances with virologists to employ native metabolomics, a powerful technique integrating non-targeted liquid chromatography tandem mass spectrometry and native mass spectrometry, to discover inhibitors of viral proteins and other anti-infective targets. [4]

This interdisciplinary approach led us to isolate bioactive natural products from embalming residues of Egyptian mummies, confirmed through ^{14}C analysis to originate from the 28th Pharaonic dynasty (407-386 B.C.). Utilizing AI-assisted NMR and LC-MS/MS methods, we elucidated their structures and identified promising compounds with selective antiviral properties against pandemic Influenza virus Type A (H1N1pdm)[5–7].

Our findings represent a pioneering effort to bridge ancient wisdom with modern science in the quest for novel anti-infective treatments, offering hope in the face of emerging infectious threats.

Keywords: natural products discovery, Egyptian mummy, artificial intelligence, antiviral

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S1.P275 Identification of natural compounds targeting the *Plasmodium falciparum* pyruvate kinases

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Pyruvate kinases (PfPyrK1 and PfPyrK2) are essential for the survival of *Plasmodium falciparum* during its erythrocytic stage. These enzymes catalyze the phosphorylation of ADP to ATP, using phosphoenolpyruvate (PEP) that is converted to pyruvate. Therefore, they represent promising therapeutic targets (Chan, Tan, and Sim 2007; Swift et al. 2020). However, to date, no effective specific inhibitors for PfPyrK1 or PfPyrK2 have been identified.

In this context, 70 molecules were screened *in silico* by evaluating their binding energies to both the oxalate (a substrate analog inhibitor) and the ATP binding sites of PfPyrK1 and PfPyrK2. Among the compounds tested, including flavones, sesquiterpene lactones, and diterpenes obtained from plant extracts or hemisynthesis, a group of polymethoxyflavones (PMFs) showed the highest affinity for these sites. The binding energies ranged from -8.516 to -6.943 kcal/mol for the oxalate site and from -9.129 to -7.624 kcal/mol for the ATP site. Following the *in silico* screening, flavones available in sufficient quantities in our chemical library were tested *in vitro* on PfPyrK1 and PfPyrK2. Apigenin, with binding energies of -7.292 and -7.646 kcal/mol for the oxalate and ATP sites respectively, exhibited interesting inhibitory activity of 54% and 45% on PfPyrK1 and PfPyrK2, respectively, at 50 µM (The IC_{50} of suramin, a reference inhibitor, were 7 µM and 3 µM for PfPyrK1 and PfPyrK2, respectively).

Other flavones, such as viscosine (with binding energies of -7.403 and -8.339 kcal/mol for the oxalate and ATP sites respectively), are being isolated from *Gardenia oudiepe*, to evaluate their *in vitro* inhibition effects on PfPyrK.

Keywords: *Malaria, in silico* screening, pyruvate kinases, polymethoxyflavones

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S1.P276 The relationship between bioactivity and taxonomy of *Euphorbia* species in South Africa

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The phytochemistry of Euphorbiaceae and Thymelaceae has recently received substantial attention due to the anticancer and anti-HIV activities of phorbol diterpenes in these families. Some of these diterpenes, such as prostratin, not only kill HIV at nanomolar concentrations but can reactivate the latent virus and have the potential to cure patients with HIV (Beans et al., 2013). Medication containing Euphorbiaceae diterpenes as the active ingredient has also reached the market for treating topical cancers in animals (Cullen et al., 2021).

Euphorbiaceae are one of the largest flowering plant families (6300 species), and as a result the selection of suitable species for phytochemical investigation may be problematic. In this project, we have focused on the identification of novel bioactive diterpenes from the genus *Euphorbia*. Southern Africa is home to 172 *Euphorbia* species, of which 74% are endemic to this area (Bruyns, 2023). To assist with selecting *Euphorbia* species for investigation, we have correlated the diterpene contents with the taxonomy of the different species. The diterpenes were identified by isolation of the compounds and/or identification by LC-MS-MS. The structures and bioactivity of the diterpenes will be discussed. The terpenes identified were phorbol esters, abietane, kaurane, and atisane diterpenoids, and these compounds were correlated with the taxonomic classification of the plants.

Keywords: Euphorbiaceae, Thymelaceae, *Euphorbia*, diterpenoid, phorbol ester

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S1.P277 Structure activity studies of anti-parasitic norditerpenes from subterranean fungi

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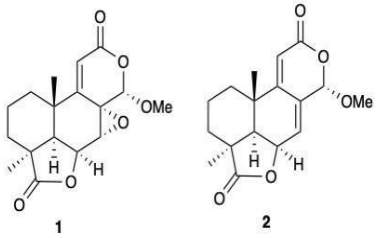
Cryptosporidiosis is a globally distributed water-borne gastrointestinal disease caused by protozoan parasites in the genus *Cryptosporidium*. *C. parvum* is one of the most important species that infects both humans and ruminant animals. The diarrheal disease is especially problematic for immunocompromised patients, and there are no vaccines or effective clinical drugs for these populations and new treatments are urgently needed.

We previously identified a suite of norditerpene lactone metabolites with anti-Cryptosporidial activity from the fungus *Oidiodendron truncatum*, isolated from the Soudan Iron Mine in northern Minnesota.[1] The two most active compounds with sub-micromolar EC50s were oidiolactones A (1) and B (**2**), which differ in both anti-parasitic potency and cytotoxicity towards host cells. In order to expand structure activity relationship studies to better understand the structural features that might lead to higher activity and minimal off target effects, we identified a *Mucor* sp. of fungus from the same subterranean environment that also produced oidiolactones A and B as well as four additional new congeners, including a norditerpene lactam derivative. We also transformed the natural oidiolactone A into eight semi-synthetic analogs and tested these together with the natural compounds against *C. parvum* infected HCT- 8 host cells. None of the new analogs or semi- synthetic derivatives were more potent than **1** or **2**, but the C-13 epimer of oidiolactone A displayed comparable activity and increased cytotoxicity. The structure activity relationships of the 21 norditerpenes will be discussed, as well as the implications for mechanism of action studies.



1 2

Fig. 1. Structures of compounds 1 and 2.

Keywords: *Cryptosporidium, Toxoplasma,* fungi, anti-parasitic, infectious disease

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S1.P278 Bioactive terpenoids from members of the Hericiaceae

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In our continued search for biologically active metabolites from cultures of rare Basidiomycota species, we identified twenty-two previously undescribed compounds from submerged and solid-state cultures of three European Basidiomycetes belonging to family *Hericiaceae* namely: *Dentipellis fragilis*, *Hericium flagellum* and *H. coralloides* (1–4). Their chemical structures were unambiguously determined based on extensive 1D and 2D NMR spectroscopy along with HR-ESI-MS analyses. The isolated compounds revealed different biological effects. Some exhibited significant activities in our standardized cell-based assays for the determination of cytotoxic and antimicrobial effects. In addition, most of the compounds demonstrated neurotrophic effects. These were determined using the rat pheochromocytoma cell (PC-12)-based assay for neurite outgrowth promotion and real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay, to assess the effects of the compounds on the expression of neurotrophins NGF and brain-derived neurotrophic factor (BDNF) on human astrocytoma cells.

Keywords: *Basidiomycetes*, NMR, HR-ESI-MS, cytotoxic, antimicrobial, neurotrophic

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S1.P279 Isolation, structural characterization and antiprotozoal activity of steroidal alkaloids from Pachysandra *terminalis*

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Trypanosoma brucei rhodesiense (*Tbr*) and *Plasmodium falciparum* (*Pf*) are protozoan parasites responsible for severe diseases, namely human African trypanosomiasis and malaria. With limited treatment options and emerging resistance, there is an urgent demand for new antiprotozoal compounds. Previous studies of our group have indicated that plants of the Buxaceae family contain aminosteroids with high activity against these parasites (Althaus et al., 2014), (Szabó et al., 2021). The alkaloid enriched fraction of *Pachysandra terminalis* Sieb. & Zucc. (Buxaceae) also showed prominent activity against *Tbr* (Flittner et al., 2021). Therefore, it is promising to further investigate the constituents of this plant and to systematically isolate and test individual compounds. An optimized extraction protocol was established to obtain an alkaloid-enriched fraction of *P. terminalis* leaves that was active against *Tbr* (IC_{50} 1.84 µg/mL) and highly active against *Pf* (IC_{50} 0.31 µg/mL). With centrifugal partition chromatography the alkaloid-enriched fraction was further divided into 18 subfractions with IC_{50} values against *Tbr* ranging from 0.50 - 7.92 µg/mL and against *Pf* from 0.17 - 1.27 µg/mL. By preparative HPLC, eight aminosteroids could be isolated so far: 5,6-Dehydro-desacyl-epipachysamine A (**1**), desacyl-epipachysamine A (**2**), epipachysamine B (**3**), pactermine A (**4**), 3*R*-pachysanaximine A (**5**), pachysamine A (**6**), *N*-methyl-desacyl-epipachysamine A (**7**) and sarcodinine (**8**). The structures were established by UHPLC/+ESI-QqTOF-MS/MS and NMR spectroscopy. To the best of our knowledge, compounds **1** and **5** are described as natural products for the first time. The activity against *Tbr* and *Pf* is shown along with the structures in **Fig. 1.**

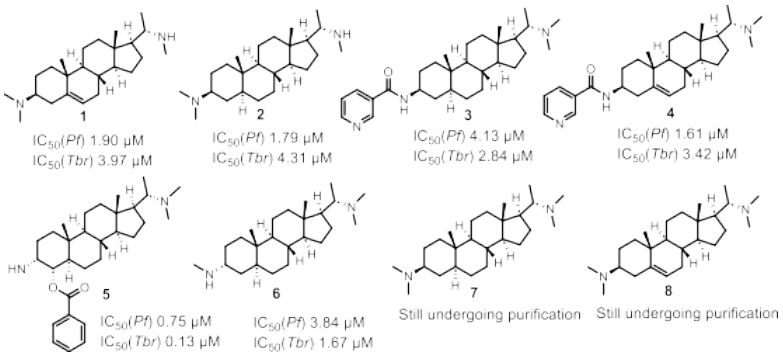

Fig. 1. Structures of the steroidal alkaloids (**1**-**8**) from the leaves of *Pachysandra terminalis*

Keywords: *Pachysandra terminalis,* Buxaceae, steroidal alkaloids, *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*

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S1.P280 Antibacterial Activity of different extracts of *Plicosepalus acacia*

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A considerable global health crisis that threatens communities has been attributed to the emergence of resistant bacterial infections. As a result, the search for novel antibiotics from different natural sources has increased. *Plicosepalus acaciae (Zuccarini) Wiens & Polhill)* is used in Saudi Arabia for treating some infectious disease such as bronchitis. The aim of this study is to evaluate the antibacterial activity of different extracts of *Plicosepalus acacia*. MIC assay was used to assess the antibacterial activity for hexane, chloroform and methanol extracts of leaves. The findings of MIC assay illustrated that all extracts showed weak or no significant activities except for the methanol extract that possessed mild to moderate activities at 256 µg/mL in compared to a positive control: In conclusion, the methanol extract of *Plicosepalus acacia* demonstrated moderate activity against *Staphylococcus aureus* and *Bacillus subtilis*, weak activity against *Escherichia coli*, and no activity against *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

Keywords: *Plicosepalus acacia*., antibacterial agents, bacterial resistance

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S1.P281 *Epimedium koreanum* attenuates Influenza A viral infection via modulation of hemagglutinin and neuraminidase

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Epimedium koreanum also known as Nakai is a popular plant in Korean and Chinese medicine for treating various ailments. In this study, the water extract of *E. Koreanum* has a significant inhibitory impact on influenza A virus (IAV) infection by directly blocking viral attachment and having a virucidal effect. We examined the effect of the *E. koreanum* water extract on viral infection using fluorescent microscopy and fluorescence-activated cell sorting analysis with a green fluorescent protein-tagged Influenza A/PR/8/34 virus. At a concentration of 100 μg/mL, the Nakai water extract decreased GFP expression by up to 90% compared to the virus-infected control. Immunofluorescence and Western blot analyses against influenza viral proteins showed that it dose-dependently decreased the viral protein expression. The Nakai extract at 100 μg/mL inhibited the H1N1 influenza virus's hemagglutinin and neuraminidase, preventing viral binding to cells. Furthermore, it exhibited a virucidal impact and inhibited the cytopathic effects of H1N1, H3N2, and influenza B virus infection. Finally, our results showed that *E. koreanum* water extract could be developed as a natural viral inhibitor against influenza virus infection.

Keywords: *Epimedium koreanum*, antiviral effect, influenza virus, virucidal effect

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S1.P282 Anti-biofilm and anti-quorum sensing activity of galloylquinic acids from *Copaifera lucens* leaves against clinical isolates of multidrug-resistant *Pseudomonas aeruginosa* in open wound infection: *in vitro* and *in vivo* study

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This presentation investigates the antibacterial, anti-biofilm, and anti-quorum sensing actions of galloylquinic acid compounds (GQAs) derived from the brazillian *Copaifera lucens* leaves against clinical isolates of multidrug-resistant (MDR) *Pseudomonas aeruginosa*. Our results showed significant inhibition zone diameters (25-40 mm) in the antibacterial activity assay, with minimal inhibitory concentrations (MIC) ranging from 1-4 µg/mL and minimal bactericidal concentrations (MBC) between 2-16 µg/mL. GQAs effectively interfered with planktonic *P. aeruginosa* isolates and inhibited their growth within pre-formed biofilms, with MBIC80 and MBEC80 values of 64 µg/mL and 128 µg/mL, respectively. Fluorescence staining and confocal microscopy revealed a substantial reduction in cell viability and biofilm thickness (62.5%) following exposure to 128 µg/mL of GQAs. Scanning electron micrographs further confirmed GQAs ability to disrupt biofilm and bacterial structures by interfering with biomass and exopolysaccharides (Fig. 1). Moreover, GQAs significantly ($p<0.05$) reduced the production of virulence factors and bacterial motility, including rhamnolipid, pyocyanin, as well as swarming and swimming motility. This reduction is attributed to GQAs downregulation (up to 89%) of quorum-sensing genes (lasI, lasR, pqsA, and pqsR) involved in biofilm formation. In conclusion, GQAs derived from *C. lucens* represent promising antibiofilm agents with the potential to enhance wound healing in biofilm-associated infections caused by MDR *P. aeruginosa* clinical isolates.

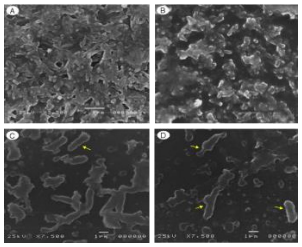

Fig. 1. Scanning electron micrographs showing biofilms of *P. aruginosa* after exposure to different concentrations of GQAs. (A) untreated preformed biofilm showing large number of the bacterial cells with intact cell walls, arranged in close contact with each other, covered with EPS matrix glycocalyx, (B) spaces were detect in the biofilm and afew distortions in the cell morphology was observed after treatment with 16 µg/mL of GQAs, (C) irregularities in cell morphology (arrow) are markedly noticed following treatment with 64 µg/mL of GQAs in addition a significant reduction in the biomass and EPS matrix, (D) severe damage was detected in the biofilm in case of the treatment with 128 µg/mL of GQAs, with leakage of cellular content and death (arrows).

Keywords: Antibacterial, *Pseudomonas aeruginosa* clinical isolates, anti-quorum sensing, anti-biofilm, antivirulence, galloylquinic acid compounds

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S1.P283 Effects of raw and boiled ginger (Zingiber officinale Roscoe) on hyperlipidemia in high cholesterol diet-induced obese rats – A comparative therapeutic study

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This presentation will cover the comparative therapeutic effects of raw and boiled ginger (Zingiber officinale Roscoe) on hyperlipidemia in high cholesterol diet-induced obese rats. Traditional herbal medicine has claimed to have successfully employed ginger rhizome to treat obesity in people all over the world (Pan et al., 2016; Gupta et al., 2015; Abdulrazaq et al., 2011).

As part of continuing search for new natural compounds and their bioactive components as potential novel drug development and illness treatment alternatives, we compared the therapeutic benefits of raw as well as boiled ginger at different percentages (2% and 4%) on hyperlipidemia in high cholesterol diet-induced obese rats. Feeding of high cholesterol diet (HCD)-induced obese rats with raw and boiled ginger for twenty-four weeks resulted in the levels of antioxidant status, lipid profile as well as 3-hydroxy-3-methylglutary-coenzymeA reductase (HMG Co-A reductase) activity to be significantly elevated ($p < 0.05$), with a concomitants reduction in activities of liver marker enzymes in the serum and liver of HCD-induced obese rats fed raw and boiled ginger in comparison with untreated obese rats. Levels Histology reports also depict the potential of the ginger to reduce abdominal fat deposits in HCD-induced obese rats fed raw and boiled ginger (Fig. 1).

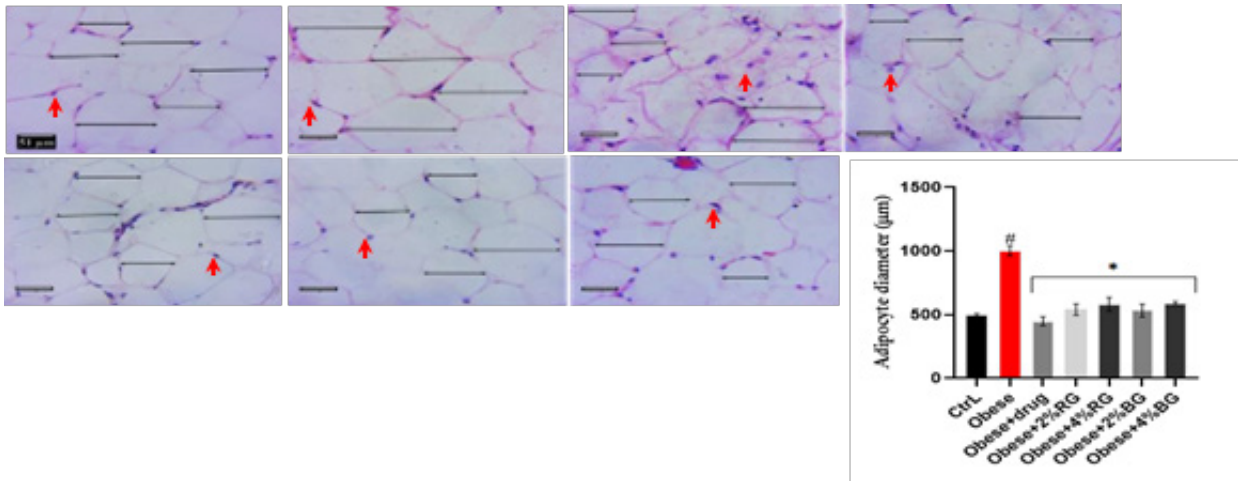

Fig. 1. Histological changes of the adipose tissue (abdominal fat)

Both raw and boiled ginger had positive effects on the parameters studied, but boiled ginger at a higher percentage (4%) had the greatest significant result in comparison with raw ginger. Therefore, consuming boiled ginger will be more effective than eating raw ginger in preventing hyperlipidemia brought on by a high-cholesterol diet.

Keywords: obesity, ginger rhizome, thermal processing, lipid profile, lipogenesis, HMG-CoA reductase

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S1.P284 Triterpenoids from the roots of *Anthocleista djalonensis* and their antiplasmodial, antimicrobial, and antitrypanosomal properties

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This study explores the phytochemical composition and antibacterial, antiplasmodial, and antitrypanosomal properties of *Anthocleista djalonensis* (A. Chev.) roots used in traditional medicine for treating malaria, and leprosy, among others. Chromatographic procedures were used to isolate the compounds from the species, and their structures were determined by 1D and 2D NMR data and compared with the literature. *In vitro*, antimicrobial properties, cell growth-inhibiting characteristics, and cytotoxicity were assessed. Bauerenone (**1**), bauerenol (**2**), and a mixture of stigmasterol and β- sitosterol (**3** & **4**) were isolated from this species. Antimicrobial effectiveness was demonstrated by the ethanol, acetone, and ethyl acetate extracts and all compounds against all tested microbes. MICs ranged from 16 to 100 µg/mL in the crude extracts and 4 to 31 µM for the isolated compounds. Significant anti-malarial activity was recorded for hexane (IC_{50}: 0.0191 µg/mL), ethyl acetate (IC_{50}: 0.0139 µg/mL), acetone (IC_{50}: 0.0168 µg/mL), ethanol (IC_{50}: 0.0159 µg/mL), and the isolated compounds (bauerenone, IC_{50}: 0.0150 µM; bauerenol, IC_{50}: 0.0138 µg/mL; a combination of β- sitosterol and stigmasterol, IC_{50}: 0.0145 µg/mL). The crude extracts (hex, IC_{50}: 0.0576; ethyl acetate, IC_{50}: 0.0318; acetone, IC_{50}: 0.0452; and ethanol, IC_{50}: 0.0313 µg/mL) with bauerenol (IC_{50}: 0.0554 µM) demonstrated substantial antitrypanosomal effects comparable to pentamidine (IC_{50}: 0.01252 µM). No toxicity was observed toward human cervical carcinoma (HeLa) cells. The study reveals that bauerenone, stigmasterol, and β-sitosterol, along with the root extracts of *Anthocleista djalonensis,* are potential candidates for integration into the natural products drug discovery programs, highlighting the need for evidence-based investigation of the species.

Keywords: *Anthocleista djalonensis,* cytotoxicity, HeLa cells, antimicrobial activity, antiparasitic activity

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S1.P285 Enzymatic Synthesis of Tryptophan-Linked DKP Dimers: Expanding Biosynthetic Routes for Antifungal Drug Discovery

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Dimeric diketopiperazine (DKP) alkaloids belong to a diverse class of natural products with an array of pharmacological applications such as anti-cancer and anti-microbial therapies. Synthesis of dimeric DKPs are of interest due to their varied biological activities and intricate structural features. Total chemical synthesis of tryptophan linked DKP dimers remains a challenging task which has motivated us to turn to a biocatalytic route. Currently, there are no catalyst-controlled approaches for accessing a variety of DKP dimerization modes from a single starting material. A previous in silico analysis of *Streptomyces* genomes revealed several bacterial DKP dimerases, which have been identified as cytochrome P450 (P450). Biochemical analysis revealed the diverse dimerization activities of the four cytochrome P450 DKP dimerases using Trp-Pro (brevianamide F) as the monomeric DKP substrate.

In this study, we explore the substrate scope of pivotal P450s, NzeB and AspB, which catalyze C–C and C–N bond formations in DKP dimer biosynthesis. Using 24 unnatural DKP substrates, which are analogs of the native substrate brevianamide F, we have expanded enzymatic control over C-H functionalization sites. This investigation not only enhances our understanding of selective dimer formation but also lead to the biosynthesis of novel small molecules with antifungal properties, crucial in combating rising fungal infections. Conducting this substrate scope study has led to the discovery of compounds effective against drug-resistant pathogen *Candida auris*.

Keywords: *Biocatalysis,* cytochrome P450, diketopiperazine, antifungals

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S1.P286 An investigation of *Origanum onites* and *Thymus fallax* in terms of the European Pharmacopoeia criteria

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The Lamiaceae family includes approximately 236 genera and 6900 to 7200 species (Tzima et al., 2018). In Turkey the Lamiaceae family is represented by 46 genera and 586 species, 260 of which are endemic, and is the third richest family in Turkey in terms of plant species and diversity, with an endemism of 44.2% (Davis, 1982). Medicinal plants belonging to this family have been used for many years in the treatment of various animal and human diseases based on their antibacterial, antioxidant, antitumoral, antifungal, analgesic and insecticidal effects (Hossain et al., 2010).

This presentation covers the determination of quality control methods and analytical studies for *Thymus fallax* (catri) and *Origanum onites* (kekik) which were collected from Şırnak and Izmir, respectively. Heavy metal analyses using the WDXRF method and the determination of ash content and loss on drying according to the European Pharmacopoeia 8.0 standards were carried out (EDQM). Furthermore, the total phenolic contents of the aqueous extracts of both plants were tested with the Folin-Ciocalteu colorimetric method to getting first step for determining their antioxidant activities (Kulisic et al., 2005).

The amounts of heavy metals determined for both species were within the limits of pharmacopoeia standards, according to WDXRF results. In addition, total ash and water amounts were determined to be 7%, 14% and 14%, 16% for *Origanum onites* and *Thymus fallax* respectively, and were determined to be in compliance with pharmacopoeia standards. Total phenolic contents (GAE µg/mL) were found to be 28.152 ± 0.389 and 41.009 ± 2.059 for *Origanum onites* and *Thymus fallax,* respectively. This is evidence that both species contain phenolic compounds that will cause them to display antioxidant activity. The data obtained within the scope of the study showed that if the plants are procured correctly and the water extracts are prepared with appropriate methods, the quality and effectiveness will increase.

Keywords: *Origanum onites, Thymus fallax,* Lamiaceae, phenolic content, WDXRF

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S1.P287 Transformation of food by-products into high value added extracts with antimicrobial properties

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The transformation of food by-products into high-value added extracts with antimicrobial properties involves several steps and processes aimed at extracting bioactive compounds from these by-products and enhancing their antimicrobial activity. The first step involves selecting appropriate food by- products that are rich in bioactive compounds and have the potential for antimicrobial properties. Examples of such by-products include fruit peels, vegetable scraps, seed husks, and spent grains from brewing. Food by-products often require pre-treatment to facilitate the extraction process. This can involve washing, drying, grinding, or any other method to prepare the by-products for extraction.

Various extraction techniques can be employed to isolate bioactive compounds from the food by- products, including supercritical fluid extraction (SFE) as a green technique. After extraction, the crude extract may undergo fractionation and purification to isolate specific bioactive compounds responsible for antimicrobial activity. Techniques such as column chromatography, thin-layer chromatography, or high-performance liquid chromatography (HPLC) can be used for this purpose. Then the obtained are evaluated for their antimicrobial activity against a range of microorganisms such as bacteria, fungi, and viruses. This can be done using standard microbiological methods, including agar diffusion assays, broth microdilution assays, or other relevant techniques.

By transforming food by-products into high-value added extracts with antimicrobial properties, not only are valuable compounds recovered and utilized, but also environmentally sustainable practices are promoted by reducing waste and creating value from otherwise discarded materials.

Keywords: bioactive compounds, by-products, food, extracts, valorization

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S1.P288 Secondary metabolites as chemotaxonomic markers in *Salix myrsinifolia* and *S. starkeana* (Salicaceae) twigs extracts

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Chemotaxonomy is based on the special metabolite profiles in plants and is, together with the morphological characters, used as a tool in plant taxonomy and species identification (Julkunen- Tiitto, 1986). *Salix starkeana* and *S. myrsinifolia* (Salicaceae) are dioecious shrubs native to the temperate biome. Species identification within the genus *Salix*, and especially when it comes to the dormant winter twigs, can be difficult when based solely on morphological characters. Thus, we subjected twig extracts of *S. starkeana* and *S. myrsinifolia* to UPLC/QTOF-MS analysis, to elucidate the relations between these *Salix* spp. and to provide an additional guide to *Salix* taxonomy and identification.

We found that the *S. myrsinifolia* twig methanol extract exhibited various resemblances with the *S. starkeana* twigs methanol extract. For example, the condensed tannin, procyanidin B1 $[M\text{-}H]^-$ 577,1348 and its isomers and the procyanidin monomers, epigallocatechin and catechin, were the main compounds in both species, although these compounds varied in quantity between the two species. Many compounds, and among them benzyl-β-primeveroside, caffeoylhexose, an isolariciresinol-pentoside isomer, salicortin, salicin-7-sulfate, naringenin and acetyl-*O*-salicortin were present in both species. Previously, salicin-7-sulfate, which is a novel salicylate in the genus *Salix*, was found only in *Salix caprea* (Lackus et al., 2020) and *Salix koryanagi* (Noleto-Dias et al., 2018).

However, some metabolites in *S. starkeana*, such as the unknown compounds at $[M\text{-}H]^-$ 495,1511, (tR 2,97 min) and at $[M\text{-}H]^-$ 453,1401 (tR 2,39 min) were found to be specific for *S. starkeana* but absent from *S. myrsinifolia*. Thus, these compounds may be used as chemotaxonomic markers to distinguish *S. starkeana* winter twigs.

It is worth to conclude that for pharmaceutical purposes, it is important to distinguish chemotypes with the most beneficial composition of therapeutic phytochemicals.

Keywords: *Salix myrsinifolia, S. starkeana,* Chemotaxonomy, UPLC-QTOF/MS, phenolic

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S1.P289 Carvacrol solution and copper nanoparticles as an effective and non-phytotoxic treatments of carrot seeds against the pathogenic bacterium *Xanthomonas hortorum* pv. *carotae*

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The effectiveness of three phenolic compounds: carvacrol, eugenol and thymol, as well as three nanomaterials based on copper, silver and silver–selenium for elimination of bacterial pathogen from carrot seeds was evaluated in two presented studies. *Xanthomonas hortorum* pv. *carotae*, causing bacterial blight, leads to serious loss in carrot production worldwide, especially in seed production because of its seed-borne character. The treatment of bacterial diseases in plant production is challenging due to the restrictions in antibiotic use. Currently available methods for suppression of *Xanthomonas hortorum* pv. *carotae* in carrot seeds do not always provide a satisfactory effect, can reduce a germination and burden an environment. Therefore, the new alternative treatments have been tested *in vitro* and *in vivo*. The lowest minimum bactericidal concentration was observed for carvacrol and thymol (0.0098–0.0196%) and silver nanoparticles (10 mg/L^{-1}). The application of tested chemicals *in vivo* showed that the biological compound carvacrol at concentration 0.0196% completely eliminated pathogenic bacterium quantified in plants germinated from infected seeds. From three nanomaterials, only use of copper nanoparticles at concentration 993 mg/L^{-1} resulted in a significant reduction of the bacterium quantity in carrot plants. The quantification was performed using real-time PCR assay targeting the hypersensitive response and pathogenicity-associated phosphatase (*hpaP*) gene. Both treatments did not reduce the seed germination percentage in comparison to non-treated controls and did not cause any visible morphological changes of plants, therefore can be considered as non-phytotoxic.

Keywords: bacterial blight, seed treatment, biological bactericide, phenolic compounds, nanomaterials

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S1.P290 Characterization of polyphenolic compounds from an antibacterial twig methanol extract of Salix *aurita*

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Polyphenols are natural and biologically active compounds found in plants, fruits and vegetables. They have numerous health benefits and serve as dietary antioxidants in promoting human health (Abdulkhaleq et al., 2017). Polyphenolic compounds that are common in *Salix* spp., such as the proanthocyanidin monomers; catechin and epicatechin, the procyanidins, and cinnamic acid and its derivatives, have been reported to be effective in the management and treatment of infectious diseases, diabetes, and cancer (Bouarab-Chibane et al., 2019; Adisakwattana, 2017). *Salix* species such as *S. aurita* L., are used traditionally in the treatment of inflammation and fever, and their efficacy has been attributed to the presence of its pharmacologically active compounds such as salicylates and polyphenols (Noleto-Dias et al., 2018).

The aim of this study was to conduct a phytochemical investigation and chemical profiling to identify the polyphenolic compounds present in a *Salix aurita* methanol twig extract using UPLC-PDA- QTOF/MS (ESI- mode). In our previous studies this extract showed good antibacterial activity. From the results, condensed tannins, flavonoids and salicylates were determined from a methanol twig extract of *S. aurita*. The chemical identification and molecular mass determination revealed procyanidin B1 (m/z 577.1346), naringenin (m/z 271.0606), salicin-7-sulfate (m/z 365.0542), salicin (m/z 285.0977), salicortin (m/z 423.1291), catechin (m/z 289.0712), luteolin (m/z 285.0399), and taxifolin (m/z 303.0505) as the major polyphenolic compounds present in the twigs of *S. aurita.* This study will pave way for our further research on the mechanism of actions of these identified polyphenols as antibacterial agents.

Keywords: *Salix aurita*, polyphenols, UPLC-PDA-QTOF/MS

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S1.P291 Antimicrobial and anti-biofilm properties of geranylated flavonoids from *Paulownia tomentosa* Steud. fruit

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Paulownia tomentosa (Thunb.) Steud. (Paulowniaceae), a traditional Chinese medicinal plant, had been used for centuries as a component of remedies for various illnesses. It is a rich source of phenolic compounds, mainly geranylated flavonoids, which are currently studied for their promising biological activities.

An increasing resistance to conventional antibiotics is a threat to the global healthcare system, and therefore it is necessary to search for new alternatives in the treatment of bacterial infections. Also, production of biofilm by certain bacteria is connected to persistence of their infections and it is difficult to eradicate them. Thus, new treatments against such long lasting infections that inhibit biofilms are needed.

This experiment was carried out on a biofilm-producing gram-positive bacteria *Staphylococcus epidermidis*. Ten *C*-geranylated flavanones and dihydroflavonols (**1** – **10**) isolated from the fruit of *P. tomentosa* were tested for their antimicrobial and anti-biofilm properties. The evaluation of antimicrobial activity was performed using the microdilution method and the anti-biofilm activity was evaluated by crystal violet staining.

As a result of our study, seven of the tested compounds showed antimicrobial activity and six of them revealed anti-biofilm activity. Diplacone (**1**), diplacol (**4**), and 3′-*O*-methyl-5′-hydroxydiplacone (**6**) showed the most potent antimicrobial activity. Diplacone (**1**), mimulone (**2**), and mimulol (**10**) were the most effective in suppressing bacterial biofilm formation. These compounds need to be further investigated for their antimicrobial or anti-biofilm activity and safety, but there is a potential for their future use in the suppression of antibiotic resistance.

Keywords: anti-biofilm activity, antimicrobial activity, geranylated flavonoids, *Paulownia tomentosa*, *Staphylococcus epidermidis*

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S1.P292 Navigating Experimental Metabolomics Knowledge Graphs for Targeted Isolation of Anti-Infective Natural Products

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The escalating threat posed by Multidrug-Resistant Tuberculosis (MDR-TB) caused by Mycobacterium tuberculosis (Mtb) has spurred the necessity to find new antibiotics to treat tuberculosis. In our study, we employed an innovative 3R infection model using Mycobacterium marinum alongside the amoeba and professional phagocyte Dictyostelium discoideum as stand-ins for Mtb and macrophages, respectively. We used this model in a high-throughput phenotypic assay which enables simultaneous assessment of a compound's impact on both the host and the pathogen (Hanna et al., 2020).

To find novel anti-infective compounds, a registered diverse collection of 1600 plant extract samples (Pierre Fabre Library, PFL) was screened using this assay. In parallel, this collection was analyzed by untargeted UHPLC-high-resolution tandem mass spectrometry (UHPLC-HRMS/MS) (Allard et al., 2023).

In a careful two-step validation process, we eventually reduced the collection to a list of 13 validated hit extracts. We then applied High-resolution HPLC microfractionation bioactivity profiling to swiftly pinpoint bioactive compounds within these selected hit extracts (Hell et al., 2022). Furthermore, by leveraging experimental metabolomic data organized within a knowledge graph (KG) (Gaudry et al., 2023), we could systematically explore the virtual chemical space generated. This approach facilitated the targeted isolation of new/bioactive structural analogues based on annotations, also in extracts not necessarily targeted in the initial bioactivity screening (Kirchhoffer et al, 2023). These were then fully characterized by NMR and electronic circular dichroism (ECD) to establish their absolute stereochemistry. The example chosen serves as a proof of concept for the use of a KG (integrating bioactivity and metabolomics) to efficiently identify bioactive NPs in collections of plant extracts.

Keywords: knowledge graph (KG), 3R infection model, novel anti-infective compounds, high-resolution tandem mass spectrometry (HRMS/MS), plant extracts library

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S1.P293 Discovery of novel terpene hydrocarbon scaffolds and their associated tailoring enzymes from *Streptomyces* bacteria

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Bacterial natural products (NPs) from the genus *Streptomyces* provide approximately 70% of antibiotics used to date (Kieser et al., 2000). Given the growing antimicrobial resistance crisis, it is critical to discover novel compounds with antibiotic activity. In this work, we look towards terpenoids, the largest class of NPs with over 100,000 characterized compounds (Rudolf et al., 2021). Terpenoids have broad bioactivities including antibacterial, cytotoxic, and anti-inflammatory properties. Historically, plants were considered the most prolific producers of terpenoids. However, genome mining reveals that bacteria, especially *Streptomyces*, have great terpenoid biosynthetic potential. We genome mined for uncharacterized bacterial terpene synthases (TSs) encoded in NP biosynthetic gene clusters (BGCs) also containing cytochrome P450 enzymes that may generate novel terpene scaffolds. These bacterial TSs of unknown function were heterologously expressed in *E. coli* containing upregulated precursor genes and a kinase system, allowing conversion of exogenously added isoprenol into terpene precursors *in vivo*. This system facilitates detection of terpenes at small scale to validate TS activity and supports reliable terpene production in large scale fermentations. *Streptomyces* normally produce complex NP mixtures; furthermore, BGCs with TSs of interest often are not expressed under laboratory conditions. Thus, expressing TSs in *E. coli* decreases complexity of fermentation extracts and increases the titer of terpenes produced. Once TS products are characterized, we can express tailoring enzymes including P450s clustered with TSs producing novel terpene scaffolds to discover highly functionalized terpenoid NPs for bioactivity testing. This poster will showcase novel terpenes isolated thus far from previously uncharacterized bacterial TSs, our heterologous expression workflow, and BGCs under study.

Keywords: biosynthesis, *Streptomyces*, heterologous expression, terpene synthases, cytochrome P450s

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S1.P294 Effects of difference LED light colors on isoflavonoid production in callus cultures of *Pueraria candollei* Wall. ex Benth. (Fabaceae)

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The tuberous root of *Pueraria candollei* Wall. ex Benth. (Fabaceae) is in high demand for dietary supplements and cosmetics, making it scarce. Callus culture is a source of secondary metabolites production (Udomsin et al. 2020). The primary factor for *in vitro* plant tissue culture is the light source. Light-emitting diode (LED) lights were introduced to plant tissue culture in recent years (Bantis et al., 2018). The current work used fluorescent, red, blue, white LED, and dark conditions to investigate the phytoestrogen compounds production in *P. candollei* callus for 14 and 28 days. Chalcone synthase (*CHS*) , isoflavone synthase (*IFS*) , isoflavone reductase (*IFR*) , hydroxyisoflavanone dehydratase (*HID*), cytochrome P81E (*CYP81E*) and prenyltransferase-1 (*PT- 1*) related biosynthesis pathway genes were determined expression level using quantitative reverse transcription-polymerase chain reaction (Suntichaikamolkul et al., 2019). The white LED condition showed the highest growth rate at day 28 increase of 1.89-fold compared to the initial culture day. White LED light stimulated a significant upregulation of *HID*, *CYP81E*, and *PT-1* genes by 1.6, 4.8, and 56-fold, respectively. Treated red LED callus extract showed daidzin, genistin, and deoxymiroestrol levels of 612.83 ± 42.80, 549.39 ± 32.72, and 1.46 ± 0.24 μg/g dry weight, respectively. The total isoflavonoids concentration of callus extract increased 3 -fold and 2-fold under red and white LED, respectively compared to fluorescent light. The presence of red and white LED lights can stimulate the formation of phytoestrogens in *P. candollei* callus, particularly in the isoflavonoids and their derivatives. However, further studies should be performed to confirm the pharmacological activity of plant extracts.

Keywords: LED light, *Pueraria candollei*, Fabaceae, gene expression, isoflavonoids

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S1.P295 Elicitation of licorice callus for improving flavonoids production

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Licorice is an herbal plant that has been used as a medicinal source since the ancient era. The current issue with this plant is the depletion of raw materials in natural cultivation (Kwon et al., 2020). The callus culture is conducting research to resolve this problem. Ononin and its aglycone, formononetin, are bioactive licorice flavonoids with well-established anti-inflammatory activity (Krittanai et al., 2021). In this study, a combination of sample pre-treatment and elicitation using methyl jasmonate (MJ) or cellulase enzyme (CL) was implemented to increase the yield of these substances in *Glycyrrhiza inflata* callus culture. The largest ononin induction was seen on day 3, with a 3- and 5.93- fold induction in MJ and CL, respectively, compared to the control group. In the case of formononetin, the highest production was observed on day 7, with a 3.22- and 5.46-fold induction over the control. The mechanism of elicitation was revealed as stimulating the antioxidant defense mechanism and stimulating the flavonoid biosynthesis genes (*CHI*, *CHS,* and *IFS*). Therefore, the combination strategies of sample pre-treatment and elicitation present in the current study can be applied for enhancing the medicinal substance in licorice, especially in the form of callus culture, which has high potential to be the future source for the production of medicinal substances.

Keywords: elicitation, methyl jasmonate, cellulase enzyme, *Glycyrrhiza inflata*, callus

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S1.P296 Chemopreventive properties of *Alchemilla vulgaris* L. extract biotransformed by the human gut microflora – preliminary study of a human cellular model of colon cancer

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Numerous scientific reports have demonstrated that substances introduced into the body are subject to metabolic changes caused by bacteria in the digestive tract (Kamada et al., 2013). Thus, we evaluated the chemical composition of lady's mantle herb extract subjected to gut biotransformation and assessed the possibility of using the obtained products for colon cancer chemoprevention. First, extraction was carried out using a eutectic mixture (urea/choline chloride). The obtained extract was exposed to the gut microflora from 3 donor faecal samples (D1-D3) in an anaerobic chamber. After incubation (24 h), the changes in the phytochemical composition of the biotransformed extract were assessed, followed by LC-MS characterization. In the next step, the impact of the extracts on human colon CCD841 CoN and HT-29 cells was examined. Raw-extract (non-biotransformed) did not affect either cancer or normal colon cells. Biotransformed extracts at concentrations of 25-250 µg/mL did not cause any changes in the viability or proliferation of colon epithelial cells, but did have a dose- dependent cytotoxic effect on HT-29 cells. LDH release from damaged HT-29 cells in response to D1, D2 and D3 at a concentration of 250 µg/mL increased by 12.6%, 25.3% and 30.0%, respectively. Moreover, biotransformed extracts at concentrations ranging from 50-250 µg/ml significantly decreased the proliferation of HT-29 cells. The strongest effect was caused by extract D3 (IC_{50} = 471 µg/mL), while the weakest change was induced by extract D1 (IC_{50} = 1440 µg/mL). The performed studies revealed the chemopreventive properties of the biotransformed extracts of *Alchemilla vulgaris* L. (Rosaceae).

Keywords: *Alchemilla vulgaris,* microbiota, colorectal cancer

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S1.P297 Chemical characterization, prebiotic and anti-diabetic effects of galactomannans from two traditional Chinese medicines

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The galactomannans of *Trigonella foenum-graecum* L. (fenugreek) and the fungus *Cordyceps militaris* L. are two TCMs that demonstrate anti-diabetic activities. Such activities might be caused by modulation of the homeostasis of gut microbiota. Among the gut microbes, *Bacteroides ovatus* is more abundant (about 1.2-fold) in healthy individuals than in diabetes patients. Another microbe, *Akkermansia muciniphila*, is known to degrade mucin and produce short-chain fatty acids (SCFAs), which aid in the growth of other bacteria and maintain healthy mucus. To chemically characterize and evaluate the prebiotic effects of the galactomannans from the two TCMs, they were column chromatographed to give 19 fractions. The major monosaccharide components of these were Man, Gal and Glc (in the ratio about 5:3:1 to 4:3:1). Among these, TFPS-1 (carbohydrates obtained from fenugreek), CMMPS-1a02 and CMFPS-1a03 (carbohydrates obtained from *C. militaris* L.), improved the growth of *B. ovatus* and *A. muciniphila* (1.3~1.5-fold) with the production of SCFAs (i.e., acetic acid, propionic acid and butyric acid, about 2~4-fold increase, respectively). To evaluate the anti- diabetic effects, these polysaccharides were evaluated by three *in vitro* assays. Among these, CMMPSs triggered the secretion of GLP-1 (STC-1 cell, 1.8-fold) and insulin (BRIN-BD11 cell, 1.5-fold); TFPS-5, CMMPS-1a02, CMFPS-1a03 showed significant inhibitory effect of DPP-4 enzyme; the combination of carbohydrates derived from *C. militaris* and cordycepin could enhance the DPP- 4 inhibitory effect.

Keywords: fenugreek, *Cordyceps militaris*, galactomannans, gut microbiota, anti-diabetic effects

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S1.P298 Phytochemical profiling of phenolic metabolites in flower buds of *Magnolia kobus* DC. and their antioxidant activity

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Some representatives of the genus *Magnolia* L. (Magnoliaceae) are popular in traditional Asian medicine systems due to their broad spectrum of bioactive specialised metabolites (Poivre et al., 2017). Following our previous research on *Magnolia* × *soulangeana* Soul.-Bod. var. 'Lennei' (Zgórka et al., 2023), dried flower buds of *M. kobus* DC. collected from two different locations in Poland, namely the Warsaw University Botanical Garden (MKW) and the Rogów Arboretum (MKR), were subjected to ultrasound-assisted extraction using 30, 50 and 70% (*V/V*) EtOH. Herbal preparations were analysed using coupled chromatographic (RP-LC), spectroscopic (PDA) and mass spectrometric (QTOF/ESI-MS/MS) techniques. The results showed similar quantitative profiles of the main phenolic compounds, including phenolic acids, phenylethanoids, flavonols and lignans. However, their total content, calculated as gallic acid equivalent (GAE), increased significantly with the elution strength of the extraction solvent and was highest in extracts obtained with 70% EtOH (60.59 and 56,76 mg GAE/g dry weight, for MKW and MKR, respectively). Interestingly, the antiradical capacity of the above-mentioned extracts, as assessed by the DPPH• assay, was strongly correlated with the total phenylethanoid content, which was about three times higher in MKR than MKW, so the former extract exhibited higher antiradical activity (IC_{50} ~25.90 µg/mL) than the latter (IC_{50} ~33.76 µg/mL). Our study showed a relationship between environmental factors (UV exposure) in the plant habitat and the content of phenolic products of biotransformation, especially phenylethanoids, which are synthesised in the flower buds of the same magnolia taxon.

Keywords: *Magnolia kobus* DC., phenolics, antioxidant properties

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S1.P299 A research of the optimization of recombinant protein expression based on plant system

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Plants are generally suitable for recombinant protein production. Recombinant proteins can be rapidly produced in 4-6 weeks using transient expression (Kapila et al., 1997). In addition, plant production systems cannot be infected by mammalian pathogens during protein production and are easy to scale up (Fischer et al., 1999). To effectively express recombinant proteins in plants, we introduce stable and transient expression systems in this study. In order to find the optimal stable expression combination for each promoter and terminator, plastid transformation was performed to confirm the level of GFP expression (Fig. 1). As a result, it was found that when the rbcL promoter-psbA terminator was used, the GFP expression level was 2 folds higher than when original combination (the rrn promoter-psbA terminator) was used.

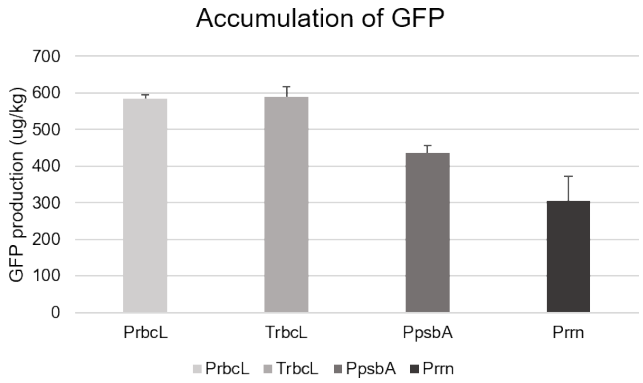

Fig. 1. Accumulation of GFP in promoter and terminator combination.

In addition, several types of vectors were designed to increase the expression level of the antigen protein of foot-and-mouth disease virus (FMDV) using transient expression. Among various combinations, the expression level of the antigen protein was high when the csVMV promoter and pinII-extensin double terminator were combined. In the future, we will compare the expression level of the antigen protein through stable expression using the rbcL promoter and transient expression using a double terminator to identify a better method of producing the antigen protein and evaluate immunogenicity.

Keywords: recombinant protein, transient expression, transgenic plant

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S1.P300 Cyanobacterial natural products: bioactive cyclic and linear peptides

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Chemical diversity and biological activity of natural products from cyanobacteria is continuing to be a subject of interest to researchers. This presentation focus on the chemistry and biology of cyanobacterial cyclic and linear peptides. Research on cyanobacterial natural products has allowed us to isolate 1) argicyclamides, a new class of cyanobactin with unique mono- and bis-prenylation on guanidine moieties from *Microcystis aeruginosa* NIES-88. We performed biochemical characterization on the new prenyltransferase, AgcF a key enzyme involved in enhancing antibiotic activity on the cyclic peptide, argicyclamide A (Phan et al., 2021); 2) nostosin G and spiroidesin B, new analogue of three to four residues containing linear peptides from *Dolichospermum* sp. NIES- 1697. We showed that nostosin G exhibited strong activity in trypsin inhibition, and the biosynthetic gene clusters shared similarities to spumigin except for their genetic organization, where an unrelated NRPS module was positioned in between two NRPS modules involved in nostosin G biosynthesis (Phan et al., 2022); 3) five new microginins and two new anabaenopeptins from *M. aeruginosa*. We also discovered a unique residue of 2-amino-5-(4'-hydroxylphenyl) pentanoic acid (Ahppa) at the ureido linkage of both new anabaenopeptins, which prompted us to investigate the biosynthetic origin of this non-proteinogenic amino acid Ahppa. We performed heterologous expression in *E. coli* and biochemical assay to show MaHphABCDE involved in double homologation of Tyr residue. These works disclosed bioactive natural products from cyanobacteria and characterized enzymes which expand the biocatalysis toolbox for prenylations and homologations.

Keywords: *Cyanobacterium*, natural products, biosynthesis, bioactivity

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S1.P301 Investigation of the effect of solid state fermentation on the release of phenolic compounds from by-products of the agro-food industry

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The main purpose of this research was to obtain valuable phenolic compounds (PC) from the by- products of the agricultural and food industry - linseed oil cake (LOC) and brewer's spent grain (BSG) - using solid state fermentation (SSF) and submerged fermentation (SmF) culture modes. The by- products used in the study are lignocellulosic materials with a proven content of PC, which are desirable in the industry due to their biological properties. Indeed, they exhibit antioxidant properties, which makes them widely used in medicine, cosmetology and food industry (Kyselka et al., 2017). Various biocatalysts (29 *Ascomycota* and *Basidiomycota* strains) were used in the screening experiment. A comparative analysis of the two culture systems was performed, as well as the selection of the most effective biocatalysts in terms of their ability to degrade lignocellulosic raw materials and release PC. Qualitative and quantitative analysis of the extracts obtained from the fermentation media allowed to identify several PC such as: ferulic acid, *p*-coumaric acid, caffeic acid, vanillic acid, veratric acid, and vanillin (Fig. 1).

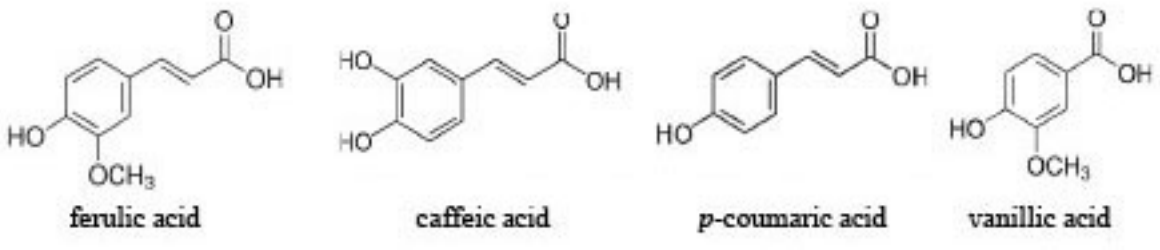

Fig. 1. Structures of identified phenolic compounds.

This study indicates that the use of biotechnological methods to obtain biologically active compounds is an attractive alternative to value difficult to recycle by-products of the agro-food industry. Moreover, it is consistent with sustainable development and the business model of using agricultural waste in the bioeconomy (Šelo et al., 2021).

Acknowledgments: The work was financed as part of the LIDER XII project financed by the National Center for Research and Development (Poland) under the contract No. LIDER/44/0228/L-12/20/NCBR/2021.

Keywords: agro-food by-products, brewer's spent grains, linseed cake, solid state fermentation, phenolic compounds

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S1.P302 Biosynthesis of gramine in barley by a cryptic oxidative rearrangement

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The indole alkaloid gramine protects barley and other grasses from insects. Early studies demonstrated that gramine is derived from tryptophan via the key intermediate aminomethylindole (AMI) by an unknown enzyme that removes C-1 and C-2 of tryptophan while forming a new C-N bond (Dieter et al., 1974, Ishikawa et al., 2023). In this study, we elucidated the biosynthetic pathway of gramine in barley by identifying the missing AMI synthase (AMIS). Co-expression of *AMIS* with a previously described *N*-methyltransferase gene in *Nicotiana benthamiana*, *Arabidopsis thaliana* and *Saccharomyces cerevisiae* enabled the production of gramine in heterologous platforms (Fig. 1).

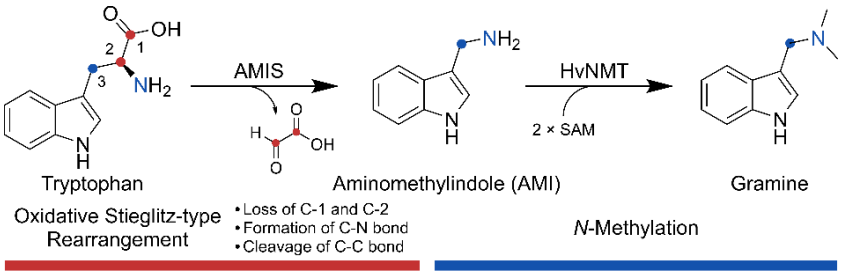

Fig. 1. Gramine biosynthetic pathway.

As a further part, we demonstrated that AMIS catalyzes an oxidative Stieglitz-type rearrangement of tryptophan by a combination of isotope labeling experiments, intermediate trapping and by-product investigation. In summary, our study reveals the genetic and biochemical basis of an unusual amino acid chain shortening in the biosynthesis of gramine. This enables further biotechnological applications utilizing this alkaloid for cereal protection.

Keywords: biosynthesis, gramine, alkaloid, oxidative rearrangement, barley

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S1.P303 Urolithin production in *ex vivo* incubations of gut microbiota – survey on different substrates and antibiotics' influence

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The most extensively explored of ellagitannins' postbiotic metabolites is urolithin A (Ryu et al., 2016). However, not every individual is able to have it produced in the gut, and certain urolithin metabotypes were distinguished (García-Villalba et al., 2022). This might be associated with the occurrence of several already isolated species producing urolithins (Iglesias-Aguirre et al., 2023). Therefore, in subsequent studies, the metabotypes need to be considered as the main variable. Nevertheless, other factors affecting gut microbiota, such as antibiotic treatment, might influence urolithin production *in vivo* as well. Thus, the authors performed a series of incubations with gut microbiota *ex vivo*, using fecal samples from donors of different metabotypes. Substrates such as *Punica granatum* juice, corilagin, ellagic acid, and urolithins M7 and C were used. The influence of three commonly used antibiotics, i.e., amoxicillin-clavulanic acid, cefuroxime, and azithromycin, was tested. The samples collected during incubations were analyzed using LC-HRMS. Using an intermediate product - urolithin C, as substrate allowed detectable concentrations of bioavailable urolithins to be obtained more quickly than in incubations with other substrates. High variability of the biotransformation outputs (product ratio) was observed among metabotype B donors. The addition of amoxicillin-clavulanic acid inhibited urolithin production in the batch, while azithromycin and cefuroxime showed less impact. The incubation of metabotype B donor microbiota with azithromycin at 25 µg/mL resulted in a biotransformation output typical to metabotype A (only urolithin A).

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Keywords: ellagitannins, urolithins, biotransformation, antibiotics, metabotypes

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S1.P304 Identification of gene cluster for biosynthesis of antiviral polyketide limocrocin

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Explosion of bacterial genome data has led to discovery of myriads of biosynthetic gene clusters (BGCs) for specialized metabolites of unknown chemical identity. There is urgent need for generic approach to elucidate such (often cryptic) BGCs. Over the last decade we focused on assessing various tools and approaches which ultimately would help us decipher the natural products hidden behind cryptic BGCs through heterologous expression. Here we report the use of *Streptomyces albidoflavus* J1074 as a platform to discover the BGC for polyketide limocrocin (LIM). LIM is an unusual linear polyene that interferes with viral reverse transcriptase that was discovered back in the 1950s (Brockmann et al., 1953; Hanajima et al., 1985). However, the genetic and biochemical logic of LIM biosynthesis remained unknown until this report. In course of the studies of *S. roseochromogenes* NRRL 3504 (Melnyk et al., 2022) our attention was attracted to exotic manumycin-like BGC within its genome. We generated NRRL 3504 cosmid library, and, through end sequencing, identified the one carrying aforementioned BGC. Its introduction into *S. albidoflavus* Del14 led to accumulation of yellow-pigmented compound. LC-MS and NMR results showed that the compound is LIM **1**. Two oxidized derivatives of **1** were also identified for the first time. Detailed bioinformatic analysis of *lim* BGC as well as *lim* gene knockouts led to the initial hypothesis about biosynthetic pathway leading to **1**. Further experiments are necessary to understand several obscure stages of LIM assembly to enable the overproduction and genetic engineering of LIM derivatives.

Keywords: *Streptomyces roseochromogenes* NRRL 3504, BGC, heterologous expression, limocrocin, reverse transcriptase inhibitor

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S1.P305 Reconstitution *of natural products biosynthetic pathways using cell-free synthetic biology*

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We report the successful cell-free reconstitution of two natural product biosynthetic pathways of divergent complexity and structural classes – teleocidin and UK-2A. To enable the construction of the teleocidin biosynthetic pathway, we discovered a direct interaction between a known pathway enzyme TleA and an uncharacterized enzyme MbtH. We also illustrated the regulatory bottleneck of an intermediate step catalyzed by TleB. We then extended our methodology to successfully reconstitute UK-2 diol, the precursor to a commercially valuable secondary metabolite. The cell-free system enabled refactoring of a complex pathway of ten enzymes to build UK-2 diol from simple building blocks. We show that our cell-free biosynthesis platform is suitable for reconstructing pathways and identifying the functions of uncharacterized genes linked to biosynthetic gene clusters (BGCs) and rate-limiting biosynthetic steps.

Keywords: cell-free synthesis, biosynthesis, BGC, teleocidin, UK-2A

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S1.P306 Biotransformation of phlorotannins from brown algae to enhance bioactivity

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The EU ban on antimicrobial growth promoters (AGPs) in animal production prompted a quest for plant-based alternatives (Yang et al., 2015). Several plant-derived compounds ("phytochemicals") have been shown to support animal health. Marine plants like brown algae rich in phlorotannins possess e.g. antioxidative, and anti-inflammatory properties (Ford et al., 2019). However, the high molecular weight of phlorotannins limits their resorption in the intestine (Catarino et al., 2021), reducing their health-promoting effects. This poster will cover the biotransformation of brown algae by fermentation and chemical hydrolysis. The disruption of phlorotannin's chemical bonds is hypothesized to yield smaller, more bioavailable oligomers, potentially increasing bioactivity. *In vitro* experiments using extracts from two brown algae species, *Ascophyllum nodosum* and *Fucus vesiculosus* provided by NUQO©, demonstrated their bioactive effects. Intracellular antioxidant activity was shown by reducing reactive oxygen species (ROS) in oxidative stressed Caco-2 cells. Treatment with the algae extracts led to significantly reduced nitric oxide (NO) production in LPS- stressed RAW264.7 cells, indicating anti-inflammatory effects. Moreover, treatment with *A. nodosum* and *F. vesiculosus* extracts led to reduced levels of pro-inflammatory cytokines IL-6, IL-8, IL-10, TNF-α in cell culture supernatants of LPS-activated THP-1 macrophages. Preliminary findings suggest that fermentation with lactic acid bacteria (LAB) and NaOH hydrolysis of *A. nodosum* enhance its antioxidant effects in oxidative-stressed Caco-2 cells. Further research is needed to elucidate the molecular mechanisms underlying this augmented bioactivity.

Keywords: phlorotannins, brown algae, bioactivity, biotransformation, fermentation

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S1.P307 Enhancing polyphenol profiles and production: a novel approach through synergistic non symbiotic fungi and organic fertilization

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Polyphenols, as naturally occurring high-value secondary metabolites, possess well-documented benefits for human health, catalyzing an augmented demand for these compounds. Attempts to economically synthesize polyphenols by alternative methods face industrial challenges, such as low yields and toxicity affecting cell growth (Ofosu et al., 2020). In this context, Mediterranean semideciduous shrubs, thriving under abiotic stress (thermal and solar radiation) without hydric stress, emerge as a potent source of pharmacologically relevant metabolites. *Cistus albidus* L. is such a species, traditionally recognized for its medicinal properties attributable to its prolific production of diverse natural metabolites under harsh Mediterranean climate conditions, with minimal resource requirements (Raus de Baviera et al., 2023). Through the implementation of a treatment strategy that combines fertilization with non-symbiotic inoculation with *Rhizophagus* sp., we have successfully augmented the production of several polyphenols of pharmacological significance. The annual average efficiency of leaves, quantified by the specific leaf area (SLA), exhibited a 57% enhancement relative to control plants, with a twofold increase observed in the spring. This enhancement was primarily manifested in the total phenolic content and antioxidant capacity, both of which saw increments exceeding 30%. Notably, the inoculation and organic fertilization regimen induced significant alterations in the compound profile, varying with the season, and predominantly affecting flavanols, flavonols, ellagitannins, and phenolic acids. It is proposed that the synergistic application of non-symbiotic fungi and adapted organic fertilization presents a novel strategy to modify the compound profile of *C. albidus*, thereby significantly elevating the production of targeted compounds.

Keywords: polyphenol production, *Cistus albidus*, non-symbiotic fungi, organic fertilization

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S1.P308 The potential role of aqueous extract from fruits of *Chaenomeles japonica* and its post-digestive fractions in inflammation-related intercellular communication

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Chaenomeles japonica (Thunb.) Lindl. ex Spach (CJ), commonly cultivated in Poland, have been also used in traditional Chinese Medicine. The fruits of CJ are rich in polyphenol compounds, especially procyanidins (Du et al., 2013; Siegien et al., 2021). The study aimed to assess the role of phytochemicals present in CJ fruit extract in intercellular communication associated with inflammation. We evaluated the effect of CJ extract on cytokine secretion in peripheral blood mononuclear cells (PBMC) and human neutrophils (PMN). To assess potential bioavailable metabolites, we performed gastrointestinal digestion (GI) *in vitro* of said extract. We compared the activity of crude fruit extract and obtained GI fractions in the human adenocarcinoma cell line (Caco- 2). Finally, we evaluated the effect of the GI fractions on the neutrophil extracellular traps (NETs) secretion in PMN. The aqueous extract from CJ fruit inhibited the secretion of all pro-inflammatory cytokines (TNF-α, interleukin(IL)-8, IL-6, IL-1β) in PMN, PBMC, and Caco-2 cells and increased the concentration of anti-inflammatory IL-10 in PBMC. The release of TNF-α in PMN was 62.1% for 100 μg/mL of CJ fruit extract, compared to samples treated with LPS. Each fraction obtained after digestion *in vitro* decreased significantly the release of IL-8 in Caco-2 cells. There are noticeable differences in the NETs secretion among the used GI fractions of CJ. In conclusion, both aqueous extract from the fruit of *C. japonica* and post-digestive fractions present inhibitory activity of cytokine secretion. The gastrointestinal digestion of the extract likely affects the composition and bioactivity of the fractions.

Keywords: *Chaenomeles japonica,* Rosaceae, cytokines, metabolites, procyanidins

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S1.P309 *Phlegmariurus carinatus in vitro* culture and study on *Lycopodium* alkaloid biosynthesis

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Phlegmariurus carinatus (Desv. ex Poir.) Ching plant of Lycopodiaceae family is a rich source of neuroactive 'huperzine A' and other Lycopodium alkaloids (LAs) and gained significant interest despite challenges such as low natural content, slow plant growth, and limited success in cultivation, *in vitro* propagation, and chemical synthesis (Xu et al., 2018). Recent biosynthetic studies have identified key genes involved (Fig. 1a; Nett et al., 2021 and 2023). As a part of our ongoing research on piperidine derived-alkaloid biosynthesis (Xu et al., 2018; Fan et al., 2021). We explored LAs biosynthesis in *in vitro* grown sporophytes of 9 plants in the Lycopodiaceae, including *P. carinatus*. It was observed that optimal sterilization techniques were essential for axenic culture establishment. Moreover, we saw slow growth, but sporophyte bifurcate within 4-6 months of invitro culture. The LCMS/MS analysis of LAs showed varying concentration across sporophytes (Fig. 1b). Huperzine A was highest in the leaves. Furthermore, the transcriptome analysis showed that the early biosynthetic genes upregulated in the young tissues of sporophyte, while the late biosynthetic genes especially the modification step seems to be upregulated in specific tissues like roots and leaves (Fig. 1c). In conclusion, this study provides a new platform for neuroactive LA's production and biosynthetic studies.

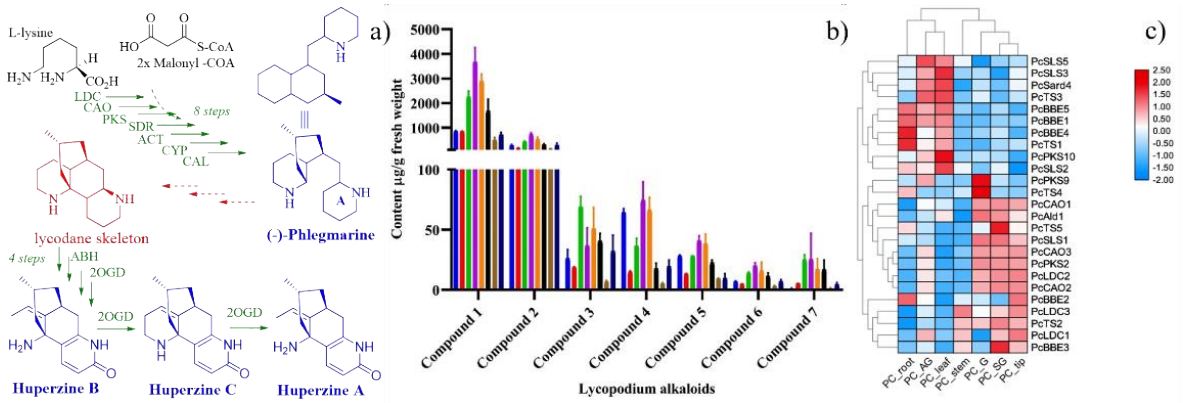

Fig. 1. Huperzine A biosynthetic pathway (a), lycopodium production (b) and differentially expressed candidate genes of *in vitro* culture (c).

Keywords: *Phlegmariurus carinatus,* huperzine A, sporophyte culture, transcriptome

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S1.P310 Biosynthesis of α-aminopyrones produced by the marine-derived fungus *Aspergillus* sp. DLM38

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α-Aminopyrones are metabolites produced in culture exclusively by *Aspergillus* spp. with cytotoxic and antiviral activity (Barnes et al., 1990; Varoglu et al., 2000; Zhang et al., 2010). Using a combination of stable-isotope feeding and gene knock-out experiments, we investigated the biosynthesis of the α-aminopyrones isopyrophen and naphthoquinoneimine, produced by *Aspergillus* sp. DLM38 (Ióca et al., 2016). ECD analysis confirmed the absolute configuration of naphthoquinoneimine. Feeding experiments with [1-^{13}C]-D-glucose unveiled the biogenetic pathways of both compounds as organized by NRPS-PKS gene cluster, reinforced by genome mining analysis. To confirm the bioinformatics results, knock-out mutants were obtained by homologous recombination, followed by comparison of the chemical profiles produced by the wild-type and mutant strains. UPLC-HRMS/MS confirmed that α-aminopyrones production was abolished in the knock-out mutant strains.

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Keywords: aminopyrones, *Aspergillus*, genome mining, biosynthesis, gene knock-out

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S1.P311 Plant tissue culture for production of bioactive metabolites in *Agrostemma githago* L.

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Flavone-*C*-glycosides (FCG) are pharmacologically active plant natural compounds with vitexin and orientin as the most studied examples found in several botanical families. One of these, distinguished by a wide diversity of flavone-*C*-glycosides, is the Caryophyllaceae (pink or carnation family) with comprises numerous species but only a few have been used medicinally, mainly due to their high content of saponins (triterpenoid glycosides – TTG) as bioactive principle or as an industrial material with surfactant properties (Jakimiuk et al. 2022). However, the regulation of FCG biosynthesis and reasons of the phytochemical profiles diversity remain largely unresolved despite pertinently suggested role of environmental factors in various species/plant systems. Here, we used plant cell, tissue and organ cultures as models to find out if FCGs and TTGs can be biosynthesized in a highly controlled environment in cells at different levels of differentiation–callus culture, cell suspensions, regenerated organs during *in vitro* stimulated development. *Agrostemma githago* L. *in vitro* cultures were established in solid and liquid media. The content of specialized metabolites was analyzed using LC-qTOF-MS and UV-HPLC and compared to plants grown conventionally. As a result, the FCGs orientin, isoorientin and vitexin were detected with at least five derivatives with higher molecular weight supposedly with additional glycosylation. The content of TTG in all tested *in vitro* cultures was significantly lower and less complex than in field-grown plants. However, both content and HPLC profile of both compound classes differed between culture systems, such as callus culture on agar medium, cell suspension, organ cultures as well as between solid and liquid media.

Acknowledgements. This research is funded by National Science Center of Poland (NCN) # 2020/39/I/NZ7/01515 (RIPSAPO).

Keywords: *Agrostemma githago,* Caryophyllaceae, *in vitro* cultures, orientin

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S1.P312 Analysis of host and gut microbial biotransformation products of kratom (*Mitragyna speciosa*) by mass spectrometry

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Despite the widespread use of botanical medicines and supplements, the metabolic fates of bioactive and other chemical constituents of these complex mixtures are without systematic characterization. This limits our understanding of the contributions of individual components to overall efficacy or toxicity. A main contributor to the biotransformation of xenobiotics in the body is the various drug- metabolizing enzymes present in the liver. Additionally, there has been significant focus on the role the gut microbiome plays in the pharmacokinetics and efficacy of drugs (Pant et al., 2023; Zimmermann et al., 2019). However, there is a severe lack of research regarding the poly- pharmacokinetics of botanical medicines in this regard. The vast metabolic repertoire of these microbes allows for entirely unique enzymatic reactions that contribute significantly to the metabolism of xenobiotics (Pant et al., 2023). In this study, pooled human liver S9 enzymatic fractions were used for *in vitro* biotransformation of pure compounds from Kratom (*Mitragyna speciosa* Korth., Rubiaceae) and used to test capabilities for identification in the Kratom extract. Additionally, fecal matter from healthy human donors was used for the *in vitro* culturing of gut microbiome communities under anaerobic conditions. Samples were analyzed by LC-MS/MS and data were analyzed with custom scripts in Python. An integrated workflow to generate and detect multiple phase-I and -II liver, and various gut microbial metabolites from a botanical mixture was established. Applications of molecular networking and various metabolomics tools will expand capabilities for the discovery of new metabolites of pharmacological interest from complex botanical mixtures.

Keywords: kratom, liver, gut microbiome, biotransformation, mass spectrometry

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S1.P313 Interactions with gut microbiota and bioavailability of natural products contained in lavender flowers

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Lavandula angustifolia Mill., also called true/English lavender, is a source of important medicinal plant material - lavender flower (*Lavandulae flos*) (Ghadim, et al. 2020). It's essential oil fraction is rich in monoterpenoids, but also, the flowers are often used in the form of self-prepared infusions to treat mild symptoms of anxiety. However, the form of an infusion usually contains small amounts of essential oil and contains a variety of polar natural products, which can contribute to the beneficial effects *in vivo* (Bazrafshan, et al. 2020). Previous studies shown that various plant materials can affect the biodiversity of gut microbiota (GM) and the GM can modify the structure of natural products contained in plant extract. This lead to the production of novel bioactive metabolites (Morris, et al. 2018). The current proposal aims to provide scientific evidence on the interaction of lavender infusion (LOI) with GM in the context of the treatment and prevention of anxiety disorders in humans. Forty three compounds of LOI were detected by UHPLC-DAD-MS including flavonoid derivatives, caffeic acid derivatives, propanoic acid derivatives, ferulic acid derivatives, glucosyl hydroxycinnamic acid and methoxycinnamic acid derivatives. The chemical composition after incubation with GM was established by the UHPLC-DAD-MS analysis.

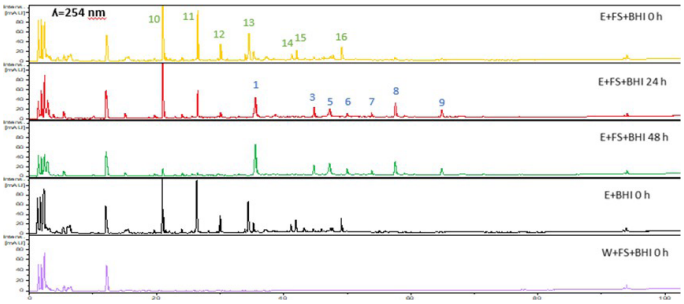

Fig. 1. Metabolites of GM and LOI.

The analysis of the GM metabolism of LOI showed at which timepoint the most diverse mixtures of metabolites are produced and which compounds undergo biotransformation (Fig.1). Up to know 9 potential metabolites were detected in analysed mixtures.

Keywords: *Lavandula officinalis*, water extract, gut microbiota, postbiotic metabolites

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S1.P314 Using banana peels in fermentation: upstream bioprocesses

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Banana (*Musa* spp., Musaceae family) is a fruit consumed worldwide (Hikal et al., 2022). Banana peels (BP), constituting 30-40% of the fruit's weight, are considered an industrial waste. This biomass presents an opportunity for more eco-friendly solutions, namely as source for biotechnological bioprocesses. BP can be considered as a lignocellulosic biomass and because of the inherent characteristics, pre-treatment becomes essential to enhance the effectiveness of enzymes on the hydrolysis of complex carbohydrates (Mahmoud et al., 2023; Mishra et al., 2020). Therefore, this study aims to determine the best pretreatment and enzymatic hydrolysis (EH) conditions to produce a fermentation substrate suitable for producing lactic acid. A diluted-acid pretreatment was used with H_2SO_4 (0.5% and 0.25%) in autoclave for 10 min at 121 ºC. After autoclaving, the pretreated material was subjected to several EH tests in which two variables were evaluated for their influence on hydrolysis yield: solid load (% dw/v), and H_2SO_4 concentration in the pretreament (% v/v). A commercial enzyme cellulase cocktail was used for the assays. Additionally, the following test were carried out; a control test in which the BP was subjected only to autoclave pretreatment (without the subsequent EH step), and another one in which only EH was performed in raw BP. The BP showed 11.42±0.09% of cellulose, 5.9±0.3% hemicellulose, 17.2±0.5% of total lignin and 44.8±0.2% of extractives. Pretreatment was essential, as shown by lower yields in the control and EH in raw BP (Figure 1). All yields surpassed 60% after pretreatment, with H_2SO_4 impacting viscosity and enhancing fluidity during hydrolysis. Acid concentration did not significantly affect yields, but 0.25% H_2SO_4 showed a slight improvement over 0.5% H2SO4 for 20% solid load. The concentration of total sugars during EH remained almost constant after 12 hours, indicating minimal further hydrolysis. Therefore, according to the results, the conditions chosen for the pretreatment and EH steps were 0.25% H_2SO_4 and 15% or 20% solid load.

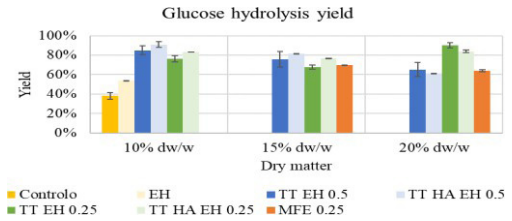

Figure 1 -. Graphical representation of the glucose yield from BP hydrolysis with different solid loads. EH - enzymatic hydrolysis; TT – thermal treatment; HA – acid treatment; 0.5 and 0.25 - acid concentration (% v/v); and MFE - BP medium for fermentation.

Keywords: by-products, pre-treatment, tropical fruit, hydrolysis, carbon source

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S1.P315 Waste to wellness: hypocholesterolemic solutions from mushroom residues, boosting circular economy

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Although statins, the primary hypocholesterolemic agents in therapeutics, are widely utilized, they are also linked with various harmful side effects. Mushrooms are rich sources of mycosterols, that reduce cholesterol absorption, polysaccharides such as β-glucans and statin-like molecules, able to lower cholesterol levels by inhibiting the 3-hydroxy-3-methyl-glutaryl CoA reductase (HMGCoA- red), a key-enzyme in the cholesterol metabolism. The Mush4Chol project aims to transform mushroom industrial biowaste into a hypocholesterolemic formulation. By exploring unsalable mushrooms and broken stems, the project seeks to develop a validated and stabilized product capable of inhibiting cholesterol synthesis and absorption. Through a combination of conventional and emerging extraction techniques, including refinement, stabilization, and encapsulation, the project aims to produce extracts rich in mycosterols, β-glucans, and statins, either as fractions or isolated compounds. The project will employ a variety of *in vitro* and *in vivo* hypocholesterolemic methodologies to explore potential mechanisms of action comprehensively. Evaluation of the probiotic *Lactobacillus reuteri's* ability to assimilate cholesterol, both alone and in synergy with the extracts, will also be conducted. Ultimately, the goal is to create a stabilized powder comprising natural hypocholesterolemic agents, offering versatile economic applications such as gelatin capsules or as an ingredient for developing functional foods. To embrace a zero-waste approach, the leftover extraction residues will be looped back into the beginning of the value chain, serving as nutrient-rich mushroom substrate, boosting circularity.

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Keywords: hypocholesterolemic agents, mushrooms, bioresidues, functional foods, circular economy

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S1.P316 Bilateral interactions between human gut microbiota and xanthohumol

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Xanthohumol (XH) is the main prenylated flavonoid found exclusively in the hop plant (*Humulus lupulus*). XH has gained attention as a potent leading molecule with promising results, positioning it as a potential agent for chemoprevention, antimicrobial, or anti-inflammatory purposes (Zanoli and Zavatti., 2008). Currently, the metabolism of gut microbiota emerges as a key regulator in the bioavailability of orally administered substances, leading to the formation of postbiotic metabolites (PMs). On the other hand, xenobiotics can substantially alter the composition of the gut microbiota. To evaluate these bilateral relationships in the context of the hop active constituent, isolated from hops XH (0.5 mg/mL) was incubated with human fecal microbiota sourced from six healthy donors under anaerobic conditions. During the 24-hour period, samples were collected and analyzed using high-resolution mass spectrometry. Subsequently, intestinal bacteria were collected and underwent 16S rRNA sequencing to evaluate changes in the α- and β-diversity indices. During the *ex vivo* incubation, the formation of certain reduced XH-derived PMs was observed. Additionally, bioinformatic analysis revealed significant changes in the abundance of a few intestinal bacteria; however, there was no negative effect on the overall diversity parameters of the microbiome. These findings highlight the critical role of gut microbiota in mediating XH's bioactivity through the production of XH-derived PMs and suggest that XH, despite its antibacterial attributes, does not lead to dysbiosis or reduce the diversity of gut bacteria at the moderate concentration.

Project financially supported by Medical University of Warsaw Grant for Young Scientists No. WF4L/1/F/MB/N/23.

Keywords: gut microbiota, xanthohumol, *Humulus lupulus*

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S1.P317 Engineering potential of the homologation pathway of L-phenylalanine and L-tyrosine

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Homologation of amino acid is a chemical transformation to add a methylene group to the amino acid side chain. Homologated amino acids (homoAAs) are not very common non-proteinogenic amino acids found in natural products (NPs), but they are seen in several NP, NP-derived, and synthetic peptide drugs. However, its potential has not been fully utilized because of a lack of knowledge of their biosynthesis and the cost of some homoAAs. This presentation will focus on the characterization and engineering of the first novel enzyme HphA in the homologation pathway for L-Phe and L-Tyr (Fig. 1). This pathway has been identified to produce homologated L-Phe (L-hPhe) and L-Tyr (L-hTyr) by one known and three novel enzymes (Koketsu et al., 2013) and studied for a mass-production of L-hPhe by metabolic engineering (Liu et al., 2020). We biochemically characterized one of the novel enzymes HphA by investigating its proposed reaction, substrate profile, and kinetics. It was also confirmed by site-directed mutagenesis studies on its metal binding site that HphA is a homologous enzyme to LeuA that is the first enzyme of L-Leu biosynthetic pathway.

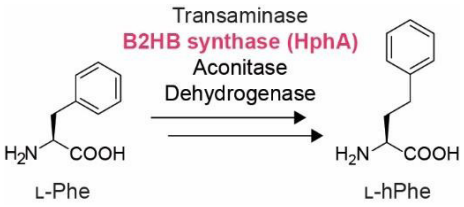

Fig. 1. Homologation of L-Phe to L-hPhe. The enzyme studied herein is colored red.

These studies and follow-up mutagenesis studies showed the engineering potential of the enzyme to homologate other amino acids that are not homologated in Nature. This study is the first step towards developing the enzymatic homologation tool of amino acids as well as peptide NPs, which has never been reported as of today.

Keywords: amino acid, biosynthesis, engineering, homologation, nonribosomal peptide

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S1.P318 Unraveling the biosynthetic pathway of aorimycin: insights from gene deletion experiments

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Aorimycin (**1**) is a novel natural product from *Streptomyces* sp. IB2014/011-12 classified as a type II polyketide (Eckert et al., 2022), containing a unique nine-ring structure as well as an epoxy functionality (Fig. 1). Understanding its biosynthetic pathway and the enzymes involved may help to expand the enzymatic tool box for combinatorial biosynthesis, increasing the chemical diversity of bioactive compounds and thus unlocking their therapeutic potential. In this study, we aimed to elucidate the role of specific oxidoreductases in the biosynthesis of aorimycin through gene deletion experiments.

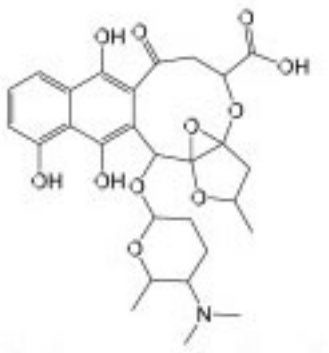

Fig. 1. Structure of aorimycin **1**.

Our investigation focused on two FMN-dependent two-component monooxygenase systems, hypothesized to be pivotal in generating the chemically intriguing structures observed in aorimycin. By deleting genes encoding for these monooxygenases and flavin reductase, we aimed to discern their individual contributions to the biosynthetic pathway. Additionally, putative intermediates and shunt products, which accumulated in the deletion mutant strains, were isolated and used for structure elucidation via NMR spectroscopy and HRESIMS as well as for *in vitro* assays. Our findings reveal the significant impact of these two-component monooxygenases on aorimycin biosynthesis, shedding light on the intricate enzymatic machinery responsible for its formation. Through detailed biochemical and structural analyses, we aimed to delineate the specific functions of these enzymes, providing valuable insights into the biosynthesis of complex natural products.

Keywords: *Streptomyces*, polyketide, knockout, oxidoreductase, two-component monooxygenase

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S1.P319 Synthetic biology platform for production of novel anticancer agents

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Anthracyclines are important anti-cancer agents, which are in clinical use worldwide. However, anthracyclines can cause irreversible dose-dependent cardiotoxicity, which limits the amount of anthracyclines that can be administered in the lifetime of the patient. In addition, the stereochemical complexity of anthracyclines complicates structural modification by organic synthesis. This research exploit advances in synthetic biology and built up a platform for production of novel biosynthetic anthracycline agents on an unprecedented scale, where further modifications to anthracyclines were addressed for discovery of cardiotoxicity-free anthracyclines. We firstly assembled artificial anthracycline biosynthesis pathways in *Streptomyces* with a BioBricks approach. The biosynthesis routes were split to four modules, including polyketide aglycone pathways, TDP-carbohydrate pathways, glycosyltransferases, and tailoring enzymes. With the synthetic biology platform, we are able to *de novo* refactor 4 typical anthracycline pathways. In addition, we have made more than 10 new anthracycline compounds by combinatorial synthesis. The bioactivity test will be carried on these new compounds to discover cardiotoxicity-free anticancer compounds. This project also provides an example of rapid large-scale systematic exploration to allow the discovery of functional gene combinations for generation of novel chemistry.

Keywords: biobricks, anthracycline, *Streptomyces*, biosynthesis, *de novo*

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S1.P320 Symbiotic relation between *Ammi visnaga* and *Rhizobium rhizogenes* in bioreactor conditions

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Ammi visnaga is a medicinal plant known by common names such as toothpick plant, toothpick herb, bisnaga, khella (Khalil, 2020). Visnagin is an important raw material in *A. visnaga* as it has a vasodilator effect that lowers blood pressure in rats (Lee et al., 2010). In the study, visnagin transformation was monitored during the bioreaction process in order to determine the visnagin level with *Rhizobium rhizogenes* inoculation. A. visnaga was propagated under tissue culture conditions with Murashige and Skoog (MS) medium containing 0.5mg.L^{-1}+ 0.1mg.L^{-1} hormones. Internodes were used to transform friable callus with MS medium containing 2.0mg.L^{-1} 2.4-D and were infected with *R. rhizogenes*. Aplikon my-control bioreactor was used to carry out the transformation conditions. Khellin levels were determined by spectrophotometric method under 390 mu (Karawya et al. 1971). In the study, no positive effect of bacterial inoculation on visnagin yield was observed.

Keywords: bioreaction, biotransformation, callus, tissue culture, medicinal plants

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S1.P321 Application of 1,2,4-triazolium in structural modification of natural products and their biological activity studies

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Nature products have been extensively used in the discovery and development of new drugs. 1,2,3- Triazoles are widely used in the fields of pesticides and medicines due to their broad-spectrum bioactive properties. The synthesis of a new class of triazole-containing natural product conjugates has gained interest in the past few years. However, these are associated with drug resistance, giving rise to the need to develop novel drugs with similar properties. In this regard, 1,2,4-triazolium appears significant due to its antibacterial, antifungal, anticancer, and antiprotozoal activities (Song et al., 2023). Some of the triazolium compounds, such as Biapenem and Cresemba, have been approved by the U. S. Food and Drug Administration (FDA) as marketed human drugs. The introduction of the cationic triazolium structure into a drug-candidate molecule can improve the aqueous solubility, efficacy, and safety of non-target organisms. In addition, triazolium salts have been employed as *N*- heterocyclic carbene catalysts (Jia et al., 2023). Therefore, we set out to synthesize different 1,2,4- triazolium compounds using 1,4-dimethyl-4*H*-1,2,4-triazolium iodide as the precursor. The synthesized triazolium molecules were evaluated for their antioxidant, antimicrobial, general toxicity, and cytotoxicity. We thus identified which of these 1,2,4-triazolium compounds have the best biological activities and suitable for the synthesis of new lead compounds. These triazolium molecules were then conjugated to royleanones previously isolated from *Plectranthus* species to improve their aqueous solubility and cytotoxicity. We also set to study the reactivity of the synthesized triazolium compounds proving the relationship between the basicity of the triazolium and the stability of the synthesised conjugate.

Acknowledgments: This work has run within the research project PTDC/QUI-QFI/1880/2020. The CQC-IMS is supported by the Fundação para a Ciência e a Tecnologia (FCT), through projects UI0313B/QUI/2020 (DOI: 10.54499/UIDB/00313/2020), UI0313P/QUI/2020 (DOI: 10.54499/UIDP/00313/2020) and LA/P/0056/2020.

Keywords: triazolium compounds, natural products, bioactivity, synthesis

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S1.P322 Biotransformation of bufadienolides from *Drimia maritima* and *in silico* biological studies

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This presentation will cover the isolation and structure elucidation and biotransformation of *Drimia maritima* extracts and *in silico* anti-influenza A properties of selected compounds. *D. maritima* (Asparagaceae) is a medicinal plant with bufadienolides as major components (Saadane et al., 2021). Bufadienolides are effective against a broad spectrum of diseases and have poor solubility deeming them unsuitable for pharmalogical applications (Bedir et al., 2021; Manganyi et al., 2021). In this study, different extracts of *D. maritima* bulbs were selected for microbial biotransformation studies. Microbial biotransformation is a process that makes use of microbial enzymes from fungi or bacteria as biocatalysts in the conversion of compounds to obtain derivatives with improved solubility. Different extracts (ethanol, 80% ethanol, water, and DCM: MeOH) were separately incubated with *Fusarium sp.* in PDA at 25°C for 5 days. The DCM: MeOH extract was used for the purification of bufadienolides using flash chromatography and preparative HPLC. NMR spectroscopy and detection with UHPLC-MS/MS confirmed the isolated compounds as bufadienolides. The binding capacity of bioactive compounds to H5N1 neuraminidase was comprehensively evaluated using molecular docking analysis with zanamivir as positive control.

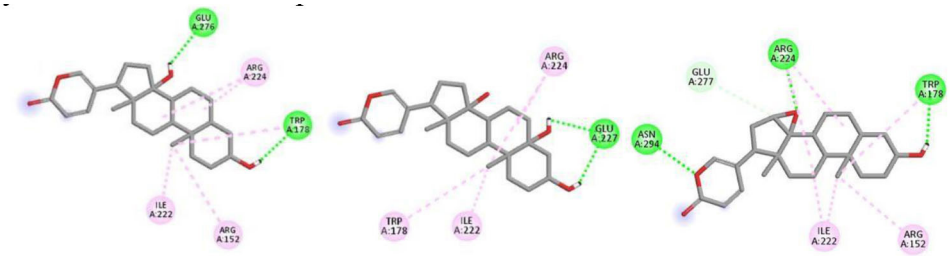

Fig. 1. Protein ligand interactions of selected bufadienolides.

The binding energies exhibited a range from -7.24 to -8.53 kcal/mol. Specifically, resibufogenin, cinobufagin, and bufalin demonstrated markedly higher binding affinities to the 3CKZ protein than zanamivir. The specific-strong interactions identified between these compounds and the protein suggest their potential effectiveness as inhibitors of influenza A neuraminidase. These insights provide a promising avenue for the development of novel antiviral therapeutic strategies.

Keywords: biotransformation, bufadiendolides, *Drimia maritima*, biological activity

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S1.P323 Diverse ketoreductases involved in tailoring of atypical angucyclines

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Soil dwelling bacteria of *Streptomyces* genus are the best producers of chemically diverse secondary metabolites, which have been sourced for the production of majority of therapeutical antibiotics. Angucyclines are the largest class of polyketide secondary metabolites produced by *Streptomyces*, and they display a tremendous diversity in their chemical structure. Typical angucyclines are characterized by a bent four-ring structure, while atypical angucyclines have undergone cleavage of the carbon scaffold leading to drastic structural rearrangements and chemical diversification (Mikhaylov et al., 2021). In this study we focused on SDR-family ketoreductases targeting positions 1, 6, 7 and 12 (Fig. 1), encoded by lugdunomycin biosynthetic gene cluster (*lug* BGC) from *Streptomyces* sp. QL37 (Wu et al., 2019), and thioangucycline BGC (*tac*) from *Streptomyces* sp. CB00072 (Cao et al., 2021), which encode for the production of C-ring cleaved angucyclinones.

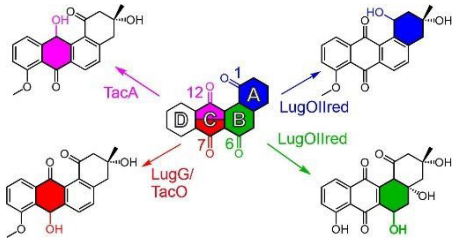

Fig. 1. SDR-family ketoreductases targeting positions 1, 6, 7 and 12.

Angucycline precursor UWM6 was converted into 8-*O*-methyltetrangomycin using 12-hydroxylase PgaE, 6-ketoreductase LugOIIred and *O*-methyltransferase LugN. We elucidated the function and substrate scope of LugG, TacO and TacA encoded by *lug* and *tac* BGCs, and characterized LugG and TacO as 7-ketoreductases through NMR-analysis of their reaction product. 8-*O*-methyltetrangomycin was converted into SM 196 A and hydranthomycin with LugG and TacA together with previously characterized 1/6-ketoreductase LugOIIred (Xiao et al., 2020). We propose that competition of many tailoring enzymes for the same substrates, and differences in enzyme promiscuity lead to a branching biosynthetic network.

Keywords: *Streptomyces,* angucycline, ketoreductase

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S1.P324 CBD biotransformation potential of endophytic fungi from *Cannabis sativa*

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Endophytic fungi refer to a group of fungi that live inside plant tissues without causing any apparent harm to the host plant and establishing a symbiotic relationship with it. Endophytic fungi are known to produce a wide variety of secondary metabolites within the tissues of their host plants, which is also the case with cannabis (Kusari et al., 2012; Scott et al., 2018). Although numerous studies suggest the biotechnological potential of endophytic fungi (Zheng et al., 2015), the ability of *Cannabis sativa* mycobiota to biotransform cannabinoids is still an unexplored field. For this purpose, ten strains of endophytic fungi, isolated from cannabis plants of the dioecious variety Carmagnola, were used in biotransformation experiments of cannabidiol (CBD). The endophytic fungus that presented the most interesting chemical biotransformation profile of CBD was *Dichotomophilus erectus*, of the Chaetomiaceae family of the phylum Ascomycota, and was subjected to large-scale liquid cultivation to isolate its main biotransformation products. In total six cannabinoids were isolated by semi- preparative high performance liquid chromatography (HPLC). Their structure elucidation was performed by 1D&2D NMR spectroscopy and by UPLC-HRESIMS. Among the compounds, three new natural products were isolated: two belonging to cannabielsoin-type cannabinoids, and one to a hydroxylated metabolite of cannabidiolaldehyde.

Keywords: cannabidiol, biotransformation, *Cannabis sativa,* endophytic fungi

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S1.P325 Structure and biosynthesis of hyellamide, a glycosylated N-acyltyrosine derivative, from the cyanobacterium *Hyella patelloides* LEGE 07179

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The chemical exploration of understudied taxa continues to be a valid strategy to reveal new natural products. Genome studies have indicated that the order Pleurocapsales is one of the most biosynthetically-rich among bacteria; however, so far, a single natural product has been reported from members of this order. Here, we report the discovery, isolation and NMR-based structure elucidation of hyellamide (**1**, Fig. 1), from the cultured cyanobacterium *Hyella patelloides* LEGE 07179. Compound **1** is an N-acylated tyrosine eneamide with an acetylated sugar moiety. While a few non- glyclosylated related metabolites had been discovered from the heterologous expression of eDNA- derived bacterial biosynthetic genes, this is the first reported example of a glycosylated version of such scaffolds. In addition, this class of metabolites had not been associated previously with cyanobacteria. Given the novelty of **1**, and having the genome of *Hyella patelloides* LEGE 07179 in hand, we sought to understand its biosynthesis. We encountered a single candidate biosynthetic gene cluster – *hye* – which contains the necessary functions to synthesize **1**, namely an acyltransferase, a glycosyltransferase, an O-acetyltransferase, and the key oxidative decarboxylase, which we propose decarboxylates the N-acylated tyrosine to generate an N-acylated eneamide.

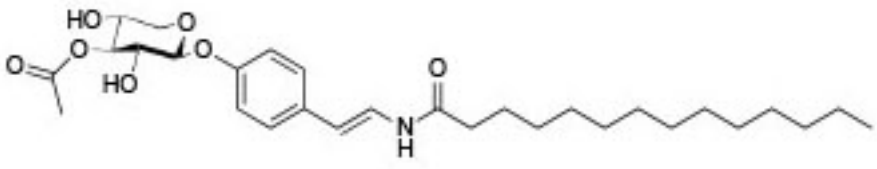

Fig. 1. Structure of hyellamide (**1**).

The discovery **1** will propel future discovery efforts in this natural-products rich order of cyanobacteria.

Keywords: cyanobacteria, biosynthesis, structure elucidation, *Hyella*

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S1.P326 Transcriptome analysis of *Triphyophyllum peltatum*, a liana producing naphthylisoquinoline alkaloids

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Triphyophyllum peltatum (Dioncophyllaceae) is a West-African liana, which produces acetogenic naphthoquinones and naphthylisoquinoline alkaloids (Feineis et al., 2023). Especially noteworthy are the naphthylisoquinoline alkaloids due to their unique structures and their promising efficacy against protozoan infections such as malaria, and against leukemia and cancer cells (Yücer et al., 2024). For further pharmacological studies, larger quantities of naphthylisoquinoline alkaloids are required, which cannot be provided from scarce natural sources or by demanding total chemical synthesis. Hence, we aim at elucidating the biosynthesis of these intriguing compounds in order to produce them in transgenic bacteria or yeast. To obtain further insight into the biosynthesis of naphthylisoquinoline alkaloids, we analyzed the transcriptome of *T. peltatum* callus cultures. *De novo* assembly of the sequences yielded 170,415 transcripts with an average size of 1494 bp. Functional annotation of the transcripts was performed with the BLAST2GO tool, and further gene ontology terms were assigned to them. In the KEGG pathway analysis, 64,608 transcripts were assigned to 22,495 KEGG pathway maps. According to this analysis, 4705 sequences are involved in the biosynthesis of secondary metabolites. In addition, a phylogenetic analysis of transcripts putatively involved in naphthoquinone and naphthylisoquinoline alkaloid biosynthesis was performed. The results of this study will increase the knowledge on the biosynthesis of these natural products and provide the base for their metabolic engineering.

Keywords: *Triphyophyllum peltatum*, naphthylisoquinoline alkaloids, antimalarial, anticancer, transcriptome analysis

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S1.P327 The roots of *Scorzonera kotschyi* Boiss. a new source for unusual dihydroisocoumarin and saponin derivatives

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Scorzonera L. is a genus of nearly 180 species that belongs to the Asteraceae family. It is naturally distributed in Asia, central and southern Europe, and northern Africa. The plants of this genus are traditionally used as vegetables or animal feed, as well as in folk medicine (Lendzion et al., 2021). In Turkey, the genus *Scorzonera* is represented by 52 species and among these, 31 species are endemic to Turkey (Coşkunçelebi et al., 2015). *Scorzonera kotscyhi* Boiss. is a perennial species native to Turkey (Chamberlain, 1975). Current research has investigated the plant's roots for their phytochemical structure. *S. kotschyi* gave three saponins that have not yet been isolated from any *Scorzonera* species one of which has an undescribed chemical structure (2α, 3β, 19α, 21β, 23- pentahydroxy urs-12-en-21-*O*-β-glucopyranoside) has not been isolated from any natural sources. Furthermore, three undescribed dihydroisocoumarin derivative which were identified as 4-hydroxy hydrangenol-4-*O*-β-glucopyranoside, 4-hydroxy-hydrangenol-4-*O*-β-rhamnosyl-glucopyranoside and 4-hydroxy-hydrangenol-4-*O*-β-apiosyl-glucopyranoside as well as one new syringic acid derivative described as syringic acid-4-*O*-[(E)-6-*O*-feruloyl]-β-glucopyranoside have also been isolated. All structures were established using spectral data, including ^{1}H, ^{13}C and 2D NMR techniques (HMBC, HSQC, COSY) and the HR-MS.

Keywords: *Scorzonera kotschyi,* Asteraceae, dihydroisocoumarin, saponin

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S1.P328 Genome-wide identification and characterization of two potential antiviral plant protein families (Major Latex Protein and Glycine-Rich Protein) in *Chelidonium majus*

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Chelidonium majus L., a prominent latex-producing plant from the Papaveraceae family, boasts a wide array of medicinal properties, such as antiparasitic, insecticidal, anti-neoplastic, antibacterial, and antiviral (Zielińska *et al.*, 2018). Despite long-standing use of *C. majus* extracts in traditional medicine for treating visible symptoms of human papillomavirus (HPV) infection, the precise molecular mechanisms underlying its therapeutic action remains elusive. In recent years, two proteins—major latex protein (MLP) and glycine-rich protein (GRP)—have garnered our attention as potential contributors to the antiviral and anticancer activities of latex (Nawrot et al., 2017). Moreover, their interaction with alkaloids could modulate therapeutic effects. The main goal of current studies is genome-wide characterization of *Chelidonium majus* GRP and MLP gene families and their antiviral and anticancer properties. To initiate the investigation, we performed hybrid *de novo* sequencing of *C. majus* genome by combining the Nanopore technology for long-read sequencing and Illumina technology for generating numerous short reads. After genome assembly and annotation, we managed to identify 72 genes encoding 91 MLP proteins and 18 genes encoding 21 GRP proteins. Using OrthoFinder, we found 13 ortholog gene clusters for MLP and 10 clusters for GRP genes in the Papaveraceae family. Moreover, we produced and purified CmGRP and CmMLP what was followed by cytotoxicity analysis of recombinant proteins against HPV-positive and HPV- negative cells. Through rigorous genome analysis and annotation approaches, we unravel the hidden intricacies of *Chelidonium majus*, providing valuable insights into its biological functions, potential therapeutic applications, and evolutionary relationships with other species within the plant kingdom.

Acknowledgements: This research was funded by the National Science Centre, Poland (grant number 2019/35/B/NZ9/03851).

Keywords: *Chelidonium majus,* latex, major latex protein, glycine-rich protein, genome-wide analysis, sequencing

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Medicinal natural products: from bench to bedside

S2.P1 Stellettin B inhibits VEGF-induced angiogenesis in human endothelial progenitor cells *in vitro* and *in vivo*

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Angiogenesis plays an important role during pathological processes, including various inflammatory diseases and tumor progression. Accumulating evidence reports that bone marrow derived endothelial progenitor cells (EPCs) regulate angiogenesis. It has been suggested that understanding the molecular targets and pharmacological functions of marine derived natural products is important for novel drug discovery. Stellettin B is a marine sponge derived triterpenoid and has been found to exert anti-cancer, anti-invasion, and anti-angiogenic activity. However, the function of stellettin B upon vascular endothelial growth factor (VEGF)-induced angiogenesis on human EPCs is undetermined. In this study, we found that stellettin B suppressed VEGF-induced EPCs tube formation in a concentration dependent manner. Furthermore, stellettin B markedly abrogated VEGF-induced microvessel formation in both CAM angiogenesis and murine Matrigel implant models. Mechanistic investigations showed stellettin B inhibited VEGF-induced phosphorylation of VEGF receptor-2 (VEGFR-2) and its downstream signals, including Akt, Erk, p38 and Src in EPCs. This is the first demonstration that stellettin B impeded VEGF-induced EPCs angiogenesis through VEGFR-2 dependent pathway on human EPCs. Stellettin B is a promising marine natural product worthy of further development for the treatment of angiogenesis related diseases.

Keywords: anti-angiogenesis, endothelial progenitor cells, marine natural products

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S2.P2 Antidiarrheal coumarins from *Psydrax schimperianus* (Rubiaceae)

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This presentation will cover the isolation, structure elucidation, and antidiarrheal evaluation of crude extracts and two coumarins, isoscopoletin, and scoparone, from *Psydrax schimperianus* (Rubiaceae). *Psydrax schimperianus* (A. Rich.) Bridson. In Ethiopia, the root of *P. schimperianus* is traditionally used to treat diarrhea (Dalle, 2019), and its leaves are used for the treatment of stomach aches and snake bites (Asfaw *et al.*, 2021). This study investigated the in vivo antidiarrheal activity of an 80% methanol crude extract and coumarins isolated from the roots of *P. schimperianus* to provide a pharmacological basis for its traditional use as an antidiarrheal agent (Dalle, 2019). Phytochemical investigation of this extract led to the isolation of two known coumarins, isoscopoletin and scoparone (Fig. 1).

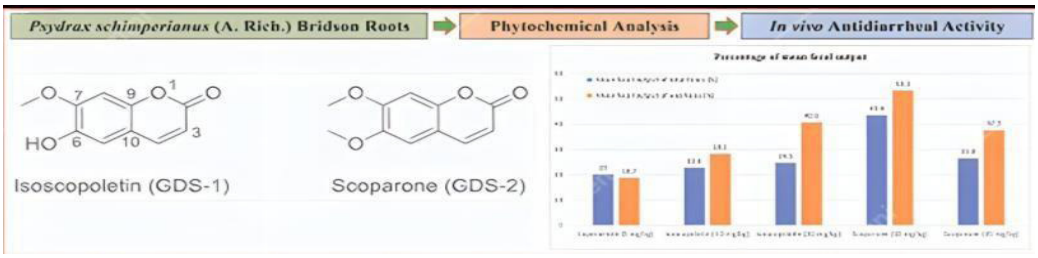

Fig.1. Antidiarrheal activities of isoscopoletin and scoparone.

The crude root extract of *P. schimperianus*, at doses of 100, 200, and 400 mg/kg, inhibited defecation by 37.5%, 46.2%, and 61.2%, respectively. At a dose of 20 mg/kg, scoparone and isoscopoletin reduced defecation by 61.2% and 66.6%, respectively. The finding of this study indicated the strong antidiarrheal activities of 80% methanol crude extract and the isolates, this study warrants further investigation of isoscopoletin and scoparone towards development as a novel treatment for diarrheal diseases.

Keywords: Coumarins, diarrhea, isoscopoletin, *Psydrax schimperianus*, scoparone, traditional medicine

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S2.P3 Ethnopharmacological validation of antiproliferative and anti-inflammatory activities of four *Curcuma* species through chemical characterization and *in-vitro* studies

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The genus *Curcuma* (Zingiberaceae) is widely used in traditional folk medicine to treat diseases caused by inflammation. Several species have remained under-explored and lack scientific validation for their claimed ethnomedicinal properties (Tushar et al., 2010). Therefore, the current study aimed to investigate the chemical composition, antiproliferative and anti-inflammatory activities of essential oil of *C. alismatifolia, C. aromatica, C. raktakanta* and *C. xanthorrhiza* rhizomes. GC-MS analysis identified a total of 90 compounds from which curzerenone (2.5-60.2%), β-curcumene (1.6-31.83%), 1,8-cineole (1.1-16.78%) and xanthorrhizol (0.6-14.12%) were the major constituents. HepG2, PC3 and MCF7 cancer cell lines were subjected for MTT assay that revealed strong *in-vitro* antiproliferative activity where *C. alismatifolia* and *C. aromatica* showed the highest selective cytotoxicity against the MCF7 and PC3 cells exhibiting an IC_{50} value of 52.86 µg/mL and 69.29 µg/mL, respectively. The toxicity of volatile oils was checked on normal 3T3-L1 cells and regarded as safe for normal human health. The *in-vitro* anti-inflammatory activity was initiated by the cytotoxicity of the essential oils (6.25–200 µg/mL) to determine the non-toxic range of further assays (Hong et al., 2021). The levels of inflammatory mediators were significantly suppressed within 100 µg/mL of essential oil in LPS-induced RAW 264.7 macrophages. *C. xanthorrhiza* showed the greatest anti-inflammation response by significantly lowering the production of NO (87%), PGE2 (91.2%) and pro-inflammatory cytokines viz., TNF-α (95.4%), IL-6 (92%) and IL-1β (88%) compared to the LPS treated group. Thus, the current studies showed that the *Curcuma* species used in traditional folk medicine might be a potential source of bioactive constituents exhibiting therapeutic properties.

Keywords: *Curcuma*, GC-MS, MTT assay, antiproliferative, anti-inflammatory

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S2.P4 *In vitro* and in silico study of protein tyrosine phosphatase 1B (PTP1B) inhibitory compounds from the leaves of *Cleistocalyx operculatus* (Myrtaceae)

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In our continuing study of potential antidiabetic agents from traditional medicinal plants, we have identified two novel lupane-type triterpenoids (**1**, **2**) with dicarboxylic groups and a new nor- taraxastane-type triterpenoid (**3**), along with fourteen previously known compounds (**4**–**17**), from the leaves of *Cleistocalyx operculatus* (Roxb.) Merr. et Perry (Myrtaceae). The structures of these compounds were elucidated through comprehensive spectroscopic methods, including IR, HRESIMS, 1D, and 2D NMR, and the known compounds were identified by comparing them with existing data in the scientific literature. We assessed all isolated compounds (**1**–**17**) for their capacity to inhibit the protein tyrosine phosphatase 1B (PTP1B) enzyme (Rines et al., 2016). Of these, compounds **6**, **9**, and **17** demonstrated significant inhibition of PTP1B. The inhibition mechanism of PTP1B by these compounds was explored via enzyme kinetics, revealing a non- competitive inhibition mechanism as shown by Lineweaver-Burk plots. Dixon plots were also used to ascertain the inhibition constants, aligning well with the observed IC_{50} values. Further understanding was developed through a structure-activity relationship study and molecular docking of the compounds with the PTP1B enzyme's crystal structure (Fig. 1).

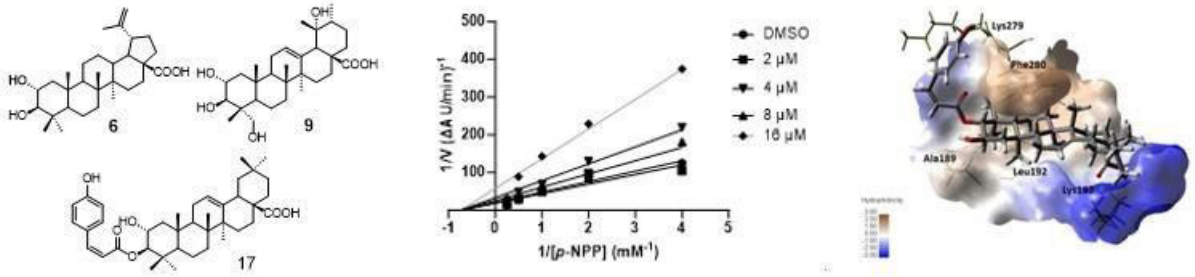

Fig. 1. Inhibitory study on compound **6**, **9**, and **17** using Lineweaver-Burk plots and molecular docking simulation on the PTP1B enzyme

Additionally, the potential of all isolates (**1**–**17**) to stimulate the uptake of 2-deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG) was investigated in differentiated 3T3-L1 adipocyte cells. Here, compounds **6**, **13**, and **17** were found to notably enhance glucose uptake in a dose-dependent manner.

Keywords: *Cleistocalyx operculatus*, Myrtaceae, protein tyrosine phosphatase 1B (PTP1B), kinetic, molecular docking, glucose uptake

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S2.P5 Revival of a traditional Austrian wound healing preparation

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Herbal preparations represent a promising therapy option in the treatment of acute and chronic wounds. However, with the ongoing increase in chronic wounds further therapy alternatives than the already used herbal formulations are highly appreciated (Vitale et al., 2022). Traditional folk medicine might hold a plethora of old knowledge about already forgotten wound healing plants. The VOLKSMED database was created to compile and preserve Austrian folk medicine and consists of more than 43,000 records about the traditional use of plants and fungi, but also other materials of animal or mineral origin (Saukel et al., 1994). It can be used to identify promising folkloric traditions, such as the preparation of a wound healing ointment made of lard and Norway spruce balm (*Picea abies*, Pinaceae). This tradition has already been supported by identifying the main constituents and *in vitro* wound healing activities (Göls et al, 2020), as well as in vivo effects on castration wounds in piglets (Prokop et al., 2023) and first case studies in patients suffering from wounds. So far, the investigations led to a monograph of a lard-based Norway spruce balm ointment in the Austrian pharmacopeia and a lard-based medical device on the Austrian market. Alternatives to lard are already actively sought and investigated to provide a more animal-friendly, stable, and standardized vehicle, which might lead to higher patient compliance (Eichenauer et al., 2023). The revival of this folkloric tradition represents an excellent example of the hidden and forgotten treasures that still can be found in Austrian folk medicine.

Keywords: Austrian folk medicine, Norway spruce balm, Picea abies, traditional preparations, wound healing

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S2.P6 Enhancing product stability by microencapsulation of a nanoemulsion of plant extracts

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Fridericia chica (Bonpl.) L.G. Lohmann (Syn. *Arrabidaea chica* Verlot) (SisGen A5489C9), standardized extract proved wound healing activity in Phase I and II clinical trials of volunteers with head and neck cancer. The Anthocyanins (carajurin), involved in the species pharmacological activity are unstable under environmental conditions. Hence, the microencapsulation of a nanoemulsion containing the association of the standardized extract with 5% of an enriched tocotrienol fraction. *F. chica* leaves' extraction at -5ºC with 70% ethanol with 0.3% citric acid provided the standardized crude extract. Further addition of 5% tocotrienol enriched fraction (70%) with high-energy using a tip sonicator gave the nanoemulsion (Rivera-Pérez et.al. 2023). Adding hydrolyzed collagen to the nanoemulsion provided a powder by spray drying. Carajurin, δ and γ tocotrienol contents were evaluated by high liquid pressure chromatography, at 470nm and 295nm, throughout 120 days, under three temperature conditions ($25^0C \pm 2^0C$), ($5^0C \pm 2^0C$), and ($40^0C \pm 2^0C$) (Sousa et al., 2022). The use of the nanoemulsion microencapsulation strategy demonstrated the ability to preserve carajurin, δ and γ tocotrienol content throughout 120 days at room temperature, providing an economically more suitable active plant pharmaceutical ingredient for use in topical formulations for wound healing purposes.

Keywords: *Fridericia chica*, *Arrabidaea chica*, carajurin, anthocyanins, stability

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S2.P7 Homoisoflavonoids from the Hyacinthaceae for use against ocular angiogenesis

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Natural products chemistry involves the extraction of potentially active compounds from plants. A class of active compounds, called homoisoflavonoids, are commonly extracted from the Hyacinthaceae family. They are regarded as promising pharmacological candidates and are often used in traditional medicine (Mulholland et al., 2013). Homoisoflavonoids exhibit antiangiogenic activity, reducing excessive formation of blood vessels. Several homoisoflavonoids have been investigated as prospective treatments for wet age-related macular degeneration, characterised by abnormal blood vessel growth. Homoisoflavonoids have potential for delivery as eye drops. Homoisoflavonoids, extracted from the Hyacinthaceae and synthesised, have been screened for antiangiogenic activity (Schwikkard et al., 2019) and have shown important structure-activity relationships. Notably, the presence and position of methoxy groups, the presence of the 3,9-double bond and the configuration at the chiral centre (C-3) are important for the activity. (Fig. 1) shows a compound extracted from *Pseudoprospero firmifolium*, showing strong antiangiogenic activity, with a GI_{50} value of 0.13 μM against Human Retinal Endothelial Cells. From this screening, we have undertaken targeted synthesis and further extractions of *Scilla* and species from the genus *Eucomis*, to increase our library of compounds for screening. Furthermore, we are investigating the possibility of growing certain bulbs commercially to extract active compounds, and comparing this to the costs of synthesising the compounds, in partnership with a UK commercial bulb grower.

Keywords: Hyacinthaceae, homoisoflavonoids, structure activity relationships, antiangiogenic activity

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S2.P8 *Pistacia lentiscus*: a plant with multiple virtues for use in human medicine as palliative in the treatment of inflammatory-related disorders

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New research trend is focusing on medicinal plants due to their wealth in various substances which can be potentially used in medicine, food and cosmetics. *Pistacia lentiscus*, a plant growing in the Mediterranean basin, is widely used in traditional medicine by rural populations for the treatment of various inflammatory-related disorders. Therefore, the present study was designed to investigate the antioxidant, anti-inflammatory, anticancer, anti-hyperuricemic and neuroprotective potential, as well as identification of active compounds, using appropriate methodology. *Pistacia lentiscus* extracts and fractions exhibited an outstanding scavenging capacity against a number of deleterious radicals while significantly enhancing superoxide dismutase and catalase activities, as well as reducing malonaldehyde levels, thus counterbalancing the oxidant status. Administration of *P. lentiscus* extracts significantly decreased ATP- and H_2O_2-induced mice paw oedema, reduced interleukin levels in cell culture and inhibited the uric acid-producing enzyme, xanthine oxidase, thereby enhancing the gouty situation of mice, mainly through a substantial reduction of uric acid levels. Additionally, *P. lentiscus* extracts showed a promising anticancer activity on human cancer cell lines, such as ovarian and liver carcinoma cells. On the other side, *P. lentiscus* extracts showed good neuro-protection and restored cognitive functions in mice, partially through inhibition of acetylcholine esterase and amyloid plaque formation and reversion of the harmful effects caused by Aluminum. Several experiments, *in vitro* and *in vivo*, including Ames' test and chromosomal aberration test indicated that *P. lentiscus* extracts are free of any genotoxicity, even showing antimutagenic action. Phytochemical investigations using preparative HPLC, HPLC-MS and NMR allowed the identification and structural elucidation of several known phenolic compounds and new anthocyanins, known to be responsible for the described biological activities, thus shedding more light on the phytochemical constituents of this plant and their action against disease. In light of the obtained results in terms of phytochemistry and biological activities, we may conclude that *Pistacia lentiscus* can be considered as a serious and safe alternative in the treatment of inflammatory conditions and deterioration of cognitive functions.

Keywords: *Pistacia lentiscus,* anti-hyperuricemia, xanthine oxidase, anti-mutagenic, phenols, anti-genotoxic

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S2.P9 Evaluation of the effective dose of the aqueous root extract of *Sphenocentrum jollyanum* Pierre: Role as an antioxidant and phosphodiesterase inhibitor for erectile function

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Improved sexual function is important in human race and with the dominance of medicinal species in the environment used in folklore as an aphrodisiac, it is necessary to understand how this plant species work and provide evidence for their mode of action (Adeleke et al., 2022; Singh et al., 2013). This study evaluated the sexual stimulating effect of aqueous extract from the root of *Sphenocentrum jollyanum* Pierre. The dose considered are 50mg/kg, 100mg/kg, 500mg/kg, 1000mg/kg body weights of the aqueous extract of the plant. The effects were assessed on sexual behavioural pattern, phosphodiesterase activity, nitric oxide level and the antioxidant status in penile tissues of rats. The aqueous root extract of *S. jollyanum* improved sexual performance, decreased the activity of phosphodiesterase while increasing the level of nitric oxide and improving the antioxidant status of the rats. Generally, the aqueous root extract at 500 mg/kg b.w administration showed a better effect of *S. jollyanum*. *Sphenocentrum jollyanum* can be considered therapeutically as an aphrodisiac.

Keywords: *Sphenocentrum jollyanum,* aphrodisiac, erectile function, phosphodiesterase

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S2.P10 Antiepileptic effects of some medicinal plant hydrosols

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Epilepsy is a common chronic neurological disorder that affects individuals across all age groups (Brillatz et al., 2020). Traditional European and Iranian folk medicines have utilized various medicinal plants to treat neurological conditions, including epilepsy (Zadali et al., 2022). In this particular study, dried *Syzygium aromaticum* L. flower buds, *Valeriana officinalis* L. roots, *Cinnamomum verum* barks, *Achillea millefolium* aerial parts, *Nigella sativa* L. fruits, *Ferula gummosa* roots, and *Heracleum persicum* seeds were subjected to hydrodistillation to extract hydrosols. The organic phase of hydrosol (OPH) was obtained by extracting the hydrosols with a solvent in a separating funnel. Gas chromatography techniques were employed to examine the content and compositions of the OPH. The anticonvulsant activity of the OPH from all the samples was tested using the PTZ epilepsy zebrafish model. The results indicated that *S. aromaticum* OPH exhibited the strongest anticonvulsant activity (Ali, 2019), reducing PTZ-induced locomotor activity at the tested concentration of 3 µg/mL ($p<0.001$). V. officinalis and H. persicum OPH also decreased locomotor activity at 100 µg/mL ($p<0.001$ for *V. officinalis* OPH and $p<0.01$ for *H. persicum* OPH). Notably, *V. officinalis* OPH at 30 µg/mL showed a significant increase in locomotor activity. In summary, this study suggests that certain hydrosols derived from medicinal plants, including *S. aromaticum*, *V. officinalis*, and *H. persicum*, exhibit potential anticonvulsant activity. The presence of specific volatile compounds in these hydrosols, such as eugenol, anethole, pulegone, borneol, and limonene, may contribute to their therapeutic effects.

Keywords: epilepsy, hydrosol, zebrafish, anticonvulsant activity, gas chromatography

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S2.P11 Alisol B 23-acetate and Alisol A 24-acetate reverse MDR cancer via ATPase modulation and membrane fluidity enhancement

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Alisol B 23-acetate and Alisol A 24-acetate (Fig. 1) are compounds isolated from *Alismatis* rhizome (Alismataceae) and belong to the triterpenoids (Feng et al., 2021). Their diverse biological effects, including oncology-related issues, were previously reported by several research groups. This study aims to investigate their modulating mechanisms on multi-drug resistant (MDR) cancer, especially the case associated with P-glycoprotein (P-gp). P-gp is an efflux pump largely expressed on the cell membrane of cancer cells, leading to the MDR problem in chemotherapeutic treatment.

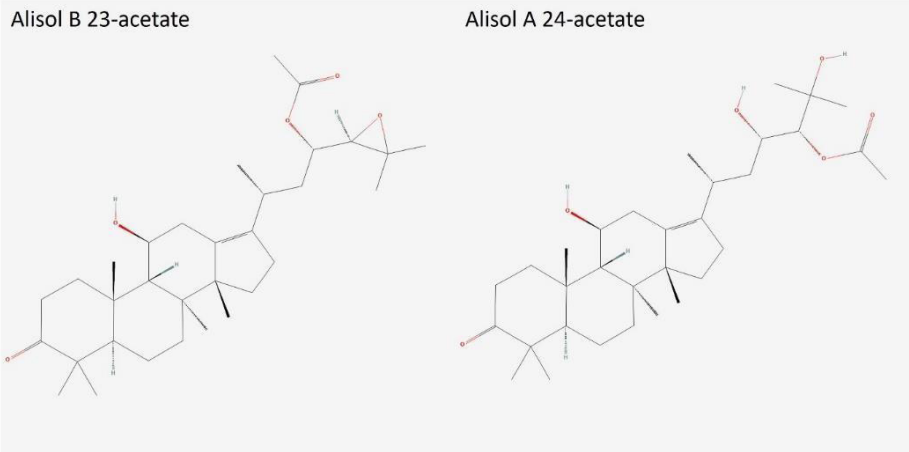

Fig. 1. Structures of Alisol B 23-acetate and Alisol A 24-acetate (images from PubChem library)

The cytotoxic IC_{50} range for Alisol B 23-acetate and Alisol A 24-acetate on the cell lines used in this study was 16.34 to 60.13 μM. The results showed that both Alisol B 23-acetate and Alisol A 24-acetate exhibited significant reversing ability at 10 μM when combined with chemotherapeutic drug doxorubicin on an extensive panel of multidrug-resistant cancer cell lines, including KB/VIN, MCF-7/DOX, and HepG2/VIN (reversal folds ranged from 3.81 to 8.14). Both compounds demonstrated prominent inhibitory effects on P-gp efflux function at 10 μM. Alisol A 24-acetate exhibited higher and dose-dependent P-gp basal ATPase activity stimulation than Alisol B 23- acetate at the dose range 1 to 50 μM. Meanwhile, Alisol A 24-acetate influenced the cell membrane fluidity more than the Alisol B 23-acetate at 10 μM, in both P-gp overexpressing cell lines Flp- In™-293/*ABCB1* and HepG2/VIN.

Keywords: *Alismatis* rhizome, Alismataceae, MDR cancer, P-gp ATPase activity, membrane fluidity

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S2.P12 The constituents and anti-inflammatory activity from *Murraya paniculata* var. *omphalocarpa* Hayata

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One undescribed coumarin, (2′*S*)-isomurralonginol 1′-*O*-(2′′*S*)-methylbutyrate (**1**) (Fig. 1), together with fourteen known coumarins, including isomurralonginol isovalerate (**2**), isomurralonginol (**3**), osthol (**4**), omphamurin (**5**), (+)-coumurrayin (**7**), 5,7-dimethoxy-8-(3′-methyl-2′-oxobutyl)coumarin (**8**), murrayanone (**9**), seselinal (**10**), (±)-7-methoxy-8-(2′-hydroxy-1′-methoxy-3-′methyl-3′-butenyl)coumarin (**11**), murragatin (**12**), mupanidin (**13**), mexoticin (**14**), and murraculatin (**15**), were isolated successively from the dried leaves of *Murraya paniculata* var. *omphalocarpa* Hayata (Rutaceae). On the other hand, we also isolated two alkaloids, γ-fargarine (**16**) and murrapanine (**17**); two coumarin-naphthoquinones, 8-naphthoherniarin (**18**) and toddacoumaquinone (**19**); three coumarins, **7**, **11**, and scopoletin (**20**); and two alkyl phenylpropanoates, methyl sinapate (**21**) and feruloyl esters (**22**), from the stem bark of the same plant. The undescribed coumarin **1** was characterized by the spectroscopic and spectrometric analytical methods, and the known compounds were identified by comparison of their physical and spectral data with those reported (Ito et al., 1990; Zhou et al., 1991). Some of the isolated compounds were evaluated for their anti-inflammatory activity (Yang et al., 2013). The results showed that IC_{50} values of **4**, **7**, **17**, and **19** (Fig. 1) for the inhibition of superoxide anion generation ranged from 2.11 to 5121 ng/mL.

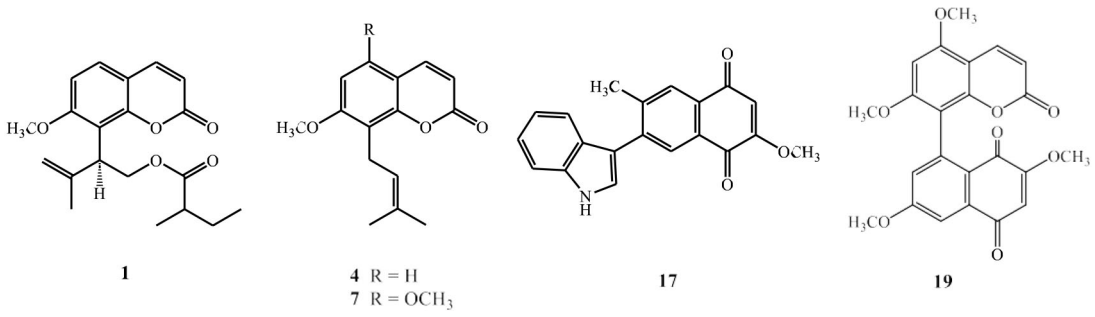

Fig. 1. Structures of **1**, **4**, **7**, **17**, and **19**

Keywords: *Murraya paniculata* var. *omphalocarpa,* Rutaceae, anti-inflammatory, superoxide anion generation

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S2.P13 Exploring Chemical Composition and α-Glucosidase Inhibitory of *Nelumbo nucifera* Seedpods

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Lotus (*Nelumbo nucifera* Gaertn.) is an aquatic plant cultivated throughout East Asia, serving various purposes such as food, as an ornamental plant, and in medicinal materials. The literature indicates that lotus exhibits a variety of biological activities, and lotus seedpods are often regarded as waste (Huang et al., 2022). More than one in 10 adults worldwide suffers from diabetes. It is a systemic metabolic disorder that is related to cardiac diseases such as heart disease and stroke. According to statistics, most of the current cases are type-2 diabetes (Tang et al., 2023). Alpha- glucosidase inhibitors are a widely used target for the treatment of type-2 diabetes. Therefore, using an alpha-glucosidase rapid screening platform to identify potential active substances from natural products is a common preliminary research method. In this study, a 95% ethanol lotus seedpod extract exhibited an alpha-glucosidase inhibitory activity of 99% at 500 μg/mL. The extract was partitioned sequentially with ethyl acetate, *n*-butanol, and water. Preliminary bioassays showed that the ethyl acetate layer had good activity against α-glucosidase, tyrosinase, and xanthine oxidase. Therefore, the ethyl acetate extract was further purified by MPLC and HPLC to obtain the pure compounds. Pure isolates were characterized as flavonoids and triterpenoids using NMR and mass spectrometry. The isolation is still ongoing and the resulting compounds will be evaluated for their alpha-glucosidase inhibitory activity. We are also continuing to use a Lab-developed UHPLC- MS/MS methods (Chen et al., 2010) employed to find additional α-glucosidase inhibitors in lotus seedpods.

Keywords: *Nelumbo nucifera* Gaertn, alpha-glucosidase, HPLC

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S2.P14 *Fragaria x ananassa* Duch aerial by-product activity against *Candida albicans*

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A *Fragaria x ananassa* Duch. is a plant native to the temperate zones of the Americas and Europe. Due to the presence of polyphenols, this plant species has antioxidant activity, with antifungal potential which the mechanisms of action involve plasma membrane and inhibition of protein synthesis disruption (Aboody & Mickymaray, 2020). The pseudofruit, popularly known as strawberry, has high benefit, with a 30% increase in global production in the last 10 years (FAOSTAT, 2024). However, approximately 7 to 20% of the production is by-product (Villamil- Galindo et al., 2022). Seeking to reduce by-product disposal with benefit, prompted this work to evaluate Fragaria's production residue for antifungal activity. An extraction pf the aerial plant parts with 70% ethanol with 0.3% citric acid provided the sample tested against strains of *Candida albicans* (ATCC 90028 and CBS 562), to determine Minimum Inhibitory Concentration (MIC) and Minimum Fungicide Concentration (MFC). The extract gave MIC of 27.9 ug/mL and 55.76 ug/mL for the *C. albicans* strains ATCC 90028 and CBS 562, respectively, and MFC values of 55.8 ug/mL for ATCC 90028, showing a moderate and strong cytostatic and cytocidal activity for this strain. The by-product's extract provided high antimicrobial activity with potential as an alternative for the treatment of candidiasis, a worldwide and recurrent disease in immunosuppressed patients. (Seleem et al., 2017).

Keywords: *Fragaria x ananassa* Duch., phenolic compounds, antifungal activity, *Candida albicans*, *by-product*

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S2.P15 Integrated *in vitro* and *in vivo* antilithiatic efficacy of a synergistic herbal formulation, URO-5 and exploration of *in silico* guided mechanism of action

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Urolithiasis, a metabolic condition caused by deposition of calculi in urinary tract has high prevalence and recurrence worldwide (Rule, 2020). In more than 60% of the clinical cases, kidney stones are made up of calcium oxalates (CaOx) and the existing treatment has limitation of side effects due to long and repeated use (Chattaraj, 2023). Therefore, a clinically validated, safe and efficacious synergistic herbal formulation (URO-5) was developed, which can be used as an adjuvant therapy. URO-5 contains five Indian medicinal plants having folklore use in urinary disease. The antilithiatic efficacy of LC-MS standardized aqueous extract was analysed, along with *in silico* guided mechanism of action. *In vitro* studies showed, that URO-5 drastically reduces the number of crystals and alters their morphology for easy passage. The inhibition (IC_{50}) of crystal nucleation and aggregation was at low concentration of 4.51 and 11.17 mg/mL. The *in vivo* activity (500 mg/kg) reveals that efflux of calcium oxalate (CaOx) stones were significant, > 80%. The decrease in tissue calcium and increase in urine calcium was also significant ($p>0.05$), at 15.14 and 74.08 µg/mg. Interestingly, URO-5 also repairs the tissue damage caused by CaOx stones. *In silico* molecular docking suggest that antilithiatic activity of URO-5 was mediated *via* calcium and oxalate dependent, and antioxidant dependent pathways (Chattaraj, 2023). The safety and toxicity profile were documented under GLP compliance and no abnormality was recorded. Double-blind, placebo-controlled, single centred clinical study advocates that URO-5 is an efficacious alternative and can be used as an adjuvant therapy in urolithiasis.

Keywords: Urolithiasis, calcium oxalate crystals, adjuvant therapy, LC-MS, molecular docking

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S2.P16 Ashwagandha improves cognition and attenuates depressive-like behavior in an Alzheimer's disease mouse model

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Withania somnifera (WS), also known as Ashwagandha, is a traditional Ayurvedic herb popularly used as a dietary supplement for effects on memory, stress, anxiety, depression and insomnia. Cognitive impairment and depression are common symptoms of Alzheimer's disease (AD), suggesting that WS may be of therapeutic value in that condition. Previous studies from our group with *Drosophila* have shown that an aqueous extract of WS root (WSAq) improves performance in a phototaxis assay and alleviates depressive symptoms. Here we sought to evaluate the effects of WSAq in a mammalian system using the 5xFAD mouse model of β-amyloid accumulation. Six- to seven-month-old male and female 5xFAD mice were treated with WSAq in their drinking water at either 0, 0.5 or 2.5 mg/mL for 4 weeks. Age-matched non-transgenic wild type littermates that received no WSAq were also included as a control group. In the fourth week of treatment mice underwent behavioral testing. The higher dose of WSAq (2.5 mg/mL) improved spatial memory and attenuated depressive-like symptoms in the 5xFAD mice. Analysis of plaque pathology as well as markers of neuroinflammation and oxidative stress is ongoing to gain mechanistic information on how WSAq could be eliciting these beneficial effects. Although future studies are needed to confirm these results in other models, these initial results suggest that WSAq does have potential as a therapeutic option for use in AD.

This work was supported by a pilot grant from the Oregon Partnership for Alzheimer's Research and the BENFRA center U19 grant U19AT010829 funded by NIH/NCCIH, USA.

Keywords: Ashwagandha, Alzheimer's disease, cognitive function, depression

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S2.P17 Chemical structure and bioactivities of a sulfated polysaccharide from precious medicinal fungi

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Antrodia cinnamomea Chang & Chou, a valuable medicinal and edible fungi, demonstrated hepatoprotective, immunomodulative, anti-tumor, anti-inflammatory and anti-oxidative activities (Xie et al., 2022). Sulfated polysaccharides (SPSs) are polysaccharides with hemi-ester sulfate groups employed to maintain the integrity of the structural skeleton of fungi, marine invertebrate connective tissues, algae and bacteria. A SPS, **3-SS**, was isolated from *A. cinnamomea*. Its chemical structure was identified by utilizing 1D and 2D NMR techniques. The anti-inflammation effects of **3-SS** on RAW264.7 macrophage cells, such as IL-6 inhibition, restoration of LPS-induced IκB protein degradation, and inhibited LPS-induced TGFRII protein degradation, were confirmed to occur via AKT, ERK1/2, and p-38. In addition, **3-SS** impaired the proliferation of H1975 lung cancer cells through EGFR/ERK/slug signaling.

Keywords: medicinal fungi, sulfated polysaccharides, structural elucidation, anti-inflammation, anti-proliferation

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S2.P18 Unlocking the therapeutic potential: Comparative analysis of tormentic acid and its ester in combating inflammation and oxidative stress

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This poster illustrates the anti-inflammatory and antioxidant properties of the pentacyclic triterpene tormentic acid (**1**) and its ester tormentic acid 3-O-p-coumaroyl ester (**2**). **1** has been shown to exhibit promising therapeutic potential, but **2** remains underexplored (Olech et al., 2021). By comparative analysis, we aim to elucidate how esterification affects the biological efficacy of such pentacyclic triterpenes. Using diverse *in-vitro* assays across various cell lines, we assessed **1** and **2**'s ability to mitigate oxidative stress by scavenging reactive oxygen and nitrogen species. Additionally, we investigated their capacity to modulate mRNA expression and protein production of pro-inflammatory cytokines upon lipopolysaccharide (LPS) challenge. Our findings revealed that **2** significantly surpassed **1** in effectively reducing levels of reactive oxygen species and nitric oxide. Moreover, we presented an extensive analysis of protein expression levels of over 100 cytokines and chemokines in THP-1 macrophages post-LPS stimulation. Addition of **1** or **2** to the cells resulted in different protein expression patterns, with **2** significantly reducing the levels of several pro-inflammatory cytokines involved in NF-κB and Prostaglandin E2 signaling (e.g., TNFα, IL-8, IL-17A and IL-2), thereby counteracting cytokine storms. These results suggest that the esterified form **2** exerts superior biological activity compared to **1**, potentially through its impact on NF-κB signaling. Future experiments will focus on analyzing their effects on TLR4 and downstream signaling pathways to further explore this hypothesis. In conclusion, elucidating the differential effects of esterification on the biological activity of pentacyclic triterpenes opens new avenues for developing effective and natural anti-inflammatory and antioxidant therapies.

Keywords: pentacyclic triterpene, esterification, tormentic acid, antioxidant, anti-inflammatory

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S2.P19 Anti-diarrheal activity of *Psidium guajava* extract and partitions in *Drosophila melanogaster*

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Diarrheal diseases are among the most common causes of death, especially in developing countries (Ugboko et al., 2020). In traditional medicine from many tropical countries, *Psidium guajava* leaves represent one of the most frequently employed plant species to treat diarrhea, however no specific compounds have been described so far. Indeed, existing anti-diarrheal models are mainly murine models requiring large concentrations of plant materials and significant quantities of mice, thus hindering a bio-guided fractionation of the molecules involved. In this study, we used a genetically modified strain of *Drosophila melanogaster* for isolating antidiarrheal compounds from guava by bio-guided fractionation (Liu and Chassagne, 2023). Previously, it was shown that the Drosophila gene *Ion transport peptide* (*ITP*) is an important endocrine regulator of water intake and excretion. With *ITP* knockdown, the frequency of defecation events is increased and the transit through the digestive tract is faster, and so results in a phenotype resembling human diarrhea (Gáliková et al., 2018). In our experiments, antidiarrheal activity was assessed using this *D. melanogaster* strain by detecting various parameters of fecal deposits (number of fecal deposits, total area of deposits, total material excreted (total IOD)). The test groups, with 10 replicates and 6 flies per replicate, received 1g/100 mL ethanol extracts while the positive control group received racecadotril. Each ethanolic extract of guava collected in New Caledonia (NC), French Polynesia (FP) and Benin (B) significantly reduced the number of fecal deposits (reduction of 61.0% (NC), 60.9% (FP), 57.1% (B)), total area of deposits (reduction of 74.2% (NC), 67.0% (FP), 65.7% (B)), and total IOD (reduction of 77.3% (NC), 63.3% (FP), 71.1% (B)), compared to the group administrated with normal food. We are working on confirming the antidiarrheal activities of the ethanolic extract of *P. guajava* using *Drosophila* with other methods, as well as identifying the bioactive component.

Keywords: *Psidium guajava, Drosophila melanogaster*, antidiarrheal, fecal deposits

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S2.P20 The influence of growth conditions on the amount of active substances in *Mentha* x *piperita* L.

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Mentha x *piperita* L. commonly known as peppermint, is a hybrid resulting from a cross between *Mentha spicata* L. (spearmint) and *Mentha aquatica* L. (watermint). Peppermint is an herbaceous rhizomatous perennial plant, native to Lithuania as well as in Europe and the Middle East which can be found wild with its parent species, typically in moist habitats. In this study was determine dependent of total phenolic, flavonoid and extractives content in extracts of Mentha x piperita L. on soil pH, humus, total nitrogen (Ntotal), plant – available/mobile phosphorus (P2O5) as well as potassium (K2O), total calcium (Ca), magnesium (Mg) and sodium (Na) concentration. Experimental fields were formed in four locations in Lithuania. Soil and perennial peppermint samples were taken at three sites of each experimental field. A total 12 samples of soil and 12 samples of tested plant were collected and analysed. The concentration bioactive compounds in plants are significantly ($P<0.05$-0.01) affected by some chemical properties of the soil: the total phenolic content reliably correlated with soil pH, Ntotal, Ca, Mg and P2O5; the flavonoid content – with soil P2O5, K2O, Mg and Na; the extractives content – with soil humus, Ntotal and Na. The obtained results are useful in the development for commercial cultivation of peppermint

Keywords: *Mentha* x *piperita* L., total phenols, flavonoids, extractives, soil chemical properties

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S2.P21 Inhalation - A promising administration route for anti-infectives from *Morus alba* root bark?

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Mulberry Diels-Alder type adducts (MDAAs) isolated from *Morus alba* root bark were identified as dual antiviral and antibacterial agents *in vitro* [Grienke et al., 2016]. However, no significant efficacy was observed for oral administration in an *in vivo* influenza A virus infection model [Langeder et al., 2023a]. Hence, the aim of this study was to assess the suitability of inhalation administration for this compound class. Two MDAA-containing extracts (MA21 and MA60, [Langeder at al., 2023b]) and their isolated MDAA stereoisomers, sanggenons C and D, were investigated for (i) cytotoxicity and (ii) permeability using the lung epithelium cell line, Calu-3, (iii) pH-dependent solubility, (iv) logP and pKa, and (v) plasma protein binding. Both extracts and pure compounds were well tolerated by Calu-3 cells up to ~800 µg/mL and ~170 µM. The apparent permeability (P_{app}) values were low (0.07–0.26 $x10^{-6}$ $cm.sec^{-1}$) compared to the control compound, metoprolol (10.3 $x10^{-6}$ $cm.sec^{-1}$ [Bosquillon et al., 2017]) which indicates the compounds could be retained in the lung following inhalation. Despite their structural similarity, sanggenon D showed a tenfold higher solubility compared to sanggenon C at a pH range of 1.2–7.4. Similarly, logP of sanggenon D was one log unit lower than sanggenon C (1.90 vs. 2.91). This study suggests that the purified sanggenons exhibit more favorable properties for pharmaceutical development compared to extracts. The low P_{app} values may indicate that membrane permeability is low, explaining the lack of efficacy in oral administration models. However, inhalation administration could be promising for treating respiratory infections.

Funding: FWF project P 35115

Keywords: *Morus alba,* Moraceae, inhalation, infections, permeability

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S2.P22 Reporting of medical and health research - An assessment of studies on medicinal plant extracts and herbal medicines registered on PROSPERO

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Studies evaluating the pharmacological effects, (clinical) efficacy or toxicity of medicinal plant extracts are constantly increasing, but the reporting quality and reproducibility of these studies remain unsatisfactory. The Consensus-based guidelines for the Phytochemical characterization of Medicinal Plant extracts (ConPhyMP), with the Open access ConPhyMP tool (Heinrich et al., 2022; Heinrich and Jalil, 2023), facilitate transparent reporting, reproducibility, and interpretations of studies. PROSPERO is an international prospective registry for systematic reviews with a health- related outcome, reducing duplication and reporting bias in studies. Using adapted ConPhyMP guidelines, we assess the reporting quality of 1727 registered studies involving medicinal plant extracts/herbal medicines on PROSPERO from inception to January 2024 (Fig. 1). Out of 65 studies included in our detailed analysis, only five report the minimum requirements (i.e., adhering to ConPhyMP guidelines), including reporting of starting materials, conducting, and reporting of phytochemical analysis of plant extracts/herbal medicines under investigation. Within 3-5 years of registration on PROSPERO, 83% of the studies remain under review, 13% are discontinued reviews, and only 4% reach publications. For specific medicinal plants, we found that *Cannabis sativa* L., *Curcuma longa* L., *Zingiber officinale* Roscoe, *Panax ginseng* C.A.Mey., and *Gingko biloba* L. have the highest registered studies on PROSPERO (n= 952, 362, 210, 176, 92, respectively), with n= 92, 32, 17, 2, 4, respectively reaching publications. There is a need for assessment and scrutiny for the registration of health-related research involving plant extracts/herbal medicines, especially for those with clinical relevance or generation of evidence- based information, which could enhance the study publications.

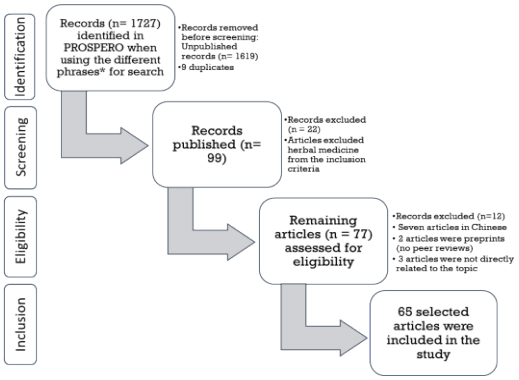

Fig. 1. Workflow chart for inclusion and exclusion of studies in the systematic review using PRISMA model (Page et al., 2021).

Keywords: PROSPERO, ConPhyMP, medicinal plant extracts, study registration, medical and health research

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S2.P23 Anti-inflammatory and antioxidant effects of *Marrubium vulgare* L. herb extracts in human immune cells and blood plasma

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Marrubium vulgare (L.) herb has been valued in traditional medicine in many regions as a cardiotonic, vasoprotective and hypotensive remedy. The monograph of *Marrubii herba* is included in the European Pharmacopeia. However, the herb is rarely used in phytotherapy, mainly due to incomplete data on its pharmacological profile in the context of cardiovascular diseases (CVDs) (Teixidor-Toneu et al., 2016; Aćimović et al., 2020). This study focused on the impact of different *Marrubii herbae* extracts (characterized by LC-MS/MS) and their active components (marrubiin, verbascoside, apigenin glucoside) on the functions of neutrophils and PBMCs. Dysregulation of immune cells is largely associated with the development of CVDs. Hence, the effect on the release of reactive oxygen species (ROS), pro-inflammatory cytokines (IL-2, IL-8, TNF-α) and enzymes (ELA-2, MMP-9) in stimulated immune cells was measured. Moreover, the influence of extracts/active compounds on human fibrinogen and other plasma components under ($ONOO^-$)- induced oxidative stress and on the expression of neutrophil adhesion molecules was evaluated. The biological activity tests were performed using immunoenzymatic, spectrophoto/fluorometric, chemiluminescence, cytometric, SDS-PAGE, and western blot methods. As a result, the tested extracts/active compounds (5-100 μg/mL, 5-50 μM) were proved to significantly decrease the levels of ROS, TNF-α, IL-2, MMP-9, some biomarkers of lipid peroxidation and protein nitration, and to protect fibrinogen against oxidative/nitrative changes in its structure and function. The results might partly support the traditional use of the plant material in CVDs and encourage further mechanistic and in vivo studies in that matter.

Keywords: *Marrubium vulgare,* immune cells, fibrinogen, human plasma

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S2.P24 Antioxidant, anti-diabetic and anti-inflammatory potential of *Sorbus commixta* (Rosaceae) leaves in cellular, cell-free and human plasma models

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Sorbus commixta Hedl. (Japanese rowan) has a long tradition of use as a herbal remedy (leaves, fruits, stem, bark) for atherosclerosis and liver or respiratory disorders (Sołtys et al., 2020). Considering the previous reports on the biological properties of *S. commixta* leaves (mainly antioxidant), as well as the available pharmacological data on anti-diabetic and anti-inflammatory potential of *S. commixta* bark and fruits, and the leaves of other *Sorbus* species (Matczak et al., 2018; Sołtys et al., 2020), we decided to evaluate these three activity directions for *S. commixta* leaves in cellular, cell-free and human plasma models (Fig. 1). As a result, we demonstrated for the first time the potent protective effects of the *S. commixta* leaf extracts against nitration and oxidation of plasma proteins and lipids under $ONOO^-$-induced oxidative stress ex vivo; the ability to inhibit the formation of advanced glycation end products and α-amylase and α-glucosidase activity in vitro; and the potential to reduce the secretion of pro-inflammatory cytokines (TNF-α, IL-1β, IL-2, IL-6) in the LPS-stimulated PBMC cells in vitro. Moreover, the phytochemical profiling of the tested extracts (qualitative and quantitative UHPLC-PDA-ESI-MS^3 and HPLC-PDA analyses) revealed the presence of seventy-eight phenolic compounds (in comparison to only 13 phenolics reported previously). Furthermore, the contribution of individual polyphenols to the tested activity was verified with chemometric tools (PCA analysis).

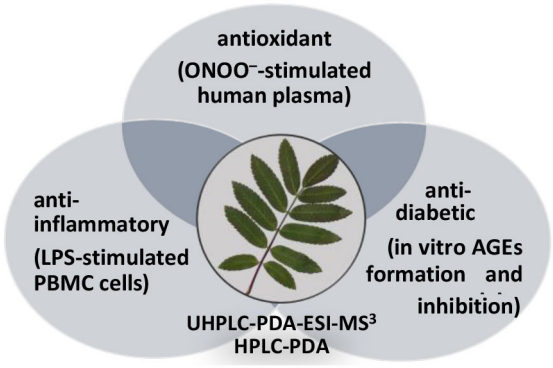

Fig. 1. Study design

Keywords: *Sorbus commixta,* leaves, antioxidant, anti-diabetic, anti-inflammatory

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S2.P25 *In vitro* antioxidant capacity screening of *Piper auritum extracts*

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Oxidative stress results from the production of unregulated reactive oxygen species (ROS). When low antioxidant levels and low regenerative capacity occur, tissue damage is produced. It has been reported that oxidative stress contributes to the progression of several diseases. Antioxidants are molecules that can donate electrons to free radicals without making them unstable; this causes the free radicals to stabilize and become less reactive, preventing, and repairing damage caused by ROS/RNS. *Piper auritum* is used to treat different ailments. To determine the antioxidant capacity *in vitro* of *Piper auritum* leaf extracts using different solvents. Aqueous extract (AQEPA), methanol extract (MEPA), ethanol extract (EEPA), acetone extract (AEPA), chloroform extract (CEPA), and hexane extract (HEPA) were obtained from *Piper auritum* leaves. The antioxidant activity *in vitro* was evaluated using the 1-1-dyphenyl-2-picrylhydrazyl (DPPH) method (Bonet and Brand- Williams, 1995), statistical ANOVA, and Tukey post hoc analysis was performed; results are presented as EC50, TEC50, and AE (IC50 x TEC50). The antiradical efficiency (AE) obtained was AQEPA:1051, MEPA:412.5, EEPA:19.74, AEPA:26.52, CEPA:33.6, and HEPA:3.71. The high AE obtained is due to the presence of anthocyanins, tannins, terpenoids, phenols, polyphenols, and flavonoids in aqueous ethanol and methanol. In acetone, phenol and flavonols are dissolved, while chloroform and hexane contain terpenoids, flavonoids, and coumarins. The presence of these secondary metabolites in the different solvents used explained the high antioxidant capacity obtained. *Piper auritum* extracts have high *in vitro* antioxidant capacity.

Keywords: *Piper auritum*, antioxidant capacity, solvents

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S2.P26 Chemical constituents and antioxidant properties of *Verbesina montanoifolia* leaf extracts

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Reactive oxygen species (ROS) are products of normal cellular metabolism, they can damage lipids, DNA, RNA, and proteins, which, in theory, contribute to the physiology of aging. Oxidative stress may play a significant role in the development of different diseases such as cancer, diabetes, cardiovascular conditions like high blood pressure, atherosclerosis and stroke, inflammatory disorders, and chronic fatigue syndrome, among others. *Verbesina montanoifolia* (VM), a shrub native to Mexico, can measure up to 2 meters in height with elongated dark green leaves and yellow flowers. Is used in Mexican traditional medicine to treat different illnesses. However, there are no reports worldwide of the components or their antioxidant capacity, so this was the aim of the present work. An aqueous extract of *Verbesina montanoifolia* leaves was obtained. The total content of phenols, polyphenols, and flavonoids was quantified. The *in vitro* antioxidant capacity was determined using the compound DPPH (1,1-diphenyl-2-picrylhydrazyl) at different times. A gas chromatography-mass spectrometry on Agilent 7000C Triple Quad w/ 7890B GC/MS/MS system with an Agilent J&W GC Column was performed to identify the components. VM extracts contained high levels of flavonoids, phenols, and polyphenols that presented antioxidant effects *in vitro.* In an HPLC analysis, the main compounds were vanillic acid, hydroxybenzoic acid, and Gallic acid. These three compounds are known for their antioxidant properties. To the best of our knowledge, this is the first study to report the chemical constituents of VMAQE with antioxidant properties.

Keywords: reactive oxygen species, oxidative stress, antioxidants, flavonoids, polyphenols

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S2.P27 Isolation and characterization of α-glucosidase inhibitors from *Ziziphora tenuior* using high- performance counter-current chromatography

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Diabetes Mellitus (DM) is a common chronic disease characterized by hyperglycemia, causing different complications. One successful approach to managing DM is to reduce postprandial hyperglycemia by inhibiting carbohydrate enzymes, such as α-glucosidase. Efforts have been made in recent years to find potent α-glucosidase inhibitors derived from medicinal plants (Phukhatmuen et al., 2020). *Ziziphora* spices from the Lamiaceae family are traditionally used for the treatment of DM (Mohammadhosseini, 2017). In this study, *Ziziphora tenuior* (L.) (Kakuti in Persian), with small leaves and pink-purple cluster flowers, was investigated for α-glucosidase inhibitory activity. The whole air-dried plant powder was extracted stepwise by hexane, EtOAc and MeOH and the extracts were evaluated for α-glucosidase inhibitory effects. The selected methanol extract was fractionated by vacuum liquid chromatography. The sub-fractions were subjected to high- performance counter-current chromatography (HPCCC) obtaining five pure compounds. Their structures were elucidated as pinocembrin-7-*O*-rutinoside (**1**), rosmarinic acid (**2**), pinocembrin (**3**), narirutin (**4**) and chlorogenic acid (**5**) using NMR spectroscopy and mass spectrometry. All the pure compounds were examined for α-glucosidase inhibitory effect (*in-vitro* anti-type 2 diabetic assay). Compound **3** exhibited the best enzyme inhibition among the others with an IC_{50} value of 3.8 ± 0.1 μM. The findings indicate that *Ziziphora tenuior* has the potential for diabetic treatment.

Keywords: anti-diabetic, α-glucosidase inhibitory activity, *Ziziphora tenuior*, HPCCC, pinocembrin

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S2.P28 Triterpenoids and flavonoids of Corncockle – a (‘n almost) forgotten weed and the (future) medicinal herb

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The botanical family *Caryophyllaceae* (pink or carnation family) comprises herbaceous species with cosmopolitan distribution and characteristic morphology. With respect to phytochemistry, *Caryophyllaceae* typically contain triterpenoid saponins and several species have been used to obtain surfactants for washing, cosmetics, food industry (halva) and in pharmaceutical technology. Some other species have been considered medicinal and some as poisonous. *Agrostemma githago* L was once a noxious weed in cereal fields but the agriculture modernization led to its eradication. Now, this plant is rare and survived mainly in fringe habitats and as ornamental. However, it also has a history as medicinal herb a few centuries ago but nowadays it is not used. Several saponins have been recently isolated with important pharmacokinetic properties (Clochard et al. 2020). A presence of orientin has been also reported in the past (Jakimiuk et al. 2022). Here, we used an LC- High-Resolution-Mass Spectrometry approach to contribute to *A. githago* phytochemistry and were able to detect 24 triterpenoid glycosides (mono- and bidesmosides with linear or branched gycosylation) where sapogenins were built of gypsogenin, quillajic acid, oleanolic acid and hydroxyoleanolic acid. In the aerial parts, the predominant compounds were orientin and isoorientin, along with 6 other flavone-*C*-glycosides, suggesting that this fast growing, high- biomass herb can provide a feasible, sustainable source of highly bioactive polyphenols, in addition to previously isolated saponins and ribosome-inactivating proteins.

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Keywords: *Agrostemma githago,* Caryophyllaceae, triterpene saponins, flavonoid C-glycosides

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S2.P29 Therapeutic effects of Menthacarin® and its individual components peppermint oil and caraway oil in an experimental colitis model

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Menthacarin® is a proprietary combination of peppermint oil (90 mg WS® 1340) and caraway oil (50 mg WS® 1520) of specified quality that is clinically used for the treatment of functional gastrointestinal disorders. Further, Menthacarin® has demonstrated to attenuate experimental colitis via anti-inflammatory effects (Alliger K. et al., 2020). However, its effect on the colonic mucus- producing cell loss, contributing to intestinal barrier dysfunction and thereby promoting intestinal inflammation, is unclear. Further, the relative contribution of the individual oils in the experimental colitis model is still not known. Therefore, mice with 3 % dextran sulfate sodium (DSS)-induced colitis were treated orally with Menthacarin® (30/60/90 mg/kg), peppermint oil (20/40/80 mg/kg), or caraway oil (10/20/40 mg/kg) for one week. Colonic tissue destruction and mucus area from Menthacarin®-treated mice were assessed in formalin-fixed paraffin-embedded Swiss rolls by Hematoxylin-Eosin and Alcian Blue periodic acid-Schiff (AB-PAS) staining, respectively. In line with a reduced body weight loss and colon length shortening, Menthacarin® (30 mg/kg) attenuated DSS-mediated mucosal destruction accompanied by an increased AB-PAS-positive area. Peppermint oil and caraway oil at the lowest dose had an ameliorative effect as determined by a reduction in body weight loss and colon length shortening. In conclusion, this data indicates that Menthacarin® promotes intestinal barrier function and shows for the first time that both, peppermint oil and caraway oil, contribute to the attenuation of experimental colitis.

Keywords: Menthacarin, peppermint, caraway, colitis, mucus

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S2.P30 *Bryophyllum pinnatum* inhibits oxytocin and vasopressin signalling in myometrial cells

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The medicinal plant *Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae) is used in the treatment of preterm contractions and was previously shown to block oxytocin (OT)-induced signals in myometrial cells, consistent with its tocolytic effect observed in patients (Plangger et al., 2006; Santos et al., 2021). Since OT activates OT receptors and V1a receptors, both expressed in the myometrium (Åkerlund et al., 1999), we aimed to study the molecular pharmacology of *B. pinnatum* press juice (BPJ) using specific receptor ligands, the human myometrial cell line hTERT- C3, and cell lines expressing recombinant human OT and V1a receptors in a fluorescent calcium experiment. We found that BPJ inhibits both OT- and vasopressin (AVP)-induced intracellular calcium ($[Ca^{2+}]_i$) increase in hTERT-C3 myometrial cells. In the same functional assay, BPJ also inhibited signals mediated by recombinant human OT and V1a receptor with a similar potency (IC_{50} about 0.5 mg/mL). We further studied the details of endogenous OT- and AVP-sensitive receptors in hTERT-C3 cells and found that OT and AVP stimulated those receptors with similar potency (EC_{50} of ~1 nM), suggesting expression of both receptor subtypes. This interpretation was corroborated by the observed antagonist potencies of atosiban and relcovaptan. However, using qPCR, we almost exclusively found expression of OT receptors suggesting a pharmacological difference between recombinant OT receptors and those expressed in hTERT-C3 cells. In conclusion, *B. pinnatum* inhibits both, OT and AVP signalling, which may point beyond its tocolytic effects to other indications involving a disbalance in the vasopressinergic system.

Keywords: *Bryophyllum pinnatum*, oxytocin, vasopressin, myometrium

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S2.P31 Didesmethylrocaglamide Cytotoxic Activity in High Grade Serous Ovarian Cancer in Preclinical *In Vitro* and *In Vivo* Models

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Didesmethylrocaglamide (DDR) (**1**) is a naturally occurring derivative of rocaglamide with potent antitumor activity isolated from *Aglaia* plant species (Fig. 1) (Chang et al., 2020). DDR displays nanomolar cytotoxic activity in high grade serous ovarian cancer (HGSOC) cell lines including OVCAR8. HGSOC is the most lethal gynecological cause of death in women and requires new treatments to help tackle chemoresistance (Feng et al., 2021). Rocaglamides are known to function as translation inhibitors and trigger apoptosis in other types of solid tumors. We found that racemic **1** induced cytotoxicity in ovarian cancer cell lines as early as 24 hours and activated caspase-3 indicating pro-apoptotic activity. In addition, **1** was cytotoxic for the PEO4 and MCF7ADR cell lines that are resistant to cisplatin and paclitaxel, respectively. In addition, we evaluated **1** in OVCAR8 xenografts and observed a reduction in tumor burden. We hypothesized that combinatorial treatments of **1** with autophagy inhibitors like phyllanthusmin-34 would enhance cell death by blocking translation and the ability to recycle amino acids for protein production through autophagy (Salvi et al., 2022). The data obtained indicates that blocking autophagy or DNA repair in combination with **1** increased cell death in the sensitive and resistant models. Its effective cytotoxicity in drug sensitive and resistant cell models suggests **1** has potential as a new therapeutic strategy against HGSOC.

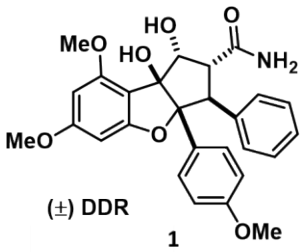

Fig. 1. Structure of (±)-didesmethylrocaglamide (**1**)

Keywords: didesmethylrocaglamide, ovarian cancer, HGSOC, cytotoxicity, chemoresistance

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S2.P32 Isobavachalcone attenuates TNF-α-induced vascular inflammation in human umbilical vein endothelial cells

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Isobavachalcone (Fig. 1) is an active molecule present in the medicinal plant *Psoralea corylifolia* Linn. and has been reported to have antioxidant, anti-inflammatory (Gao et al., 2019), antibacterial (Wang et al., 2021) and anticancer activities (He et al., 2021). However, to our knowledge, studies on the protective effects of isobavachalcone on vascular inflammation have not been reported. Vascular inflammation activated by pro-inflammatory cytokines is an inflammatory response that occurs in the early stages of atherosclerosis. Endothelial dysfunction in vascular inflammation begins with the expression of cell surface adhesion molecules by pro-inflammatory cytokines. In this study, we investigated the effects of isobavachalcone on inflammatory responses in vascular inflammation induced by the tumor necrosis factor-α (TNF-α) in human umbilical vein endothelial cells (HUVECs). TNF-α is critical for increasing the expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in endothelial cells, and activates macrophages and neutrophils. Isobavachalcone decreased the expression of TNF-α-induced ICAM-1 and VCAM-1 in HUVECs, and the adhesion of monocytes to the vascular endothelium and the adhesion of monocytes to the vascular endothelium. In addition, isobavachalcone decreased the phosphorylation of the NF-κB (necrosis factor κB) p65 subunit. The result of this study demonstrate that isobavachalcone could prevents TNF-α-induced vascular inflammation and has the potential to protect against the early progression of atherosclerosis

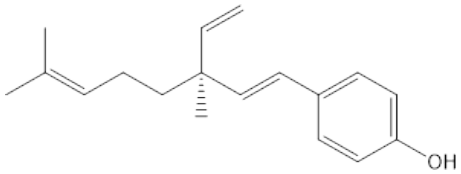

Fig. 1. Structure of Isobavachalcone

Keywords: isobavachalcone, vascular inflammation, vascular cell adhesion molecule 1 (VCAM-1), induced intercellular adhesion molecule 1 (ICAM-1), NF-κB

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S2.P33 Synergistic therapeutic potential of quercetin, curcumin, and berberine combination in triple negative breast cancer: insights from *in vitro* models

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In this study, we explored the therapeutic potential of QUE, CUR, and BBR in *in vitro* TNBC models. Our hypothesis posited that combining these polyphenols might enhance their efficacy in TNBCs compared to individual treatments. Using MDA-MB-468 and MDA-MB-231 as TNBC cell line models, we assessed the impact of individual phytochemicals and their combined treatment ($COM^{QUE+CUR+BBR}$) on Epithelial Mesenchymal Transition (EMT), migration potential, cancer stem cells (CSCs), clonogenic/3D matrigel growth, and drug resistance in TNBC cells. Our results revealed that the combination treatment ($COM^{QUE+CUR+BBR}$) exerted significant and synergistic effects on EMT proteins and cell migration, surpassing the impact of individual treatments. Furthermore, $COMQ^{UE+CUR+BBR}$ treatment significantly reduced the $CD44^{+}/CD24^{-}$ population, clonogenic potential, and inherent cisplatin resistance in TNBC cells. Notably, all combination experiments utilized 1/10th of the respective IC_{50} concentrations, highlighting the robust efficacy of these phytochemicals in TNBC models. These findings represent the first evidence that the combination of QUE, CUR, and BBR holds promise for TNBC treatment, showcasing its anti- proliferative, EMT-inhibitory, and antagonistic cancer stemness functions.

Keywords: breast cancer, TNBC, quercetin, curcumin, berberine, EMT

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S2.P34 Quercetin aims at the HuR-signaling axis to impede the migration of Triple Negative Breast Cancer (TNBC): Association with metabolic processes

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In our present research, our focus was on evaluating the influence of a natural compound, specifically Quercetin, a flavonoid, on the proliferation and migration of Triple Negative Breast Cancers (TNBCs). Our particular interest centered around examining the effects of quercetin on the RNA binding protein HuR, given previous indications that quercetin can disrupt HuR-mRNA interactions. Utilizing online meta-analysis, we delved into the role of HuR in TNBC datasets. Subsequently, we validated HuR protein expression in TNBC cell lines, specifically MDA-MB-231 and MDA-MB-468. We determined the IC_{50} of quercetin in TNBC cell lines through MTT assays and assessed protein expression using western blotting. Additionally, we investigated cell migration and colony-forming potential in TNBC cells treated with quercetin. Our findings highlighted that HuR overexpression correlated with reduced overall survival in TNBC patients. Correspondingly, both TNBC cell lines exhibited heightened expression of HuR proteins. Quercetin treatment significantly suppressed HuR protein expression in TNBC cells. Further analysis revealed an active HuR-PFKP-LDHA signaling pathway in TNBC cells, and inhibiting this pathway led to reduced migration and clonogenic potential in TNBC cells. In summary, our research demonstrated that an elevated HuR-PFKP-LDHA axis is associated with TNBC progression and recurrence. The ability of quercetin to inhibit these proteins suggests its potential as an anticancer agent for treating aggressive TNBCs.

Keywords: breast cancer, TNBC, HuR, lactate, cell migration

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S2.P35 Biological activities of the polar and nonpolar extracts of Philippine Bamboo species

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Ethanol (E) and hexane (H) extracts of the shoots and outer covering (C) of three (3) bamboo species namely, *Dendrocalamus asper* (Da), *Bambusa blumeana* (Bb), and *Gigantochloa levis* (Gl) were subjected to an array of tests to determine their antibacterial, antidiabetic, anti-inflammatory, antioxidant, and angiotensin-converting enzyme (ACE) inhibition activity. Among the outer covering extracts, GlCE and BbCH exhibited the lowest MIC for both *Bacillus subtilis* and *Pseudomonas aeruginosa* at 0.780 µg/mL. At 800 µg/mL, DaE inhibited alpha amylase activity at

82.86 % followed by GlE and BbE with 79.31 and 75.95% inhibition, respectively. GlE exhibited the lowest IC_{50} (i. e. 2.97 µg/mL), whereas IC_{50} values for DaE and BbE are 3.03 and 3.13 µg/mL, respectively. The results show that these bamboo extracts have antidiabetic potential. Extracts from the bamboo shoots outer covering did not exhibit anti-inflammatory activity based on the standard 50% inhibition activity limit using 15-LOX inhibition assay. The highest % inhibition was that of DaCE (7.75%) followed by GlCE (6.19%) then BbCE (5.54%) at a concentration of 33.33 µg/mL. DPPH assay resulted to GlCH exhibiting the highest % Radical Scavenging Activity (RSA, 65.96%) followed by GlE at 58.13%. BbE, exhibited the lowest EC_{50} at 92.53 µg/mL. GlCH, which exhibited the highest % RSA, is the most potent antioxidant among the samples with EC_{50} value of 82.85 µg/mL. GlCE and BbCH show remarkable ACE inhibition activity with values 0.00044 and 0.00282 nmol/min, respectively.

Keywords: bamboo shoots, bamboo shoot covering, *Dendrocalamus asper, Bambusa blumeana, Gigantochloa levis*

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S2.P36 Terpenoids and polyphenols from *Carpesium cernuum* (Asteraceae) of European origin

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Carpesium cernuum L., the most widespread species of the genus *Carpesium* (Inuleae-Inulinae, Asteraceae), in the Far East and India found use as a folk remedy and as a food plant. To the best of our knowledge, the plant has not been medicinally used in Europe. Phytochemical examination of the plant material of European origin (Romania), cultivated in the Garden of Medicinal Plants, Maj Institute of Pharmacology PAS in Kraków, led to the isolation of a series of known monoterpenoid thymol derivatives and a new dihydrobenzofuran **1**, an analog of eupatobenzofuran (Chen et al., 2011), from the roots of plants. Aerial parts of the plants were rich in germacranolides with 4β,8α- dihydroxy-5β-isobutyryloxy-9β-(3-methylbutyryloxy)-3-oxo-germacran-7β,12α-olide, a sesquiterpene lactone of cytotoxic activity (Kłeczek et al., 2021), as one of the major constituents. An UHPLC-PAD MS^n analysis of hydroalcoholic extracts from the roots and aerial parts of *C. cernuum* revealed the presence of numerous caffeic acid conjugates (mainly caffeoylquinic and caffeoylhexaric acids) formerly unknown as constituents of *C. cernuum*.

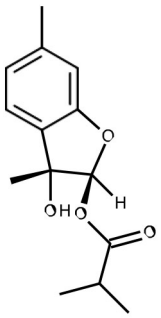

Fig. 1. Structure of a new dihydrobenzofuran derivative **1**

One of the isolated thymol derivatives - 8-hydroxy-9,10-diisobutyryloxythymol - was assayed for cytotoxicity and potential neuroprotective activity using undifferentiated and differentiated SH- SY5Y neuroblastoma cells. The compound at concentrations higher than 25 μM significantly reduced viability of both differentiated and undifferentiated cells. At a concentration range of 1-10 μM the thymol derivative provided partial protection against H_2O_2-induced cell damage in undifferentiated neuroblastoma cells.

Keywords: *Carpesium cernuum,* Asteraceae, dihydrobenzofuran, hydroxycinnamates, thymol derivatives

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S2.P37 Diet-induced differences in gut microbiota determine the responder or non-responder of traditional herbal medicine

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Daiokanzoto (DKT), commonly used clinically to treat constipation, is a traditional herbal medicine comprising rhubarb and glycyrrhiza. The purgative action of DKT is primarily due to sennoside A, the main laxative constituent in rhubarb, which is metabolized by gut microbiota. It is widely acknowledged that dietary habits influence gut microbiota. Therefore, we hypothesize that these habits may influence the purgative activity of DKT, thereby categorizing individuals as either responders or non-responders to DKT treatment. In this study, we manipulated gut microbiota of mice by feeding them a high-carbohydrate or high-fiber diet. To examine the connection between laxatives and gut microbiota, we monitored the gut microbiome before and after administering laxatives. Mice on the high-carbohydrate diet exhibited significantly high purgative activity with DKT. This correlated with increased Enterobacteriaceae abundance due to reduced immunoglobulin A regulation. However, the high-fiber diet greatly reduced DKT efficacy. Additionally, rhein 8-*O*- β-D-glucopyranoside, gallate, and tannin, which are DKT constituents, contributed to the difference between responders and non-responders. These results demonstrate that diet-induced differences in gut microbiota determine the effectiveness of DKT. This is intriguing, given that Oriental medicines are typically tailored to specific functional states, or "patterns." These results reveal that the usefulness of DKT depends on multiple constituents. Furthermore, dietary habits influence gut microbiota, impacting the success of this traditional medicine.

Keywords: daiokanzoto, gut microbiota, immunoglobulin A, short-chain fatty acids, diet

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S2.P38 Antioxidant activity and cell viability assay on L6 myotubes of different parts of *Mitragyna speciosa*

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Mitragyna speciosa Korth., commonly known as kratom (Rubiaceae), has been used in traditional medicine to alleviate several ailments such as diarrhea, cough, muscle pain, and some metabolic diseases like diabetes, hypertension, and dyslipidemia. Among its indole alkaloids, mitragynine is the principal component interacting with opioid receptors (Assanangkornchai et al., 2009; Nakaphan et al., 2016). In this study, different parts of kratom, including leaves extracted with methanol (LM) and water (LW), and branch (BM) and flower (FM) extracted solely with methanol were examined for their total phenolic and flavonoid contents and antioxidant activity. *In vitro* antioxidant activity was assessed including 2,2-diphenyl-1-picryl-hydrazil (DPPH) radical scavenging, ferric reduction (FRAP), and measurement of lipid peroxidation (TBARS). The results demonstrated that LM and LW contained high levels of total phenolic and flavonoid contents. In contrast, BM exhibited high total phenolic content but lower flavonoid content, which differs from the levels observed in FM. Among the different parts, leaves, whether LM or LW, exhibited the better antioxidant activity, followed by branch and flower, respectively. The impact of kratom on L6 myotube viability was evaluated using MTT assay. After 24 h of incubation with various concentrations (6.25-100 µg/mL), all extracts, except LW, did not induce cytotoxic effect within the range of 6.25-50 µg/mL. However, at 100 µg/mL, LM, BM, and FM decreased cell viability to less than 80%, whereas LW did not exhibit cytotoxicity. This study suggests that kratom leaves, particularly LW, could be a promising source of natural antioxidants deserving further research and development.

Keywords: *Mitragyna speciosa,* kratom, antioxidant, cell viability

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S2.P39 pH-dependent membrane permeabilisation through saponins and how it can enhance endosomal escape

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Biologicals such as antibodies, enzymes and nucleic acids are arousing interest in pharmaceutical industry and academia. This rapidly growing field could be the key to cure diseases that were considered untreatable for a long time, with the addition that their therapeutic effect is limited through their poor cellular uptake. After endocytosis of these large molecules, they are subject to endosomal entrapment. The consequence: only a small amount of the applied biological reaches its cellular target. One approach for optimizing their cellular delivery is to lower the integrity of endosomal membranes through amphiphilic substances such as saponins. The saponin Agrostemmoside E (or rather AG1856), derived from the common corncockle (*Agrostemma githago* L.), displays such an enhancing effect on the endosomal escape. The main limitation of using saponins as transfection-enhancers is their cytotoxicity, caused by an unselective lysis of cytoplasmic membranes. This work investigated the influence of pH on the membrane- permeabilizing activity of triterpensaponins. The idea was to apply Agrostemmoside E in a concentration that would not affect cytoplasmic membranes at physiological pH, but only the endosomes as soon as they reach a specific acidity. Agrostemmoside E displayed such a pH- dependent behavior, showing enhanced membranolytic activity in acidic conditions. The data was generated via hemolysis- and LDH-assays. Saponin-concentrations and incubation-times for a selective endosomal escape were suggested as well as a possible biochemical explanation for the observed pH-sensitivity.

Keywords: *Agrostemma githago*, endosomal escape, saponin, pH-dependency, membranolysis

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S2.P40 Cytotoxic potential of saponins and fruit extracts from two genotypes of *Sechium edule* (Cucurbitaceae)

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Sechium edule (chayote) is a species with a wide distribution in the world and Mexico is the center of origin and domestication (Newstron, 1991). In Mexico, *S. edule* is known to have cytotoxic and anticancer potential (Soto-Hernández et al., 2016), even hybrids and wild relatives have this characteristic (Aguiñiga-Sánchez et al., 2015). Within the genetic improvement of chayote, genotypes with bitter taste are associated with a high content of saponins, which are associated with antineoplastic processes (Koczurkiewicz et al., 2015). As part of the phytochemical characterization of the different *S. edule* genotypes obtained by hybridization, our research evaluated two segregating genotypes, 633-M11 and 387-M16. For this purpose, freeze-dried juice extracts and methanolic extracts of the pulp residue (bagasse) of fruits of both chayote genotypes were analyzed. The extraction of saponins from the lyophilized juice of 633-M11 was also carried out. Using HPLC-DAD, the presence of cucurbitacins (CuB, CuD, CuE, CuI, CuIIA), phenolic acids (β- resorcylic acid) and flavonoids (apigenin, catechin, rutin, naringenin and phloretin) were determined, and HPTLC was used to determine the content of total saponins. The juice and bagasse extracts of 633-M11 and 387-M16 showed significant cytotoxic activity on the prostate cancer cell line DU-145 in MTT assays. The lowest IC50 of aqueous juice extract was of 633-M11 was 4.3±1.2 µg/mL while that 387-M16 was 0.47±0.1 µg/mL in the same type of extract. Bagasse extracts of both genotypes and that of 633-M11 total saponins showed cytotoxic effect on DU-145 cells, however, it was not as significant as that of juice extracts.

Keywords: *Sechium edule, Cucurbitaceae*, cancer, cytotoxicity, saponins

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S2.P41 Studies *in vitro* and in vivo of anti-tumor activity of extracts derived from *Plukenetia volubilis* and *Moringa oleifera* in gastric tumor

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Gastric cancer is the fifth most common cancer and the third most common cause of cancer death globally. Perioperative or adjuvant chemotherapy improves survival in patients with stage 1B or higher cancers. *M. oleifera* and *Plukenetia volubilis* are reported to enhance a broad range of biological functions including anti-tumor and antiproliferative activity (Anwar et al., 2007; Nascimento et al., 2013). With the aim of establishing a herbal extract for anti-tumor benefits, in this work we screened *in vitro* the effect of crude extracts (obtained with different solvents) from the leaves of the two plants and the seed oil from *P. volubilis* in the cytotoxicity, anti-proliferative activity, viability, cell cycle, apoptosis, membrane potential and cell migration of AGS (CRL-1739) tumor gastric cell line. The effect of the oil of *P. volubilis* in the progression of gastric tumor was also evaluated in a mouse model of gastric cancer in C57Bl/6 mice. All extracts evaluated were cytotoxic at concentrations (CC_{50}) between 90 and 160 µg/m l (Fig 1). The seed oil from *P. volubilis* showed 95% of mortality at 1% concentration (CC_{50} = 0.86%). Cell proliferation was inhibited by 30%. At the cytotoxic dose, all extracts affected the cell cycle, observing an accumulation of AGS cells in the G0/G1 phase and induce DNA fragmentation as a mechanism of cell death. The ethanolic extract of *M. oleifera* leaf inhibits cell migration also.

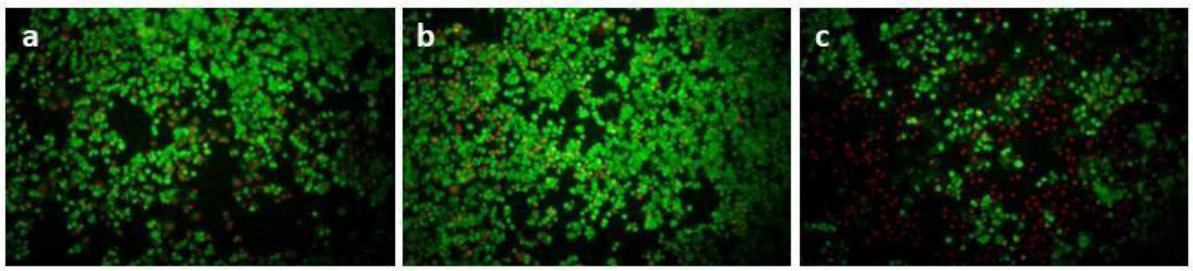

Fig. 1. AGS cells exposed to cyclohexane extract of *P. volubilis* (a), cell culture medium (b) and dimethyl sulfoxide

The effect of the seed oil from *P. volubilis* in the progression of gastric tumors in C57Bl6 mice are under study.

Funding: Colombian Ministry of Science and Technology (Grant CT923-2019)

Keywords: Cell proliferation, cytotoxicity, apoptosis, DNA fragmentation, cell cycle

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S2.P42 Differential modulatory effects of *Populus balsamifera* (Salicaceae) and *Pinus Spp.*(Pinaceae) hydroethanolic extracts on IL-6 and TNF production by activated human peripheral blood mononuclear cells

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Boswellia species are an example of plants whose harvest for western medicinal use is harmful to the species and has a high carbon footprint (Bongers, et al., 2019). This research contributes to the characterization of medicinal properties of local plants that can be sustainably harvested to reduce reliance on natural products requiring long-distance shipping. This study reports differential modulatory effects of commercially available extracts of *P. balsamifera* (Salacaceae) resinous buds and *Pinus Spp*. (Pinaceae) resin on human peripheral blood mononuclear cells (PBMC) stimulated without or with lipopolysaccharide (LPS). Both species are prolific in the United States, and harvest of their resins does not harm the trees. Both resins have documented use by indigenous peoples (Turner, 2014). PBMC were pre-treated with extracts at concentrations of 10-475 µg/mL for 2 hours. Cells were left unstimulated, or stimulated with 95 ng/mL LPS and incubated for 8-14 hours. Supernatants were harvested and IL-6 and TNF concentrations determined by ELISA. Cytotoxicity was determined by XTT assay.

Fig. 1. *Populus balsamifera*. K. Lund

Pinus spp. extract moderately decreased TNF and IL-6 release in a concentration-dependent manner. *P. balsamifera* extract induced significant TNF secretion beyond controls in a concentration-dependent manner and did not induce TNF in the absence of LPS. The TNF production enhancement is a novel finding, as previous studies have reported anti-inflammatory activity *in vitro* (e.g., Pannucci et al., 2022). Characterization of the extracts via HPLC, along with fractionation and identification of constituents responsible for the effects are warranted.

Keywords: *Populus balsamifera, Pinus Spp.,* cytokines, cytotoxicity, PBMC

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S2.P43 Optimization of extraction conditions for salidroside isolated from *Acer tegmentosum*

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Salidroside is a major phenolic glycoside of *Acer tegmentosum* (Aceraceae) and known to be a hepatoprotective compound (Park et al., 2006; Kwon et al., 2011; Linlin et al., 2019). In this study, extraction conditions were optimized for maximum yield of salidroside from *A. tegmentosum*. For optimization, three extraction factors such as ethanol concentration (%), extraction temperature (°C), and solvent to material ratio (mg/mL) were tested and optimized for maximum yield of salidroside using response surface methodology (RSM). The optimal condition was obtained as an ethanol concentration of 53.4%, an extraction temperature at 67.11°C and a solvent to material ratio (mg/mL), 195.55 mg/m l. The salidroside yield under optimal conditions was found to be 1.59 mg/g dried samples, which were well-matched with the predicted value of 1.56 mg/g dried samples (Fig 1). These results will provide useful information about optimized extraction conditions for the development of salidroside as hepatoprotective therapeutics.

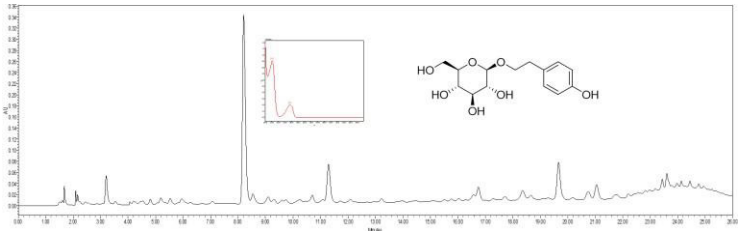

Fig. 1. HPLC chromatogram of *A. tegmentosum* extract prepared under optimal extraction conditions for salidroside.

Keywords: *Acer tegmentosum*, box–behnken design, response surface methodology, salidroside

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S2.P44 *Eleutherococcus senticosus* fruits intract - no toxicity in a balb/c mouse model

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Eleutherococcus senticosus (Rupr. et Maxim.) Maxim. fruits and roots are used to treat immune- related diseases. Because of the overexploitation of the roots, the species is considered to be endangered therefore, the aerial parts of *E. senticosus* might be explored as a new sustainable source of compounds with an immunostimulative activity (Graczyk et al., 2021). This study was aimed to evaluate the safety profile (hepatotoxic, blood parameters) of the *E. senticosus* fruits intract to support the use of the fruits in folk medicine and in a modern phytotherapy in the EU's countries. Mice Balb/c have been treated for 5 days with water (group I) and the intract in doses 750 (group II) and 1500 (group III) mg/kg b.w. The body weight was measured every day, euthanasia on the eighth day and the activity of ALTL and ASTL was assayed. Additionally, the level of creatinine, urea and total protein as well as blood morphology were measured. There were no statistically significant differences in the body weight, ALT and AST activity between groups I, II and III, a slight decrease in urea level and total protein was observed. A decrease in total leukocytes count was observed (7.9, 7.8 and 5.8 x10^3 uL, resp.) with a percentage lymphocytes' increase (80.2, 81.8 and 82.6, resp.). Our observations justify the traditional use of the fruits and in the doses used, the intract is rather safe. On the basis of the results obtained, the dose 750 mg/kg b.w. is considered for the next research.

Keywords: *Eleutherococcus senticosus,* fruits, Balb/c mouse, toxicity

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S2.P45 Potential molecular mechanisms of Silexan® identified by RNA-Sequencing in a mouse model of chronic social stress-induced excessive aversion sensitivity

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Silexan® is a proprietary essential oil from *Lavandula angustifolia* approved for the treatment of temporary anxious mood. Silexan® has also shown antidepressant properties in a clinical study (Bartova et al., 2023). To further evaluate potential molecular mechanisms of Silexan® contributing to its activity we used a chronic social stress (CSS) mouse model inducing excessive Pavlovian aversion learning and memory, as a validated *in vivo* model for excessive aversion sensitivity. Silexan® (3 and 30 mg/kg per oral) dose-dependently reversed CSS-induced excessive Pavlovian aversion memory with similar efficacy as the SSRI escitalopram (3 mg/kg). Afterwards hippocampal RNA was isolated for RNA-Sequencing from the control (unstressed)-vehicle group, the CSS-vehicle group, the CSS-Silexan® 30 mg/kg group and the CSS-escitalopram group (n=5 per group). From 27,676 genes, 51 genes were both downregulated by CSS and upregulated by Silexan®. These included genes that, according to the literature, are altered in rodents following stress e.g. *Gucy1a2* (guanylate cyclase 1 soluble subunit alpha 2), *Ncam2* and *Ube3a*. Genes with a potential link to depression and anxiety in rodents and humans not affected by CSS but upregulated by Silexan® were e.g. *Hrtp1b*, *Pld5*, and *Scn5a*. For genes upregulated by CSS and downregulated by Silexan®, no obvious association with depression and anxiety was identified in the literature. In summary, such RNA sequencing data might prove useful to establish first hypotheses on potential modes of action of Silexan® in the treatment of excessive aversion processing in stress-related psychiatric disorders such as anxiety and depressive disorders.

Keywords: Silexan®, depression, hippocampal gene expression

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S2.P46 The *Urtica dioica* root extract component in WS®1541 diminishes parameters of epithelial mesenchymal transition in human benign hyperplastic prostate epithelial cells

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Epithelial mesenchymal transition (EMT) contributes to the pathogenesis of benign prostate enlargement (BPE). In a human hyperplastic prostate epithelial cell line (BPH-1), we evaluated the effect of the extract combination WS®1541 and its single components WS®1473 and WS®1031 on various EMT-parameters. WS®1541 is a proprietary combination of ethanolic extracts from *Sabal serrulata* fruits (WS®1473) and *Urtica dioica* roots (WS®1031) clinically used for treatment of lower urinary tract symptoms attributed to BPE. We treated BPH-1 cells with different concentrations of the three extracts and evaluated cell migration as a marker for EMT. The extract combination WS®1541 inhibited migration which was solely achieved by the presence of WS®1031, as WS®1473 had no effect on this parameter. Since TGF-β is a key driver of EMT, we examined the expression of the three TGF-β isoforms and its two receptors. WS®1541 diminished expression of all target genes. This effect was primarily mediated by the *Urtica dioica* root extract WS®1031, whereas the *Sabal serrulata* fruit extract WS®1473 only reduced expression of *TGFB2* and *TGFBR2*. Demonstrated by phalloidin staining, F-actin polymerization was not affected by either treatment. Inhibition of the migratory phenotype of BPH-1 cells by the combination product WS®1541 is conferred by the activity of the *Urtica dioica* root extract WS®1031. This effect may be due to a reduced expression of relevant TGF-β isoforms and their receptors. This mechanism could contribute to the clinical efficacy of WS®1541.

Keywords: WS®1473, benign prostate enlargement, *Urtica dioica, Sabal serrulata,* epithelial mesenchymal transition

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S2.P47 Antitumor activity of evodiamine analogs by targeting PKCβI in gemcitabine-resistant pancreatic cancer cells

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Evodiamine, an indoloquinazolidine alkaloid, is a major component of the traditional herbal medicine *Evodia rutaecarpa* (Yu et al., 2013), which is widely used in Asian countries to alleviate vomiting, pain, and diarrhea. Evodiamine exerts various biological activities, including anti- inflammatory, anti-obesity, and antimicrobial effects. Accumulating evidences reported the antiproliferative and antitumor activities of evodiamine against various cancers through the metastasis inhibition, apoptosis induction, and cell cycle arrest. In cancer cells, protein kinase C βI (PKCβI) plays a crucial role in diverse cellular processes, including cell proliferation, invasion, and apoptotic pathways. In this present study, we created a scaffold to develop PKCβI inhibitors using evodiamine-based synthetic molecules.

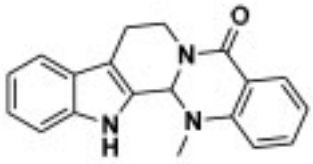

Fig. 1. Structure of evodiamine

Based on various biological activities of evodiamine, the scaffold was synthesized and investigated for antiproliferative and antitumor activities against gemcitabine-resistant pancreatic cancer cell line PANC-1 (PANC-GR) cells. In addition, target validation experiment was employed to elucidate the molecular mechanism and verifying the target to overcoming gemcitabine resistance. Mechanistically, evodiamine analogs suppress the PKCβI expression, which subsequently inhibits the proliferation of PANC-GR cells *in vitro* and tumor growth in PANC-GR cells-implanted xenograft mouse model.

Keywords: *Evodia rutaecarpa,* synthetic analogs, antitumor activity, gemcitabine-resistant pancreatic cancer

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S2.P48 Perceived changes in anxiety symptom burden during treatment with *Bryophyllum pinnatum*: a prospective, non-randomised, observational study

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Bryophyllum preparations are widely used in anthroposophic medicine, a form of integrative medicine. Prospective studies have revealed positive effects of treatment with Bryophyllum 50% tablets on sleep quality (Lambrigger-Steiner et al., 2014; Mirzayeva et al., 2023). These improvements are corroborated by preclinical studies showing that fractions from B. pinnatum leaves can prolong induced sleeping time in rodents, indicating a CNS depressant action (Pal et al., 2010). A recent study showed anxiolytic and psychoactive effects in young zebrafish (Martins Fernandes Pereira et al., 2022). We set to find out if patients perceive improvements of their anxiety symptoms during treatment with Bryophyllum 50% tablets. A total of 51 patients with anxiety symptoms were recruited among the waiting list from the Department of Psychiatry and Psychosomatics at the Klinik Arlesheim. During the three-week study, patients were treated with Bryophyllum 50 % tablets (3x2 per day) and filled out a questionnaire at baseline, after two and three weeks of tablet intake. The primary endpoint of the study was the change in anxiety symptoms measured with the Beck Anxiety Inventory (BAI). Depression, sleep quality, stress, health-related quality of life and internal coherence were assessed as well. Results show a statistically significant and clinically relevant decrease in the BAI-score between baseline and after two (and three) weeks of treatment. Additional improvements were observed in the secondary endpoints; tolerability and compliance were very good. We conclude that Bryophyllum 50% tablets have beneficial effects on anxiety-related symptoms and should be considered as a well-tolerated treatment for their management.

Keywords: *Bryophyllum pinnatum,* anxiety symptoms, Beck Anxiety Inventory, sleep quality

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S2.P49 Effects of orally applied sanggenons C and D in BALB/c mice

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As part of our continuing search for bioactive natural products, a standardized root bark extract (MA60) from *Morus alba* revealed as promising agent. *In vitro* activities against inflammation, influenza viruses, SARS-CoV-2, *S. aureus*, and *S. pneumoniae* were previously determined (Grienke et al., 2016; Langeder et al., 2023a; Wasilewicz et al., 2023). Major active constituents of MA60 are the sanggenons C and D. This presentation will cover a preclinical study conducted in female BALB/c mice to evaluate whether *in vitro* results are transferable to an *in vivo* setting. Oral application of MA60 was selected to determine i) the maximum tolerated dose (MTD) in an acute toxicity study and ii) its potential against influenza virus in an efficacy study. Moreover, inflammatory parameters were monitored. A further aim was to evaluate whether there is a correlation between the obtained results and the amount of sanggenons C and D in serum and tissues by quantitation using a validated UPLC-ESI-MS method. The MTD was reached at 100 mg/kg. In the efficacy study, the treatment effects were moderate. Dose-dependent quantities of sanggenon C in serum and sanggenon D in liver samples were detected. Only very low concentrations of sanggenons C and D were determined in lung samples and none of these compounds was found in spleen samples. There was no compound accumulation when MA60 was administered repeatedly (Langeder et al., 2023b). In conclusion, due to low serum concentrations after oral application once daily, alternative application routes, in particular inhalation or intranasal administration are currently pursued.

Funding: Wilhelm-Doerenkamp-Foundation, Switzerland (NATVANTAGE Grant 2018); Austrian Science Fund (FWF P34028 and P35115)

Keywords: *Morus alba,* sanggenon, influenza virus*, in vivo*, quantitation

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S2.P50 Novel natural product inhibitors targeting oncogenic MAPK/ERK and PI3K/AKT signaling in melanoma: From large library screening to target identification

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Malignant melanoma is the deadliest type of skin cancer with unmatched mutation rates arising in both MAPK/ERK and PI3K/AKT signaling pathways. Although specific inhibitors of these critical pathways show spectacular initial results, most patients relapse within just a few months. Combination therapy can improve overall survival rates, but the currently available options are limited (Tanda et al., 2020). Therefore, novel inhibitors targeting oncogenic ERK and AKT signaling in melanoma are urgently needed. To tackle this issue, our in-house library of crude plant extracts was combined with an innovative high-content screen (HCS) that quantifies downstream inhibitory activity at ERK and AKT level. HPLC-based activity profiling of the active hits and subsequent targeted isolation of the bioactive constituents was performed (Dürr et al., 2022). To further explore the coverage of chemical space for such inhibitors, we also screened large pure compound libraries by accessing high-throughput screening pipelines through an EU- OPENSCREEN program. To this end, we screened 2,576 plant extracts and additional 25,696 pure natural and synthetic compounds. A total of 46 active compounds were confirmed as downstream inhibitors of ERK and/or AKT with IC_{50} values in the low micromolar range. The current challenge aims towards target identification of the most promising hits. Several key kinases of the ERK-AKT network were produced through different cloning techniques and heterologous expression systems. Our strategy further includes the assessment of physical binding as well as enzymatic inhibition. Ultimately, we envisage to develop these newly discovered inhibitors into lead compounds for future drug development.

Keywords: melanoma, high-content screening, EU-OPENSCREEN, MAPK/ERK and PI3K/AKT signaling, target identification

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S2.P51 Screening for natural products inhibiting PI3K/AKT pathways in melanoma: investigation of *Mammea americana* EtOAc extract

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Malignant melanoma is the deadliest skin cancer and its incidence has risen significantly over the past decades. Mutations are frequently observed in the PI3K/AKT and MAPK/ERK pathways, leading to abnormal cell proliferation. Targeted therapy showed spectacular initial results, but drug resistance appears after a few months only. Combination therapy has shown improvement in the progression-free and overall patient survival and additional compounds are urgently needed to complete the arsenal of available drugs. To address this issue, our natural product discovery platform was combined with an innovated high-content screening (HCS) assay to quantify AKT and ERK signaling in melanoma cells (Dürr et al., 2022). Here, the EtOAc extract from Mammea americana inhibited the PI3/AKT signaling pathway and was therefore selected for scaled-up isolation of the active constituents. To this end, an HPLC-based activity profiling approach was used, which led to the identification of novel betulinic acid derivatives (**1**-**10**) and known coumarins (**11**-**19**). Upon closer inspection, the coumarin theraphin B (**15**), was the only compound displaying a weak activity with an IC50 of 37 ± 5.9 µM. In contrast, although betulinic acid has been reported to have anti-tumor activity in mice (Pisha et al., 1995), the analogues **1**-**10** did not show any inhibition of AKT and ERK, suggesting a different mechanism unrelated to the AKT signaling pathway to explain the in vivo activity.

Keywords: *Mammea americana,* melanoma, high-content screening, coumarin, ECD

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S2.P52 Exploring the anti-inflammatory potential of an herbal mixture preparation and individual herbal extracts on inflammatory bowel disease

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Patients affected by inflammatory bowel disease (IBD) often suffer of compromised motility due to mucosal inflammation. The herbal mixture preparation found in Digestodoron® is traditionally used for treatment of gastrointestinal dysmotility disorders. This study aimed to investigate the anti- inflammatory properties of the herbal mixture and its four individual extracts—*Dryopteris filix-mas* (DF), *Phyllitis scolopendrium* (PS), *Polypodium vulgare* (PV) and *Salix mixture with alba/purpurea/viminalis* (SM)). Dry extracts were derived from Digestodoron® or from the individual ethanolic tinctures. Pro-inflammatory leukotrienes and prostaglandins, as well as the activation of the NF-κB pathway, are hallmark indicators of inflammation. NF-κB activation was evaluated *via* a reporter assay using the human Jurkat cell line. Inhibition analysis of the pain- related enzymes 5-lipoxygenase (5-LO) and cyclooxygenase-2 (COX-2) as part of the arachidonic acid pathway was performed using recombinant human enzymes. The whole extract, along with individual extracts PS and SM, notably supressed 5-LO enzyme (IC_{50}: 12.6, 60.6, 16.6 µg/mL, respectively). In contrast, PV and DF showed comparatively lower inhibition effects (IC_{50}: 223.8 and 648.6 µg/mL, respectively). In terms of COX-2 enzyme suppression, the whole and all individual extracts exhibited effectiveness (IC_{50}: 12.6 (mixture), 25.5 (DF), 14.3 (PS), 7.9 (PV) and 2.7 (SM) µg/mL). Moreover, NF-κB activation was hindered by the whole extract and exclusively by the SM extract (IC_{50}: 223.1 and 79.6 µg/mL). These findings suggest that Digestodoron®, with its potent anti-inflammatory and analgesic properties, could be a promising therapeutic option for individuals suffering from IBD. Ongoing research aims to elucidate further the potential therapeutic benefits of Digestodoron®.

Keywords: herbal preparation, NF-κB, 5-lipoxygenase, cyclooxygenase-2, anti-inflammatory, inflammatory bowel disease

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S2.P54 Extracts of *Sisymbrium officinalis* and *Plantago lanceolata* reduced inflammation induced by lipoteichoic acid from *Streptococcus pyogenes* in human tonsil epithelial cells

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Pharyngitis is an inflammation of the pharynx caused by viral, bacterial, or non-infectious triggers. *Sisymbrium officinalis* (L.) Scop (SO) and *Plantago lanceolata* L. (PL) leaf extracts are used in traditional medicine for the treatment of sore throat and inflammation of the upper respiratory tract. In this study, the anti-inflammatory effects of SO and PL dry extracts were evaluated in an *in vitro* model of streptococcal pharyngitis. Human primary tonsil epithelial cells were stimulated with lipoteichoic acid (LTA) from *Streptococcus pyogenes* (10 mg/mL) in the presence or absence of SO (12.5, 25 and 50 mg/mL). In similar experiments, tonsil cells were treated with PL (25, 50 and 100 mg/mL) prior to LTA stimulation. Culture supernatants were collected after 24 h and analysed for levels of PGE_2 using enzyme immunoassay (EIA) kit. Secretion of pro-inflammatory cytokines (TNFα and IL-6) were measured using ELISA. Results showed that pre-treatment of tonsil epithelial cells with SO produced significant ($p<0.05$) and concentration-dependent reduction in LTA-induced production of PGE_2, TNFα and IL-6. The highest concentration of the extract reduced inflammatory mediator production by ~60%. Results also showed that PL significantly ($p<0.05$) reduced LTA-induced increased production of PGE_2, TNFα and IL-6, with 100 mg/mL of the extract reducing elevated production of inflammatory mediators by ~70%. Cell viability experiments revealed no significant reduction in viability of tonsil epithelial cells at concentrations of extracts investigated. These results suggest that *Sisymbrium officinalis* and *Plantago lanceolata* are anti-inflammatory in pharyngitis caused by *Streptococcus pyogenes* and should be further investigated for healthcare applications.

Keywords: inflammation, pharyngitis, upper respiratory tract infections, *P. lanceolata, S. officinalis*

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S2.P55 Protein kinase activity of chromatographic fractions of *Acacia auriculiformis* stem bark as potentials for anti-cancer drug discovery

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The incidence of deaths from cancer has been on the rise globally with reported cases of 10 million deaths in 2020 that is about one in six deaths and over one million new cases occur each year in Africa, with about 700,000 deaths reported. Data estimates showed a considerable increase in cancer mortality to nearly one million deaths per year by 2030. As a result of the negative side effects of the current treatment regimen plan which includes chemotherapy or a combination of radiotherapy and chemotherapy, this has justified the continuous search for new therapeutic agents from natural sources including medicinal plants. In this present work, we report the inhibitory activity of the chromatographic fractions of the ethyl acetate soluble of the stem bark extract of *Acacia auriculiformis* against some disease related protein kinases. The ethyl acetate fraction was subjected to chromatographic separation on silica gel and sephadex LH-20 to afford H1, H2 and H3 respectively. H1 was identified as Betulin using spectroscopic technique, while H2 and H3 gave positive test for flavonoid. H1-H3 were then subjected to kinase enzymatic assays performed using the ADP Glo assay kit in a 384- well plates. The result of the primary screening showed that H1 and H2 inhibited four and six of the kinases tested, while the best activity was exhibited by H3 against all the kinases tested. The result of the IC_{50} revealed similar trend as H3 showed the best activity out of the three fractions with an IC_{50} of 0.086 µg/mL against Haspin and 0.46 µg/mL against CDK 9 kinases, while H2 gave an $1C_{50}$ of 0.71 against CDK 9 but was in active against the haspin kinase. The kinase activity of H2 and H3 were more than H1 (Betulin) previously reported as a multi target kinase inhibitor suggesting the potentials of H3 as a lead in the discovery of haspin and CDK 9 inhibitors which can be exploited in the search for anticancer drug.

Keywords: *Acacia auriculiformis*, chromatographic fractions, kinase activity, IC_{50}

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S2.P56 Comparative protein kinase inhibitory activity of three *Acacia* species growing in Nigeria

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Significant improvements have been made in the field of drug discovery targets against cancer over the years, which have necessitated the search for novel chemical entities for the therapeutic treatment and management of cancer from natural sources. In this present work, we report herein the kinase inhibitory effect of the crude chloroform extract and the partitioned fractions of the hydroalcoholic stem bark extract of *Acacia ataxacantha* against a panel of disease related protein kinases and the comparison of the activity with that of *Acacia nilotica* and *Acacia auriculiformis*. The extract and the fractions of the stem bark of *Acacia ataxacantha* were subjected to Kinase enzymatic activities performed in 384-well plates using the ADP-Glo assay kit. The result showed that chloroform extract was inactive, while the ethyl acetate fraction gave the highest activity against haspin kinase with IC_{50} of 0.1 µg/mL, followed by aurora B kinase with IC_{50} of 0.45 µg/mL, while against CDK2, CDK5 and CDK9 kinases, the IC_{50} were 0.6, 0.88 and 0.9 µg/mL respectively. The n-butanol fraction gave an IC_{50} of 0.59 µg/mL and 0.7µg/mL against haspin and aurora B Kinases respectively. Comparison with *Acacia nilotica* and *Acacia auriculiformis* showed that the ethyl acetate fraction of *acacia ataxacantha* is the most active. The results showed the potentials of the ethyl acetate fraction of *Acacia ataxacantha* as source of inhibitors against Haspin, aurora B and CDK kinases which might serve as lead for antimitotic agent against cancer.

Keywords: *Acacia* species, protein kinases, ethyl acetate fraction, IC_{50}, anticancer agent

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S2.P57 Diversification of kratom alkaloids using biocatalysis for opioid use disorders

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This presentation will cover the diversification of the main indole alkaloids in the kratom plant (Fig. 1). Mitragynine, a plant-derived alkaloid natural product, has captured attention due to its intriguing analgesic properties, positioning it as a promising candidate for a safer alternative to existing mu- opioid receptor (MOR) agonists (Kruegel et al., 2016). We are exploring the creation of new analogs of mitragynine through late-stage hydroxylation and halogenation, which enables further interrogation of chemical space through additional functionalization. Two complementary strategies to identify and optimize biocatalysts capable of site-selectively modifying mitragynine have been employed. First, we are utilizing directed evolution on the 4V flavin dependent halogenase to enhance chlorination of mitragynine at variant positions on the indole ring. Directed evolution is being conducted to generate a diverse library of enzyme variants with improved activity and selectivity. Secondly, computational approaches, including density functional theory (DFT), inverse docking, and molecular dynamics (MD) simulations have been incorporated into our workflow to identify novel cytochrome P450s capable of late-stage C-H functionalization. Finally, the bioactivity of our analogs at the MOR will be assessed through M-SPOTIT and PathHunter β- galactosidase enzyme-complementation assays, providing detailed insights into their signaling and potential side effects (Kroning et al., 2021). We plan to investigate additional bioactive kratom alkaloids like speciogynine, paynantheine, and corynoxine for their synergistic potential. Through this comprehensive approach we strive to develop novel, improved mitragynine and related compound derivatives with improved analgesic properties.

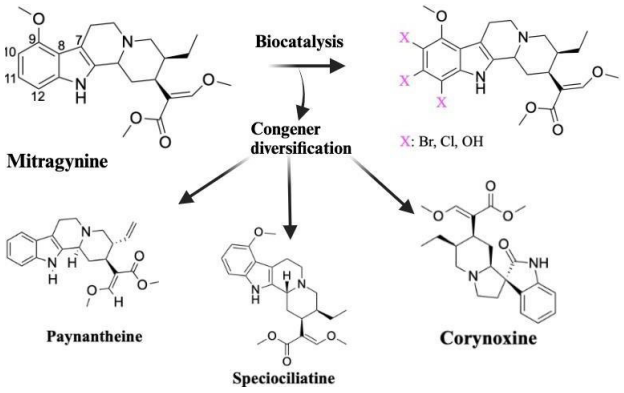

Fig. 1. Overview of strategy for kratom alkaloid structural diversification

Keywords: biocatalysis, C-H functionalization, mitragynine, MOR, directed evolution

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S2.P58 Study of *Carpesium abrotanoides* in dextran sulfate sodium-induced IBD and Caco-2 cells

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The genus *Carpesium* consists of approximately 21 species worldwide (Zhang et al., 2015). This species is particularly widespread in Asia and is used as a traditional treatment for various conditions such as colds, fever, insects, bruises, and inflammation (Zhang et al., 2015). Among them, research results have reported that *Carpesium abrotanoides* species has excellent anti- inflammatory, anti-cancer, and antioxidant effects (Lee et al., 2013). However, no studies have yet been reported on the effects of this natural product on inflammatory bowel disease. Therefore, in this study, we investigated the protective effects of *C. abrotanoids* on dextran sulfate sodium (DSS)-induced experimental colitis. Using an acute colitis model *in vivo*, we identified the possible mechanisms of anti-inflammatory activity and *C. abrotanoids* in C57BL/6 mice. *In vitro*, further studies were conducted to identify the molecular mechanisms of *C. abrotanoids* for the inflammatory response using Caco-2, a human intestinal epithelial cell. As a result, in C57BL/6 mice, *C. abrotanoids* alleviated disease activity index (DAI), colon length shortening, and inhibited pro-inflammatory cytokine production in blood and tissue. In addition, *C. abrotanoids* significantly reduced the secretion of pro-inflammatory cytokines such as TNF-a and IL-6 at LPS-induced Caco- 2 cell levels. This study suggests that *C. abrotanoids* could be a potential agent for the treatment of inflammatory bowel disease.

Keywords: *Carpesium abrotanoides,* dextran sulfate sodium, inflammatory bowel disease, caco-2 cell, anti-inflammatory effect

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S2.P59 *In vitro* co-culture analysis of Caribbean medicinal plant extracts for bacterial vaginosis

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Bacterial vaginosis (BV) is the most common gynecological infection in reproductive-age women globally. BV is directly caused by dysbiosis of the vaginal microbiome – when beneficial *Lactobacillus* species are no longer dominant and are replaced by harmful anaerobic bacteria such as *Gardnerella vaginalis*. In Caribbean cultures, women use plants topically, such as *Argemone mexicana*, to treat BV and other vaginal infections. Until now, there has been very little research into how botanical extracts affect both beneficial and harmful bacteria in the vaginal microbiota, especially as these are often prepared in different ways for the same health condition. The aim of this study is to test the effect of botanical preparations using an *in vitro* co-culture assay with beneficial *Lactobacillus* species and BV-causing *G. vaginalis*. We hypothesized that chemical differences across five preparations would influence the ratio of beneficial and pathogenic bacteria. The different *A. mexicana* extractions (methanol, ethanol, water, acidified water, and heated water) were tested using an *in vitro* co-culture method with *G. vaginalis* and one of three vaginal *Lactobacillus* species. Additionally, UPLC-TQD-MS was used to quantify known antibacterial alkaloids, including berberine, protopine, and allocryptopine, across different extraction methods. Water extractions that had no antibacterial effect in monoculture suppressed the growth of *G. vaginalis* in co-culture with *Lactobacillus*. The results of this study provide practical information for women who use *A. mexicana* globally.

Keywords: vaginal microbiota, ethnobotany, women's health, caribbean medicinal plants

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S2.P60 Pharmacokinetic dynamics of the hybrid *Sechium* H387 07 and phytochemical characterization of its fruit

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Recently, a hybrid of *Sechium edule* named H387 07 has demonstrated potential as an antiproliferative, cytotoxic, and apoptotic agent in cancer cell lines, including leukemia and breast cancer. This effect is primarily attributed to the phytochemical content of phenols, flavonoids, and cucurbitacins present in the extract (Aguiñiga-Sánchez et al., 2015; Cadena-Iñiguez et al., 2022). This study aimed to assess the phytochemical content of the hybrid *Sechium* H387 07 and compare it with its segregants, 387M20 and 387M16. Additionally, the study sought to determine its antioxidant activity. On the other hand, the pharmacokinetic profile of cucurbitacins, phenolic acids, and flavonoids was investigated following the administration of the H387 07 hybrid extract at varying doses to mice. The obtained results indicated that segregant 387M16 showed a concentration of phytochemicals comparable to that of the hybrid *Sechium* H387 07. Consequently, 387M16 emerges as a promising candidate for subsequent bioassays. Furthermore, various pharmacokinetic parameters for apigenin, florentin, CuB, CuE, and CuI were determined. Notably, the study highlighted the presence of CuB and CuIIA due to their elevated concentration in the serum, with the latter being described for the first time in the extract of the *Sechium* hybrid.

Keywords: Pharmacokinetics, Sechium, hybrid, secondary metabolites

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S2.P61 Neolignans and diarylnonanoid derivatives from the seed of *Myristica fragrans* Houtt. and their cytotoxic activities against human ovarian cancer cell lines

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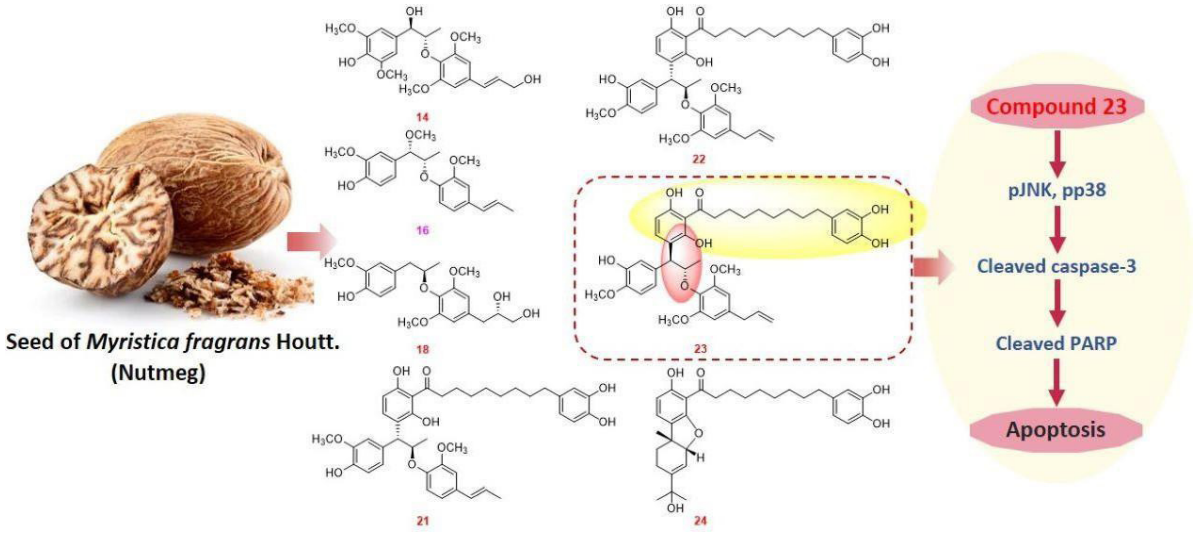
Two new neolignans (myrifralignans F–G), four new diarylnonanoid derivatives (myrifragranones A–D), and 18 known compounds from the seed of *Myristica fragrans* Houtt. (nutmeg) were isolated and structurally elucidated. The absolute configurations of these secondary metabolites were accurately determined using the electronic circular dichroism method. The cytotoxic activities of these isolated compounds against cisplatin-sensitive and resistant human ovarian cancer cell lines were evaluated. In particular, the new compound myrifragranone C (**23**) exhibited cytotoxicity against all test cancer cell lines A2780, TOV-112D, and SK-OV3 with IC_{50} values of 14.1, 16.9, and 33.4 μM, respectively. Furthermore, compound **23** induced the death of A2780 and SK-OV3 cancer cells via apoptosis. Western blotting revealed that compound **23** significantly increased the expression of cleaved caspase-3 and poly-ADP ribose polymerase and promoted apoptosis via the mitogen-activated protein kinase signaling pathway.



Keywords: *Myristica fragrans*, nutmeg, myrifralignan, myrifragranone, ovarian cancer

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S2.P62 The ameliorative effects of *Moschus* and its major compound L-muscone on traumatic brain injury mice model focusing on the mechanisms of action

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Moschus, a dried secretion from musk cyst of male musk deer (*Moschus moschiferus* Linnaeus) has been used in Korean medicine to treat neurological and traumatic symptoms with the effect of resuscitation. It has been reported to reduce ischemic brain damage and chronic stress-induced functional deficits. L-muscone, a major component of Moschus, was also reported to have neuroprotective effects. However, there are few studies on its effects and mechanisms in traumatic brain injury (TBI). TBI was induced by controlled cortical impact (depth 2.5 mm; velocity 2 m/s; duration 300 ms) in mice. Moschus (10 and 100 mg/kg) and L-muscone (0.5 and 5 mg/kg) were suspended in corn oil and administered twice a day for 8 days after TBI. Brain damage, hemorrhage volume, Evans blue leakage, behavioral deficits were evaluated. In addition, blood brain barrier (BBB) compartments, pathological factors, and water channel were quantified. Moschus and L- muscone reduced brain damage, hemorrhage and BBB permeability along with functional improvements. Especially, they restored cognitive function to normal level. They also increased BBB compartments including zonula occludens-1, claudin, and occludin, decreased aquaporin-4, Heme oxygenase-1 and matrix-metalloproteinases -2 & -9 expressions. These results provide a scientific basis for the clinical use of Moschus in traditional medicine and suggest that L-muscone might be the main compound in the effects of Moschus on TBI, and that Moschus and L-muscone might be candidates for TBI therapeutic agents. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (Ministry of Science and ICT) (No. 2020R1A2C1008603).

Keywords: *Moschus*, L-muscone, traumatic brain injury, blood brain barrier, cognitive function

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S2.P63 Cycloartane-type triterpenoids from *Combretum quadrangulare* (Combretaceae) and their potential for the treatment of hypercholesterolemia

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This presentation will cover the isolation, structure elucidation and biological evaluation of cycloartane-type triterpenoids including representative compound (**1**) (Fig. **1**). *Combretum quadrangulare* Kurz. (Combretaceae) has been traditionally used in Southeast Asian countries as an herbal medicine with antipyretic, antidysenteric and hepatoprotective properties (Banskota et al., 2003).

Previous studies (An et al., 2023; Won and Son et al., 2023) have reported acyclic and cyclic triterpenoid scaffolds as PCSK9 inhibitors. As part of our continuing search for PCSK9 inhibitory compounds from plants, we selected *C. quadrangulare*, known for its richness in cycloartane-type triterpenoids. Utilizing spectroscopic analysis, we successfully isolated 16 cycloartane-type triterpenoids, all of which underwent structural assignments and testing via ELISA bioassays.

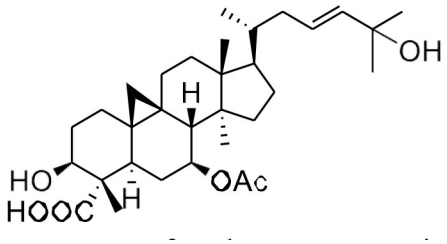

Fig. 1. The structure of cycloartane-type triterpenoid **1**

Moreover, the exploration of the structure-activity relationship of cycloartane-type triterpenoids among the 16 isolates was conducted. Among these compounds, compound **1** was found to be the most potent in PCSK9 inhibitory activity, compared to berberine—a commonly utilized positive control in studies of PCSK9 inhibitory effects. These findings suggest that cycloartane-type triterpenoids could present a promising scaffold for the optimal treatment of hypercholesterolemia. Acknowledgments: This research was supported by grants from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR202207102) and National Research Foundation of Korea (NRF), funded by the Korean government (MSIT) (NRF-2022R1A2C1010084).

Keywords: *Combretum quadrangulare,* combretaceae, cycloartane-type triterpenoid, PCSK9, hypercholesterolemia

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S2.P64 Bitter-tasting plant extracts stimulate mechanisms of gastric acid secretion of immortalized human parietal cells via TAS2Rs depending on their polyphenol content

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Bitter taste receptors (TAS2Rs) are not only involved in taste perception, but also in many extra- oral mechanisms. Previous work by our group has shown that bitter substances stimulate the proton secretion (Liszt et al., 2017) and also have anti-inflammatory effects (Tiroch et al., 2021 & 2023).

In this work, an herbal product and the nine different ethanolic plant extracts of which it is composed, namely tinctures of *Artemisia absinthium*, *Achillea millefolium*, *Centaurium erythraea*, *Cichorium intybus*, *Gentiana lutea*, *Juniperus communis*, *Peucedanum ostruthium*, *Salvia officinalis*, *Taraxacum officinalis*, were investigated for their effects on cellular proton secretion in immortalized human parietal cells (HGT-1). Quantitation of polyphenol and flavonoid contents were performed by means of Folin-Ciocalteu reagent and Al^{3+} complex formation. A TAS2R- dependent effect was studied by RT-qPCR experiments and validated by knock-out (ko) approaches. Seven of the investigated extracts as well as the mixture of all nine extracts led to a concentration-dependent stimulation of proton secretion, with a correlation between the respective polyphenol and flavonoid contents and the effect size ($p \leq 0.0001$). In accordance with the literature, the investigation of gene expression revealed regulation of *TAS2R4/5/39*, among others (Soares et al., 2013). Experiments with TAS2R4ko and TAS2R39ko cells showed a reduction of stimulatory effects on proton secretion ($p \leq 0.05$), validating an involvement of these TAS2Rs. In summary, depending on their polyphenol and flavonoid content, bitter plant extracts lead to a stimulation of proton secretion by the bitter taste receptors TAS2R4 and TAS2R39, which are therefore involved in digestion-promoting mechanisms.

Keywords: bitter plant extracts, TAS2R, human parietal cells, proton secretion, polyphenols

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S2.P65 Anti-inflammatory testing of some isolated sesquiterpene lactones and methoxylated flavonoids from *Achillea millefolium* L. in an ICAM-1 *in vitro* assay

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Achillea millefolium is a medicinal plant of the Asteraceae family used in both traditional and conventional medicine (Radušiene et al., 2005) mostly in form of alcoholic, hydroalcoholic, and aqueous extract, or infusion and decoction (Dias et al., 2013). Its main secondary metabolites are flavonoids, essential oil, phenolic acids, and sesquiterpene lactones including also proazulenes. The most important therapeutic indications for internal use are loss of appetite, dyspeptic disorders, and the treatment of cramps in dysmenorrhea, and for external use inflammations of the skin and mucous membranes (reviewed in Hutchins et al., 2021). Since the mechanism of action of the anti- inflammatory effect and the substances responsible for this are not known in detail, seven isolates from previous work on *Achillea millefolium* were investigated in an intracellular adhesion molecule-1 (ICAM-1) *in vitro* test (Freischmidt et al., 2012). Thereby, four sesquiterpene lactones of the guaianolide type, namely 8-desacetyl-8-angeloyl-4-epi-matricine, 8a-acetyl-2b,4a,10b- trihydroxy-6bH,7aH,11bH-1(5)-guaien-12,6a-olide, 8a-isopentyl-2b,4b,10b-trihydroxy- 6bH,7aH,11bH-1(5)-guaien-12,6a-olide, and 8a-angeloxyl-2b,4b,10b-trihydroxy-6bH,7aH,11bH- 1(5)-guaien-12,6a-olide, and two methoxylated flavonoids, pectolinarigenin and artemetin, reduced significantly the ICAM-1 (expression in a human endothelial cell line (HMEC-1) in a concentration range from 25-75 µM after stimulation with TNFα. Pectolinarigenin proved to be the most effective substance with a reduction of ICAM-1 expression of 35.5%, while the most effective sesquiterpene lactone only showed an inhibition of 21.3%. These results indicate a slight influence of the tested isolates in the inflammatory process. However, to fully understand the anti-inflammatory effect of these compounds, further approaches regarding their effects in the ICAM-1/NF-kB related pathways are necessary.

Keywords: *Achillea millefolium,* Sesquiterpene lactones, methylated flavonoids, anti-inflammatory, ICAM-1

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S2.P66 Evaluation of aromatase enzyme inhibition by extracts of *Bergenia crassifolia* (Saxifragaceae)

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Rhizomes and leaves of Bergenia crassifolia (L.) Fritsch (Saxifragaceae) are commonly used in the traditional medicine of several countries to treat a variety of diseases, such as gastrointestinal disorders, fevers and gynaecological conditions (Shikov et al., 2014; Koul et al., 2020). The plant contains several bioactive constituents like arbutin, bergenin, flavonoids and tannins. These compounds have been credited with some of the reported pharmacological activities of B. crassifolia, which include antimicrobial, antihypertensive, and anti-inflammatory effects (Shikov et al., 2010; Shikov et al., 2014). The aim of this ongoing study is to evaluate the ability of B. crassifolia extracts to inhibit aromatase (CYP19A1), a key enzyme in the biosynthesis of oestrogen, which is associated with different diseases, including breast cancer and endometriosis (Nelson et al., 2001). For this purpose, we established an aromatase enzyme inhibition assay, partially modelled on the basis of previously published methods (El-Kersh et al., 2021). Aromatase inhibition is assessed by measuring the fluorescence intensity of fluorescein benzyl ester, the aromatase metabolization product of dibenzylfluorescein (DBF). To optimise and streamline the assay, several different concentrations and combinations of substrate (DBF), enzyme (CypExpress™ 19A1), cofactors (NADPH and G6P) and positive control (ketoconazole) were evaluated before testing Bergenia extracts, which were obtained by accelerated solvent extraction (ASE) of flowers, leaves, and rhizomes with methanol. Initial results indicate that all three extracts exhibited a high degree of aromatase enzyme inhibition. Investigations to identify the constituents responsible for this activity are in progress.

Keywords: *Bergenia crassifolia,* endometriosis, aromatase, CYP19A1, inhibition

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S2.P67 Assessment of the anticonvulsant activity of *Myrica gale* L., a traditional irish plant in ethnoveterinary medicine, using a 4-AP induced seizure, electrophysiological model, *in vitro*

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The bogland species *Myrica gale* (MG) has ethnobotanical reports of its use for the treatment of 'fits in dogs' (Jackson, 2014) with 'fits' in Irish ethnography referring to 'seizures' (epilepsy). Other literature reports that many plant oils and their metabolites, especially terpenes, exhibit anticonvulsant activity. Seizures can be reduced or induced *via* inhalation of certain essential oils (EOs) (Tyagi et al., 2003; Yamada et al., 1994). This study assessed the acute anti-epileptic potential of two Irish MG EOs (EO1M, EO15C) and a commercial EO (COM) using *in vitro* electrophysiology. Extracellular local field potential (LFP) recordings measured ictal-like events (ILEs) in adult *Rattus norvegicus* brain slices (400µm). Application of 4-aminopyridine (26.7nM) elicited baseline ILE activity. Subsequently EOs were applied to the tissue *via* perfusate (0.5%v/v). LFP recordings of ILE activity were recorded from the superficial layers (II – III) of the medial entorhinal cortex. EO effects on ictal duration (ID), inter-ictal duration (IID), first spike amplitude (FSA), power spectrum density (PSD) and number of seizures (NoS), were analysed. Application of EO1M significantly reduced ID, IID, NoS, PSD (n=9, $P < 0.01$) and FSA ($P < 0.05$). EO15C significantly reduced ID, IID and NoS (n=9, $P < 0.01$). COM did not demonstrate significant effects on ID, IID, NoS, PSD or FSA (n=9, $P > 0.01$). MG extracted EOs can reduce ILEs in this model and our results support traditional ethnographic use. Qualitative and quantitative metabolomic differences between samples contribute to the variation in activity observed.

Keywords: *Myrica gale,* anticonvulsant, ILE, 4-aminopyridine, essential oils

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S2.P68 Centrifugal partition chromatography as a tool for the isolation of polyphenols from ginger rhizomes

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Zingiber officinale Rosc. (Zingiberaceae) belongs to the most widely consumed spices around the world. Its vast application in traditional medicine as an antiemetic, antibacterial, anti-inflammatory or bile production enhancing agent still triggers numerous studies on its metabolites (Kukula-Koch et al., 2019). The study aimed to optimise the protocol for the recovery of ginger polyphenols using modern extraction techniques, like supercritical solvent extraction (SFE), accelerated solvent extraction (ASE), ultrasound-assisted extraction (UAE), but also to perform an efficient fractionation of the ginger oleoresin obtained by supercritical fluid extraction (SFE) using centrifugal partition chromatography (CPC). A successful separation of the two major groups of ginger secondary metabolites, namely phenolic components and terpenes remains a difficult task to achieve. In the study, the applied gradient of hexane, ethyl acetate, buthanol and water enabled a fast recovery of the major phenolic components from the preparative CPC instrument equipped with a one-liter-volumed column, directly from the crude oleoresin. The performed optimisation of the biphasic solvent composition, rotation speed and solvent flow allowed for the selection of beneficial settings for the isolation of different polyphenols. The undertaken tasks allow for a large scale recovery of single polyphenols of ginger for food, cosmetic and pharmaceutical applications as both SFE and CPC can be operated in preparative or industrial scales.

Acknowledgements: The research was funded by the Polish National Science Centre project OPUS No 2021/41/B/NZ4/00337

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S2.P69 Comparative assessment of the antioxidant capacity of Domat olive leaf and Sari Yaprak olive leaf

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Oxidative stress can cause several degenerative conditions, including inflammation, cardiovascular diseases, and cancer. Organisms have inherent mechanisms to reduce oxidative stress, but it is not entirely effective. Thus, they can benefit from additional antioxidants. Many fruits, vegetables, and plant leaves have antioxidant properties. Olive leaves are particularly interesting because they have been used in traditional treatments throughout the Mediterranean (Lawrendiadis, 1961), and their health benefits have been supported by scientific studies (Reece et al., 1991).On the other hand, different olive leaves cultivars showed different antioxidant activity (El et al., 2009).

This presentation will cover the antioxidant capacity of two different *Olea europaea* Domat olives cultivars and Sari Yaprak olive leaves collected from Izmir, Turkey. Domat olive leaves showed higher antioxidant capacity when compared with 21 other olive cultivars (Orak et al., 2012). The Sari Yaprak olive cultivar is exclusively grown in Turgutlu and Manisa, Turkey. In addition, how the antioxidant capacity of sari yaprak olive leaves was affected by geographical features was tested. Different cultivars of olive leaves were extracted with 80% methanol. Their antioxidant capacities were compared with DPPH radical scavenging activity, CUPRAC (cupric reducing antioxidant capacity), and TOAC (total antioxidant capacity) assays. Their phytochemical properties were compared with total phenolic content and total flavonoid content. Sari Yaprak olive leaves showed higher total flavonoid content than Domat olive leaves, whilst Domat olive leaves had higher antioxidant activity on CUPRAC than Sari Yaprak olive leaves. Furthermore, environmental conditions such as altitude, climate, and light affected the total phytochemical assays and antioxidant activities of Sari Yaprak olive leaves.

Keywords: Olea europaea, Domat, Sari Yaprak, Phenolic Profile, Antioxidant

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S2.P70 Syringin accelerated wound healing and reduced inflammation in human skin fibroblastst and keratinocytes *in vitro*

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Previous studies have shown that syringin, a trans-sinapyl alcohol glucoside isolated from common lilac (*Syringa vulgaris* L., Oleaceae) bark, stimulates the secretion of transforming growth factor (TGFβ) by human monocytes/macrophages and inhibits tumor necrosis factor (TNFα) release (Filipek, 2019). TGFß is an important factor accelerating the regeneration process of damaged skin. It stimulates skin cell migration and proliferation, accelerating the process of restoring tissue continuity at the site of injury. On the other hand, a prolonged inflammatory process at the site of skin damage impedes the healing process. The aim of this study was to investigate the effects of syringin on the wound healing process *in vitro* using human dermal fibroblasts (NHDF) and keratinocytes (HaCaT) cell lines. In the presence of syringin (12.5 – 100 μM), the cells showed an increase in the invasion potential in scratch assay. A significant increase of migration through a porous membrane in modified Boyden's chambers in skin fibroblasts stimulated with syringin was also observed. Syringin stimulated TGFβ secretion by fibroblasts and keratinocytes and inhibited secretion of pro-inflammatory cytokines (IL-6, IL-8). Since the wound healing process can be impaired by prolonged inflammation, which hinders the transition to the proliferative phase, the presented action of syringin justifies its use as a potential agent for accelerating the healing of difficult wounds and ulcers.

Keywords: syringin, *Syringa vulgaris*, wound healing, migration, scratch assay

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S2.P71 Exploring the Impact of Different Heat Extraction Processes on the phytochemical profile of *Cichorium intybus* Extracts

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A wide array of extraction methods is described in pharmacopoeias for producing medicinal products from plant material, varying in extraction medium and temperature. Focusing on *Cichorium intybus*, we explored differences in extracts prepared at various heat levels, from room temperature to boiling point. *Cichorium intybus* extracts are used in Weleda AG's medicinal products for digestive complaints, atopic dermatitis, and allergic asthma, among others. Initially, we screened six *Cichorium intybus* planta total extracts, prepared from fresh, flowering, whole plants, using semi-quantitative analysis. In particular, four hydroethanolic and two aqueous extracts were prepared, including maceration, digestion, infusion, decoction, pressed juice, and rhythmic (Rh) extractions (Table 1). The extracts underwent filtration and lyophilization. Semi-quantitative analysis via UHPLC-HR-QTOF-MS/MS identified 32 secondary metabolites, such as flavonoids, polyphenols, acid esters, carboxylic and caffeic acids, and sesquiterpene lactones. Several metabolites are known for their anti-inflammatory, antioxidant, antimicrobial, and/or hepatoprotective properties, as well as more specific activities such as anti-allergenic, gastroprotective and lung protective effects. Notably, the infusion, followed by two other hydroethanolic extracts (decoction and digestion), showed the largest area under the curve (AUC) for over 60% of the detected metabolites, predominantly flavonoids, acid esters, and caffeic acids. In contrast, maceration, pressed juice, and Rh extraction exhibited smaller or undetectable AUCs for these metabolites, with higher AUC observed for carboxylic acids. These findings suggest that the therapeutic potential of *Cichorium intybus* may vary depending on the extraction methods and the extracted compounds. Subsequent *in vitro* cell models will assess the impact of these methods on pharmacological activities.

Table 1. Overview of the extraction methods for six *Cichorium intybus* extracts.

Sample	Extract medium	Temperature	Extraction time	Method
Maceration	Hydroethanolic	≤25°C	30 days	Ph.Eur. 1.1.7
Digestion	Hydroethanolic	37°C	1 hour	Ph.Eur. 1.2.5
Infusion	Hydroethanolic	Boiling point	5 minutes	Ph.Eur. 1.2.13
Decoction	Hydroethanolic	Boiling point (100°C)	30 minutes	Ph.Eur. 1.2.11
Pressed juice	Aqueous	≤25°C	Fresh plant	n.a.
Rhythmic	Aqueous	Alternating between 4°C (2-6°C) and 37°C (35- 39°C)	35 days	Ph Eur 1.5.1

Keywords: *Cichorium intybus*, plant extraction methods, metabolites, semi-quantitative analysis

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S2.P72 Modulation of Gastric Motility by a Traditional Herbal Preparation in Functional Dyspepsia: Implications of Muscarinic Receptor Inhibition

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This study investigates the impact of Amara-Drops, a traditional herbal remedy for gastrointestinal dysmotility, on fundus accommodation in functional dyspepsia (FD). FD is characterized by impaired gastric accommodation after food intake, however, its etiology is poorly understood. Amara-Drops consist of a mixture of nine hydroethanolic herbal extracts, including *Juniperus communis* (JC), *Peucedanum ostruthium* (PO), and *Salvia officinalis* (SO). We examined the mechanisms of Amara-Drops' dry and individual extracts on gastric motility *ex vivo*/*in vitro* and their implication for muscarinic receptors, crucial in gastrointestinal disorders. Muscle strips from guinea-pig (GP) gastric fundus were exposed to the whole extract, resulting in significant relaxation at the highest concentrations tested ($p < 0.001$). Additionally, a significant relaxation was observed in Carbachol-induced contracted muscle strips ($p < 0.05$), indicating potential modulation of the M2 receptor. *In vitro* studies revealed binding of the whole extract to both M2 and M3 receptors (IC_{50}: 249 and 380 µg/mL, respectively), with selective impairment of M2 receptor activity (IC_{50}: 219 µg/mL) by inhibiting intracellular cAMP release. JC, SO, and PO extracts showed inhibitory effects on M2 receptor activity (IC_{50}: 32, 20.1, and 20.8 µg/mL, respectively). Additionally, PO extract was found to bind to M2 receptors (IC_{50}: 137 µg/mL) and to induce relaxation in Carbachol-induced contracted muscle strips ($p < 0.001$). These results suggest that the pharmaceutical product Amara- Drops, particularly through *Peucedanum ostruthium* extract, modulates gastric motility by inhibiting M2 receptor activity. This finding offers a molecular explanation for the therapeutic use of Amara-Drops to address impaired gastric accommodation in FD.

Keywords: Herbal preparation, gastrointestinal dysmotility, fundus accommodation, muscarinic receptors, Carbachol-induced contraction

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S2.P73 *In vitro* liver metabolism of four biflavonoids and their corresponding monomers

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Biflavonoids gained more attention during the last years. These secondary plant metabolites can be found in some well-known medical plants such as *Ginkgo biloba* L. (Šamec et al., 2022) or *Hypericum perforatum* L. (Carrubba et al., 2021) and may contribute to their pharmacological effects. The physiological role of biflavonoids in plants is still discussed but might be related to protection against UV radiation or predators as they are located in plant leaves (Šamec et al., 2022). Pharmacologically, these dimeric flavonoids are known for a wide range of effects such as their anti-inflammatory, antioxidant and antiviral properties (He et al., 2021). Recently, their application and therapeutic benefits in microbial diseases, Alzheimer's disease or metabolism-related diseases such as type2-diabetes was discussed (Menezes et al., 2021). Information on human liver metabolism of flavone dimers especially compared to their corresponding monomers are lacking. Thus, we investigated the metabolic stability of amentoflavone, I3,II8-biapigenin, bilobetin, hinokiflavone, apigenin and acacetin by incubation with human liver S9 fraction. Additional to the microsomal fraction of the liver, the S9 fraction contains the cytosolic fraction as well. Hence, it provides both phase I and phase II enzymes (Richardson et al., 2016). The received metabolites were identified using UHPLC-DAD-MS or HPLC-DAD-MS/MS revealing phase II glucuronidation products as main metabolites for all flavonoids. Interestingly glucuronidation pattern and number of metabolites varied for each compound based on the subunits and their linkage.

Keywords: biflavonoids, flavones, liver metabolism, S9 fraction

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S2.P74 Screening of flavonoids and phenolic compounds in *Betula litwinowii* Doluch. by LC-Q- TOF/MS and determination of their antioxidant potential

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Birch tree (*Betula* L.) is a tree-shaped plant distributed in the Northern Hemisphere, and its leaves, buds, bark and essential oil are traditionally used for many purposes (Rastogi et al., 2015). To the best of our knowledge, the flavonoid and phenolic compounds of *Betula litwinowii* have not been investigated before. Therefore, the aim of this study is to reveal the flavonoids and phenolic compounds found in the buds and leaves of *B.litwinowii* collected from Van, Türkiye, to determine the total phenolic (TP) and flavonoid contents (TF) and to evaluate the antioxidant activity capacity. Qualification of compounds in aqueous alcohol extracts (80/20:v/v) was performed using a LC-Q- TOF/MS system equipped with an electrospray ionization interface operating in positive and negative ion mode (Sevimli-Gur et al., 2021). According to METLIN library results, extracts prepared from leaves and buds contain quinic acid, quercetin, quercetin 3-*O*-glucoside, quercetin 3- *O*-malonylglucoside, 5-desoxyquercetin, 3-*O*-methylquercetin, rutin, luteolin, diosmetin, genkwanin, gallic acid, epigallocatechin, epicatechin, catechin 7-β-D-xylopyranoside, chlorogenic acid, apigenin, sinapic acid, salidroside, naringenin, maslinic acid, kaempferol, kaempferol-*3*- glucuronide, 3-methylkaempferol, 8-hydroxykaempferol, caffeic acid, betulonic acid, betulinic acid, 4-*O*-methylgallate, 4-hydroxybenzaldehyde, 3,4-dihydroxybenzoic acid, procyanidin B2, and myricetin tetramethyl ether. The TP were 220,65 ± 3.83 mg gallic acid equivalent (GAE)/g extract and 243,65 ± 4.27 mg GAE/g extract for the leaves and buds respectively while the TF were 33,44 ± 2.13 and 49,6 ± 2.75 mg quercetin/g extract. The antioxidant capacity of the extracts was evaluated using the DPPH, ABTS, and FRAP techniques.

Keywords: *Betula litwinowii,* Betulaceae, LC-Q-TOF/MS

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S2.P75 The extracts of *Capparis cartilaginea* Decne attenuate inflammasome activation in LPS- stimulated THP-1 macrophages

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Capparis cartilaginea Decne. (CC), originating from arid regions in Asia and the Mediterranean basin, is recognized in traditional medicine for its efficacy in treating inflammatory conditions like rheumatoid arthritis, osteoarthritis, and gout through the common use of its leaf tea (Tounekti et al. 2019). However, the underlying anti-inflammatory mechanism of CC is still unclear. Recently, leucine-rich repeat protein-3 (NLRP3) inflammasome activation has been demonstrated to be essential in the pathogenesis of arthritis (Yin et al., 2022). In the present study, we aimed to investigate the effects of CC extract, tea, oil, and their isolated compounds (Alsharif et al., 2023) on NLRP3 inflammasome activation in LPS/ATP-stimulated THP-1 cells. The levels of inflammatory cytokines and NLRP3 inflammasome-related proteins were detected by ELISA and western blotting (WB), respectively. We found that CC extract significantly reduced NLRP3 expression, ASC speck formation, and caspase-1 cleavage, and therefore, IL-1β and IL-18 secretion in THP-1 cells. The extract also inhibited NLRP3 and caspase-1 activation in LPS/ATP-stimulated THP-1 cells. Furthermore, mitogen-activated protein kinase (MAPK) activation in P. aeruginosa LPS/ATP stimulated THP-1 was also attenuated by the extract and some of the tested compounds. These results support the use of this medicinal plant to treat NLRP3 inflammasome-driven inflammatory diseases such as arthritis.

Keywords: *Capparis cartilaginea,* capparaceae, flavonoids, NLRP3 Inflammasome

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S2.P76 *Pistacia chinensis* Bunge subsp. *falcata* (Becc. ex Martelli) aqueous extract possesses acetylcholinesterase and monoamine oxidase inhibitory activities

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Plants in the genus *Pistacia* represent valuable natural resources for neuroprotection research based on ethnomedicinal knowledge (Al-Saghir and Porter 2005). Research findings have consistently affirmed the significance of bioactive compounds found in *Pistacia* for addressing various central nervous system disorders, including Alzheimer's disease, Parkinson's disease, multiple sclerosis, cerebral ischemia, depression, and anxiety (Moeini, Memariani et al. 2019). *Pistacia chinensis* Bunge subsp. *falcata (*Becc. ex Martelli*)* (*P. chinensis*) belongs to the Anacardiaceae family and has received limited study on phytochemistry and biological activities compared to other members (Crutcher, Schroeder et al. 2023). This study aimed 1- to characterise the phenolic compounds in *P. chinensis* extract using high-performance liquid chromatography coupled with electrospray ionisation mass spectrometry (HPLC-ESI-QTOF-MS) and 2- to evaluate the neuroprotective potential of the extract through the inhibition of CNS enzymes such as acetylcholinesterase (AchE), and monoamine oxidases (MAO-A and MAO-B). *P. chinensis* was found to be rich in phenolic acids and to a lesser extent in flavonoids, which explains its significant DPPH antioxidant activity (IC_{50} of 23.37 ± 0.63 µg/mL). The extract was able to inhibit MAO-B and AchE. The anti- monoamine oxidase, anticholinesterase, and antioxidant properties exhibited by *P. chinensis* extract could contribute to its neuroprotective abilities. Thus, the plant can potentially serve as a source of raw material for the industry of functional foods and nutraceuticals targeting the management of neurodegenerative diseases.

Keywords: *Pistacia chinensis*, phenolic compounds, flavonoids, neuroprotective activity, neurodegenerative diseases

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S2.P77 Cyclotides from Bolivian plants

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Cyclotides are a family of circular peptides derived from plants that possess a significant range of biological activities, such uterotonic, antimicrobial, immunosuppressive, anti-HIV and cytotoxic (Niyomploy et al., 2018). They are cyclic compounds with around 28-37 amino acids (AA) where the N and C terminals are bound together by a peptide bond. They also contain six cysteine residues which for three disulfide bonds to form a Cyclic Cystine knot (CCK) (Burman et al., 2014). Currently there are many publications that give account of the properties of these compounds (Jennings et al., 2001; Svagnard et al., 2004) and there is an ongoing interest in finding new structures with different properties, which can be achieved either by synthetic modifications or by looking for cyclotides in different species that have not been studied before. In the current study, we aim for the latter option. Given the vast Bolivian biodiversity and because of the unique environmental characteristics of certain regions of the country (Ibisch et al., 2003), we believe that the plants that grow in these regions could develop compounds of interest. Therefore, different samples of species from the Violaceae and Rubiaceae families have been collected in Bolivia. These species have not been studied yet and they could represent a source of new structures of cyclotides.

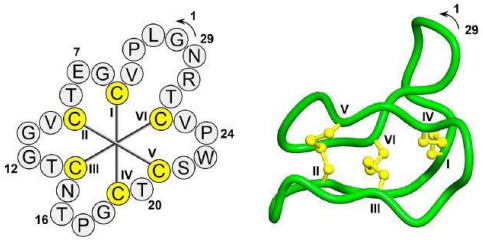

Fig. 1. Sequence and representation of the cyclotide Kalata B1. (De Veer et a., 2014)

So far, from the eleven samples that have shown positive content of cyclotides, the compounds found in *Viola boliviana* have been isolated and processed for sequencing through mass spectrometry (MS). The cyclotides were reduced, alkylated and digested with enzymes for their structural elucidation. As for now, the structure of Kalata B1, Kalata S and Vpub A has been elucidated from one sample of *Viola boliviana.* Although these are known structures, we hope that further analysis will bring unknown structures, since there are more masses to be identified.

Keywords: cyclotide, *Viola boliviana,* kalata, Violaceae, Bolivia

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S2.P78 Cytotoxicity assessment of *Rosa pisiformis* (Christ) D. Sosn. (Rosaceae) endemic to Turkiye

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Interest in medicinal plants grows due to their low cost, availability, and minimal side effects. Among diverse medicinal plants, the genus *Rosa* L. is known to possess various species with promising anticancer activity (Fayaz et al., 2024). However, research exploring the bioactivity potential of *Rosa pisiformis* (Christ) D. Sosn. (Rosaceae), a Turkish endemic species (Kültür, 2003), remains limited. This study aims to evaluate the cytotoxic activity of *R. pisiformis* extracts against various cancer cell lines using the MTT assay (Mosmann, 1983). In this context, MCF-7 (breast cancer), HT-29 (colon cancer), and A-549 (lung cancer) cells were treated with ethanolic and hydroalcoholic (ethanol 80% v/v) extracts of leaves and fruits of the plant collected from Gumushane/Turkiye. Experiments were triplicated, with 5-Fluorouracil (5-FU) as the positive control. Cell viability was determined by comparing the formazan concentrations of the treated cells with those of the untreated control. The ethanolic fruit extract of R. pisiformis exhibited the most promising results among thetested extracts (Table 1). In the MCF-7 cell line, it exhibited an IC_{50} value of 14.37 ± 0.05µg/mL compared to 5-FU's IC_{50} value of 2.86 ± 0.30 µg/mL, indicating significant but lesspotent cytotoxicity compared to 5-FU. While most effective against MCF-7, it also displayedmoderate activity against HT-29 and A549 cell lines, with IC_{50} values of 36.22 ± 0.13 µg/mL and 48.84 ± 0.28 µg/mL, respectively.

Table 1. Cytotoxic activity of ethanolic and hydroalcoholic extracts of *Rosa pisiformis*

Extracts	MCF-7 (IC_{50}, µM)	HT-29 (IC_{50}, µM)	A549 (IC_{50}, µM)
Ethanolic fruit extract (100%)	14,37±0,05	36,22±0,13	48,84±0,28
Hydroalcoholic fruit extract (80:20; v/v)	33,07±0,20	84,82±0,24	78,44±0,23
Ethanolic leaf extract (100%)	54,84±0,25	89,23±0,23	110,34±0,23
Hydroalcoholic leaf extract (80:20; v/v)	40,99±0,32	90,15±0,28	52,99±0,37
5-FU	22±2,33	25,98±3,1	44,21±3,54

This is the first report revealing the potent cytotoxicity of *R. pisiformis* against MCF-7 breast cancer cells, suggesting its potential for further investigation as a therapeutic candidate.

Keywords: *Rosa pisiformis,* MTT, cytotoxicity, cancer

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S2.P79 Confirming (–)-epicatechin's biological signature; universal modulation of NOS in chronic disease

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Cardiovascular disease (CVD), common in those suffering from diabetes, is the leading cause of global mortality (WHO, 2021). Our work shows that botanical flavonoid (–)-epicatechin stimulates vasodilation and insulin secretion (Chun et al., 2022). As insulin regulates vessel reactivity, we hypothesized that (–)-epicatechin acts globally by modulating enzyme nitric oxide synthase (NOS), a regulator of vessel reactivity (eNOS) and pancreatic inflammation (iNOS), critical to insulin production. We administered 1 mg/kg/day of (–)-epicatechin to female Wistar rats for 7 days, then injected caerulein to induce pancreatitis. We measured glucose tolerance prior to caerulein treatment, and vascular reactivity, pancreatic iNOS and insulin production post-sacrifice. (–)- Epicatechin significantly lowered glucose area under the curve (AUC) as compared with control during a glucose tolerance test (4,458 ± 1167 AUC vs. 7,973 ± 1,177 AUC, $p<0.05$). Caerulein treatment resulted in diminished percentage of vasodilation response to acetylcholine (ACh, upstream of eNOS), restored by (–)-epicatechin (55.3 ± 8.2% vs. 69.5 ± 2.7%, 10 μM ACh, $p<0.05$). (–)-Epicatechin treatment resulted in lower expression of iNOS in (–)-epicatechin-treated animals as compared with controls (0.0091 ± 0.0039 colorimetric intensity units [CIU] vs. 0.0542 ± 0.0232 CIU, $p<0.05$), and elevated expression in animals treated with both (–)-epicatechin and caerulein as compared with caerulein alone (0.5353 ± 0.0649 CIU vs. 0.2710 ± 0.1068 CIU, $p<0.05$). (–)-Epicatechin restores caerulein-damaged vasodilation and modulates pancreatic iNOS expression. These data support our hypothesis that (–)-epicatechin globally modifies NOS activity, suggesting its potential use in chronic disease.

Keywords: cardiovascular disease, diabetes, vasculature, flavonoid, nitric oxide synthase

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S2.P80 Analytical development on *Pulsatilla* species: comparison of two species

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This presentation will cover the analytical development and the validation of a method to assay the protoanemonin content in different species of *Pulsatilla*. Protoanemonin is one of the most characteristic compounds of Pulsatilla species and is considered as a toxic marker. In order to obtain a precise quantification of this compound in a hydro-alcoholic extract of the plant, an analytical method using liquid chromatography was set and a validation of the method was performed for two different species and protoanemonin contents were compared. The different results, particularly the accuracy profile of the methods is presented. Botanical characteristics and other constituents such as flavonoids and phenolic compounds are also given a complete comparison of Pulsatilla species. The methodology presented to set the botanical characterization and the analytical method can be considered as appropriate to elaborate pharmacopoeial monographs.

Keywords: *Pulsatilla pratensis* Mill., *Pulsatilla vulgaris* Mill., analytical method, development, validation, accuracy profile, protoanemonin, HPLC, flavonoids, phenolic compounds

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S2.P81 Wound healing effects of *Arnica montana* formulations administered by different routes in mice

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This presentation will cover the use of different preparations of Arnica in wound healing. *Arnica montana* L. (*A. montana*) is a traditionally therapeutic plant, known for antalgic and anti- inflammatory properties (French Pharmacopeia 11th Edition, 2013; Iannitti et *al.*, 2016). The plant is used to treat skin bruises, sprains, contusions, and hematomas but, to our knowledge was never tested so far in incisional wounds in mice. Blinded treatments of *A. montana* 5C or *A. montana* mother tincture gel at 7% (w/w) and their respective controls given by systemic or topical routes were performed daily during 20 days. An External Macroscopic Global score (EMG) taking into account the length, the width, the swelling and the visibility of the wound, were performed in order to evaluate the progression of wound healing. The inflammatory cell infiltration, necollagen, re- epithelialization and angiogenesis were quantified by histological and immunohistochemical analyses. At Day 2, both treatments significantly decreased EMG scores faster compared with their placebos. Moreover, *A. montana* 5C and *A. montana* gel significantly accelerated complete wound closure by 1.7 or 3.1 days and reduced inflammatory cell infiltrations by 40% or 55.6% respectively compared with placebos. Finally, *A. montana* 5C significantlly improved the formation of neocollagen, re-epithelialization and angiogenesis while *A. montana* gel only favoured re- epithelialization. *A. montana* 5C does not impact cell viability in primary cells (Paumier et *al.*, 2022). In conclusion, we showed that independent of the route of administration, *A. montana* acts in a similar way on the several overlapping phases of wound healing.

Keywords: *Arnica montana* L., inflammatory response, wound healing, mouse

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S2.P82 Effect of *Tormentillae tinctura* and its postbiotic metabolites on human intestinal epithelium cellular model

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Tormentillae tinctura (TT) has been used for centuries to treat gastrointestinal tract ailments (Melzig et. al., 2020). Due to its tannin-reach composition, TT may have a beneficial effect on the intestinal epithelium. Thanks to these properties, TT could offer an opportunity for novel approaches in the therapy of leaky gut syndrome, which is a serious ailment that reduces the quality of life (Granica et. al., 2020). The presented research aimed to determine the effect of TT and its postbiotic metabolites (TTMs) on the integration and stability of the intestinal barrier. TTMs were obtained by incubation of TT with human fecal slurries from 3 healthy donors. After incubation, TTMs were analyzed (UHPLC-DAD-MSn) and isolated (solid phase extraction). As a cellular model, a Caco-2 monolayer with induced tight junction (TJ) disruption by bacterial toxins was used. The influence of TT and TTMs on cytotoxicity, transepithelial electrical resistance (TEER) value, and TJ proteins production (western blot) and expression (qPCR) on Caco-2 intestinal epithelium was determined. It was observed that TT and TTMs increased the TEER value and modulated the expression and production of selected TJ proteins, thereby beneficially influencing the stability and integrity of the intestinal epithelium.

Acknowledgments: The project has been funded by the Polish National Science Centre research grant Sonatina 5 2021/40/C/NZ7/00231.

Keywords: intestinal epithelium, leaky gut, postbiotic metabolites, *Tormentillae tinctura*

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S2.P83 Effects of the Korean Herbal Prescription, Bojanggunbi-tang, on indomethacin--induced Small Bowel Injury focusing on mechanisms of action

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Bojanggunbi-tang (BGT) is a well-known combination of ancient herbal medicines in Korea and has been widely used for treating gastrointestinal symptoms. The effects and mechanisms of action of BGT on non-steroidal anti-inflammatory drug-induced small intestinal injury (NSI) was evaluated by using a murine model of indomethacin-induced NSI. Indomethacin (15 mg/kg) was subcutaneously injected to induce NSI mice model. BGT was administered twice, 30 min before and 6 h after the induction of NSI, at doses of 50, 150, and 450 mg/kg, while the positive control received sodium alginate. Body weight, small intestine length, macroscopic damages, and histological damages were assessed 24 h after induction. Proinflammatory cytokines, tight junction proteins and gut commensal microbiota were investigated to reveal the mechanisms of action. BGT alleviated the shortening of small intestine, ulcer, and inflammation scores. In addition, BGT inhibited loss of the tight junction proteins including zonula occludens-1, occludin and claudin-1, the proinflammatory cytokines including interleukin (IL)-6 and IL-1b, and the gut commensal microbiota including *E. coli*. In conclusion, BGT ameliorated NSI via protective effects on tight junction, and anti-inflammatory and anti-ulcerative properties. The current study suggests that BGT could be a therapeutic option for NSI.

Acknowledgments: This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MSIT) (No. 2022R1C1C1004937).

Keywords: non-steroidal anti-inflammatory drugs, small intestinal injury, inflammation, tight junction, bojanggunbi-tang

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S2.P84 Interaction of an herbal multi-component medicine for treatment of digestive disorders with the growth of selected intestinal lactic acid bacteria

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The multi-component herbal medicine Amara-Drops is indicated for treatment of moderate digestive disorders like fullness, heartburn, nausea and disturbed gastrointestinal motility. It is composed of nine different hydroethanolic herbal extracts, *Artemisia absinthium* (AA), *Centaurium erythraea* (CE), *Cichorium intybus* (CI), *Gentiana lutea* (GL), *Juniperus communis* (JC), *Millefolii herba* (MH), *Peucedanum ostruthium* (PO), *Salvia officinalis* (SO) and *Taraxacum* (T). A well functioning digestion is inevitably linked to a healthy intestinal microbiota. Thus, medication should not interfere with intestinal commensal microbes. We aimed to evaluate the interaction of the herbal extracts with *Lactiplantibacillus plantarum* and *Ligilactobacillus salivarius* using an *in vitro* approach. Dry extracts were prepared from mother tinctures of extracts used for production of the final medicinal product Amara-Drops. The antibiotic tetracycline was used as reference compound (15µg/mL). Bacteria were incubated in liquid culture under constant shaking at 37°C for up to 7 h together with test samples or controls. The growth of *L. plantarum* and *L. salivarius* was not affected at concentrations <5 mg/mL. Only JC and SO showed antibacterial effects towards *L. plantarum* and *L. salivarius* at very high concentrations between 5-10 mg/mL. JC and SO showed now effect at 1 mg/mL. The reference antibiotic completely inhibited the growth of tested bacterial strains (Tab. 1).

Tab. 1: Overview of differential effects of herbal extracts from Armara-Drops on growth of bacterial strains *in vitro*. = growth promotion; = no effect; = growth inhibition; = no effect at low concentration (1 mg/ml), inhibition at high concentration (5-10 mg/ml).

	Amara extract mixture	*Artemisia absinthium*	*Centaurium erythraea*	*Cichorium intybus*	*Gentiana lutea*	*Juniperus communis*	*Millefolii herba*	*Peucedanum ostruthium*	*Salvia officinalis*	*Taraxacum*	Tetracycline (15µg/ml)
L. plantarum											
L. salivarius											

These results indicate that Amara-Drops are compatible with the intestinal commensal bacterial strains *L. plantarum* and *L. salivarius* and might be considered as microbiome-friendly. Additional studies are required to further characterise the compatibility of Amara-Drops with intestinal microbiota.

Keywords: Amara, lactobacilli, intestinal microbiome, microbiome friendly

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S2.P85 *Vangueria infausta* Burch (wild medlar) extract improves semen quality by regulating testosterone levels and oxidative stress in testicular Leydig cells

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Recently, men's sexual reproductive challenges have emerged as a public health problem. The utilization of medicinal plants for the treatment and management of male infertility could provide an alternative to available chemical drugs. This study aims to identify the bioactive compounds of *Vangueria infausta* Burch (VI) plant and investigate the antioxidant, cytotoxic, and androgenic activities of acetone (A), ethanol (E), methanol (M), and water (W) extracts against TM3 Leydig cells. Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis revealed the presence of rutin, quercetin glucoside, nicotiflorin, quercitrin, quinic acid, caffeoylquinic acid (1, 2, and 3), 13S-Hydroxy-9Z,11E,15Z-octadecatrienoic acid, 12,13-Dihydroxy-9Z octadecenoic acid, and dicaffeoylquinic acid. Quantitative phytochemical analysis showed that the VI@A had the highest total flavonoid content (TFC) (221.95 mg QE/g) and proanthocyanidins content (PAC) (202.71 mg CE/g), while the VI@E had the highest total phenolic content (TPC) (83.76 mg 24 GAE/g). Also, the VI@M and VI@A extracts had the highest potential to scavenge DPPH and ABTS (482.44 and 0.36 µmol TE/g, respectively), while the VI@E induce strong ferric ion- reducing power (0.27 µmol TE/g). Besides, all four extracts of VI inhibited TM3 cell growth with increasing dosage while glutathione, lipid peroxidation, and DNA fragmentation pattern provided insights relating to the antioxidant effects on cells. Moreover, the enzyme-linked immunosorbent assay showed that VI@E and VI@W extracts increased the production of testosterone from TM3 cells in the presence of human chorionic gonadotropin (hCG). These findings provide the scientific basis for the use of *V. infausta* as a source of natural antioxidants with androgenic effects.

Keywords: *Vangueria infausta* Burch, antioxidant activity, cytotoxicity, testosterone, TM3 Leydig cells

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S2.P86 Phytochemical, antioxidant, and androgenic effects of multi-solvent extracts from *Strychnos spinosa* Lam on mouse testicular TM3 Leydig cells

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Men's sexual and reproductive health challenges including infertility, lack of libido, and erectile dysfunction are of concern in the last few decades. The utilization of medicinal plant extracts in their treatment and prevention could be beneficial. This study evaluated the antioxidant content, phytocompounds, and androgenic activity of *Strychnos spinosa* (SS) leaf extracts from different solvents (water W, methanol M, ethanol E, and acetone A) on TM3 Leydig cells. Ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis UPLC-Q-TOF-MS analysis of the plant material further revealed the presence of 13 bioactive compounds mainly polyphenols and other components such as caffeoylquinic acid (1, 2, and 3), dicaffeoylquinic acid (1, 2, and 3), 13S- Hydroxy-9Z,11E,15Z-octadecatrienoic acid, and 12,13-Dihydroxy-9Z-octadecenoic acid. Specifically, the SS-A extract contained the highest total phenolic content (TPC) (419.04 mg GAE/g), total flavonoid content (TFC) (145.46 mg QE/g), and proanthocyanidins content (PAC) (131.43 mg CE/g) compared to other extracts. Besides, the SS-M extract had the highest ABTS scavenging potential (0.3759 µmol TE/g) while the SS-W extract had the highest DPPH radical scavenging ability (542.78 µmol TE/g) and strong ferric-reducing power (FRAP) activity (0.2098 µmol TE/g). All four extracts of SS decreased the viability of TM3 cells in a dose-dependent manner while the mitochondrial membrane potential and DNA fragmentation pattern provided insights into the underlying mechanism of cell inhibition. Additionally, treatment with extracts enhanced testosterone production by TM3 cells in the presence of human chorionic gonadotropin (hCG). In summary, the fascinating androgenic properties displayed by SS extracts highlighted their potential natural remedy in sexual health treatment

Keywords: antioxidants, phytochemicals, *Strychnos spinosa* Lam., testosterone, TM3 Leydig cells

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S2.P87 Unlocking longevity through the use of plant adaptogens: lifespan extension by Maral root (*Rhaponticum carthamoides* (Willd.) Iljin) extract in *Caenorhabditis elegans*

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As human life expectancy continues to rise, the burden of age-related diseases and diminished healthspan in late-life years remains a significant challenge. Consequently, there is a growing need for effective strategies to enhance both the quality and duration of life (Guarente et al., 2024). Longevity-promoting interventions, particularly those derived from natural sources, are gaining attention due to their potential to address age-related health issues (Todorova et al., 2024). *Rhaponticum carthamoides* (Willd.) Iljin, commonly known as Maral root or Russian leuzea, is a renowned adaptogenic plant in Siberian traditional medicine (Todorova et al., 2023). The chemically diverse root extract of this plant represents a unique reservoir of bioactive compounds, including flavonoids and phytoecdysteroids. Notably, Maral root has been linked to fatigue reduction, improved strength, immunomodulatory and anti-aging properties (Todorova et al., 2023). Building upon this premise, our study explores the potential of Maral root to modulate the aging processes, utilizing *Caenorhabditis elegans* as a model system. To comprehensively evaluate the impact of Maral root on aging, we employed a range of approaches, including behavioral and stress assays. The results of our investigation demonstrate not only an extension of lifespan but also a notable enhancement in healthspan, accompanied by improved fitness in the nematode population. The observed effects underscore Maral root's potential as a promising natural adaptogen capable of mitigating the decline associated with aging. These findings contribute valuable insights to the growing body of research focused on natural interventions for promoting longevity and combating age-related health challenges.

Keywords: longevity, adaptogens, *Caenorhabditis elegans,* maral root*, Rhaponticum carthamoides*

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S2.P88 Revealing the veil of barberry alkaloids: high-throughput non-targeted UHPLC-DIA-HRMS profiling enhanced by solid phase extraction based on mixed-mode sorbent

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Several isoquinoline alkaloids have been identified as antiviral agents with broad-spectrum activity against animal and human coronaviruses (Fielding et al., 2020). Based on our previous research, this study focuses on examining isoquinoline alkaloids within the genus *Berberis* L. (Berberidaceae). It's worth noting that a number of these alkaloids have shown promising efficacy against the SARS-CoV-2 spike protein in pseudovirus neutralization assays. Our study is focused on exploring and elucidating the phytochemical profiles of isoquinoline alkaloids, emphasizing the diversity of their structures and quantitative variations within this genus. Our research is focused on devising an analytical approach for the high-throughput comprehensive profiling of more than 20 isoquinoline alkaloids via UHPLC-HRMS with the qTOF platform. We evaluated multiple stationary phases during the method optimization process (BEH C18, CSH C18, Biphenyl, ACE C18-PFP) and experimented with different mobile phase compositions. This included variations in organic components (ACN, MeOH) and water components such as 10 mM ammonium formate at pH 3, 4, 5, and 9, along with concentrations of formic acid (0.02%, 0.1%, 0.5%) and ammonium hydroxide (0.02%, 0.1%, 0.5%). We designed a solid-phase extraction (SPE) procedure for sample preparation utilizing a mixed-mode polymeric sorbent with weak cation and reverse phase properties. This purification method effectively removes undesirable acidic and neutral secondary metabolites from the barberry crude extract, leading to improved performance of UHPLC-HRMS analysis. Our comprehensive methodology was effectively employed for non-targeted UHPLC- DIA-HRMS screening of various barberry species.

Keywords: *Berberis* spp., Berberidaceae, isoquinoline alkaloids, WCX-SPE, UHPLC-DIA-HRMS

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S2.P89 *Viscum album* extracts in the perioperative oncological setting: evidence from bench to bedside

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This presentation will show preclinical and clinical evidence on the use of aqueous extracts from *Viscum album* (L.) to reduce postoperative immunosuppression in the oncological setting. Postoperative immunosuppression, especially a reduced cell-mediated immunity, is often seen after surgery and is associated with worse outcome, i.e. delayed wound healing, infections, sepsis, multiple organ failure and cancer recurrence (Lachmann et al., 2018). Aqueous extracts from the European mistletoe *Viscum album* (L.) contain mistletoe lectins, viscotoxins, flavonoids and polyphenols (Urech et Baumgartner, 2015). They stimulate the maturation of dendritic cells, activate natural killer cells and have anti-inflammatory properties by specifically inhibiting cyclooxygenase-2 *in vitro* (Oei et al., 2019). Given parenterally before tumour surgery, mistletoe extracts reduce natural killer cell and neutrophil suppression (Oei et al., 2019). A recent meta- analysis found no safety signals in 10 controlled trials using mistletoe extracts in the perioperative oncological setting (Cogo et al., 2023). The two included randomized controlled trials reporting on physical function showed an improvement in the mistletoe extract group. These results support the evidence from a randomized controlled trial performed in 220 postoperative pancreatic cancer patients, in which mistletoe extracts improved physical function in a clinically and statistically significant manner, together with seven of nine EORTC QLQ-C30 symptom scales (Tröger et al., 2014). Two follow-up randomized controlled trials have been performed in the same population with 290 participants each (EudraCT numbers: 2014-002386-30, 2014-004552-64) and are expected to be published soon.

Keywords: *Viscum album,* immunomodulation, anti-inflammation, cancer surgery, physical function

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S2.P90 Potential benefits of *Malva sylvestris* in treating dry-eye pathology *in vitro*

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Given to the exposure to the pollution, digital lifestyle, and ultraviolet (UV) radiation, the worldwide prevalence of dry eye disease (DED) has increased (Patel et al., 2023). The grittiness and burning sensations of DED deteriorate one's quality of life immensely, making it a common cause of visits to ophthalmologists (Tovar et al., 2023). This study investigated the medical potential of *Malva sylvestris* flower extracts via *in vitro* cellular systems capable of simulating DED pathology.

M. sylvestris flower extract, its mucilage and polyphenol fractions facilitated 2D wound healing in human corneal epithelial cell line (HCE-T) and reduced intracellular reactive oxygen species when exposed to UVB at 30 µg/mL. Significant superoxide radical-scavenging properties were found to be prominent in flower extract and polyphenol fraction. The LPS-exposed monocytic (THP-1) cells when treated with polyphenol fraction lowered NF-κB expression at 50 µg/mL in a dose-dependent manner as well as significantly lowered pro-inflammatory cytokines (IL-6, IL-8, TNF-α) in the supernatants. The fraction was also demonstrated to diminish calcium influx in Jurkat cells. Moreover, one of the most crucial factors in DED development is a reduction of the tear film's lipid layer. Stimulation of lipid production was observable following the exposure to the flower extracts and mucilage fraction in an immortalized human meibomian gland epithelial cell (IHMGEC). Overall, the results indicate that the tested plant extracts interfere with the function of cells involved in DED pathology, thus providing a rationale for their potential therapeutic use. However, further investigation is needed to fully validate this hypothesis.

Keywords: *Malva sylvestris,* dry eye disease, reactive oxygen species, cytokines, lipid production

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S2.P91 The relationship among essential oil composition, morphological and climatic diversification - the case of *Teucrium montanum* from the Balkan Peninsula

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Teucrium montanum L. (Lamiaceae) is a small, evergreen, semi-woody shrub that has a wide ecological preference and grows in various environments. The Balkan Peninsula is characterized by a complex geological history and very heterogeneous climatic conditions in a relatively small area. *Teucrium montanum* shows high morphological and anatomical variability in the Balkan Peninsula and seven morphological groups have been recognized (Zbiljić et al., 2023). The aim of our work was to determine whether the phytochemical variability of essential oils follows morphological diversification and to determine the influence of climatic conditions. Isolated essential oils from 46 populations were analysed by GC/FID/MS and multivariate statistical analysis. The main compounds in the investigated oils were: sesquiterpenes germacrene D-4-ol, shyobunol, *cis*- sesquiterpene hydrate, α-bisabolol, germacrene D and monoterpene limonene. Principal component analysis (PCA) showed significant chemical variability in the analysed samples, with the morphological group "montanum" showing the greatest changes, similar to the pronounced morphological variations of this group. In addition, there were specific compounds for the morphological groups that led to a clear separation in the discriminant analysis (CDA). Certain morphological groups ("parnassicum", "skadarensis" and "pannonicum") can be easily distinguished according to both phytochemical and morphological characteristics. The biosynthesis of various compounds was influenced by the climatic conditions. The lack of water caused the increase of limonene content in the population influenced by arid continental climate and of cryptomerione and (*E*)-nerolidol affected by arid submediterranean climate. However, it was found that phytochemical diversification followed morphological rather than climatic variability.

Keywords: *Teucrium montanum*, Lamiaceae, essential oil, morphological variability, climatic conditions

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S2.P92 Salicylic alcohol content in the leaves of different *Salix* species and individuals: impact on pharmaceutical potential

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The genus *Salix* belongs to the family of Salicaceae and comprises around 500 different species (Meier et al., 1998). The bark is used traditionally in European countries due to its antiphlogistic, antipyretic, and analgesic efficacy (März et al., 2002). The Ph. Eur. does not define a species for the pharmaceutical use of Willow bark, but a minimum content of 1.5% total salicylic derivatives is required (Ph. Eur., 2023). The salicylic alcohols are metabolized *in vivo* to salicylic acid, the pharmacologically active form (März et al., 2002). Aim of the present study was whether *Salix* leaves could also be of pharmaceutical interest concerning their salicylic alcohol content. For that, the leaves of 12 different *Salix* species *(Salix aurita, S. bicolor, S. caesia, S. caprea, S. cinerea, S. daphnoides, S. fragilis, S. hastata, S. lapponum, S. purpurea, S. viminalis, S.* x *sepulcralis)* with 42 individuals were collected in the months of May-September from 2018-2019. The samples were analyzed for their phenolic profile and especially salicylic alcohol content using an RP18-UPLC®- PDA method (Wiesneth, 2017). Furthermore, inter-individual differences and differences between the sexes within a species were also of interest. *S. caesia, S. caprea, S. daphnoides*, *S. hastata, S. lapponum, S. viminalis,* and *S.* x *sepulcralis* contained hardly any relevant amounts of salicylic alcohol derivatives whereas *S. aurita, S. bicolor, S. cinerea, S. fragilis,* and *S. purpurea* contained more than 1.5%. Barely any differences were found between the sexes. This indicates that also the leaves of some *Salix* species could be of pharmaceutical interest.

Keywords: *Salix*, salicaceae, salicylic alcohol, Ph. Eur., European Pharmacopoeia

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S2.P93 Plant metabolites as glucose-induced insulin secretion modulators

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Type 2 diabetes mellitus (T2DM) is a disease that associates insulin resistance and pancreatic β-cell dysfunction. Based on the current understanding of the pathophysiology of this disease, multiple anti-diabetic therapies have been developed to improve glycemic control and slow disease progression. Nevertheless, they have limited effectiveness and numerous side effects. In this context, plant extracts and their active constituents are considered an important area of seeking new anti-diabetic treatments (Irondi et al., 2015). Research suggests that bioactive compounds (i.e., polyphenols) found in plants may help slow the aging process and reduce the risk of many chronic diseases, including type 2 diabetes mellitus. In this context, plant secondary metabolites are likely good candidates to protect β cells against dysfunction and regulate insulin secretion since several of these well-known antioxidant compounds are now widely regarded as pharmacological agents that regulate target proteins and signaling pathways in pancreatic β cell (Kasole et al., 2019). In this study flavonoids, lignans and coumarins (at 20 μmol/L) were screened for their ability to modulate β-cell function on INS-1 cell model in glucose-stimulating conditions. Insulin release was quantified by the homogeneous time-resolved fluorescence method. Some of the flavonoids and coumarins increased glucose-induced insulin secretion, while no such effect was observed for lignans. Modulation of the pancreatic β-cell function by plant secondary metabolites was highly dependent on their structure, suggesting that this activity is based on molecule-protein interaction rather than on antioxidative potential. Structure-activity relationship conclusions were drawn from this, leading to the identification of key substitution patterns.

Keywords: diabetes, insulin secretion, coumarins, flavonoids, lignans

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S2.P94 Antioxidative capacity of European tree bark extracts

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Tree bark was found to be a rich natural source for active antioxidant compounds (Dudonné et al. 2009) but there is little information on the effects of European tree bark. Within the presented study, we analyzed the potential of different tree bark extracts derived from European trees (oak, beech, birch, alder, pine and bird cherry) for their antioxidant capacity. Total phenolic content (TPC) and radical scavenging ability (DPPH) of the extracts was determined as well as their potential to reduce oxidative stress in a human keratinocyte cell line. For the latter, reactive oxygen species (ROS) production (using the DCFDA assay), lipid peroxidation (using the TBARS assay), GSH/GSSG ratio, and protein carbonylation were compared between cells treated with different concentrations of bark extracts and untreated cells. Within the chemical assays, oak bark extracts had the strongest anti-oxidant potential with a TPC of 366.5 µgGAE/mg and an DPPH inhibition of 76 %. For all wood extracts, a significantly lowered generation of ROS could be shown with the DCFDA method in the range of 50 to 600 µg/mL (except for beech at 50 µg/mL) compared to the untreated cells. The TBARS assay shows a strong tendency for alder bark extract to be the most promising agent, but this is currently being verified. GSH/GSSG ratio and protein carbonylation are still to be examined. Combined with their antimicrobial and wound-healing activity (Emrich et al. 2022), tree bark extracts could be promising compounds in the treatment of various skin diseases (Häsler Gunnarsdottir et al. 2023).

Keywords: European bark extracts, antioxidative capacity, skin applications

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S2.P95 Exploring the potential of ADAPT-232: plant adaptogens for healthy aging, and longevity in *Caenorhabditis elegans*

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In the ongoing scientific exploration of strategies to promote longevity and rejuvenation, there is a burgeoning interest in supplements aimed at enhancing healthspan and extending lifespan (Todorova et al., 2024). Plant adaptogens, revered for their multifaceted physiological benefits, have garnered attention as prospective contributors to this research trajectory (Vasileva et al., 2019). Among the most recognized adaptogens are the extracts from *Eleutherococcus senticosus* root, *Schisandra chinensis* berry, and *Rhodiola rosea* root, which constitute the herbal medicinal product ADAPT-323. This supplement has been investigated for its energy-boosting and stress-protective properties (Aslanyan et al., 2010; Panossian et al., 2021). We conducted an assessment of the potential of ADAPT-232 to modulate healthy aging and increase energy using *Caenorhabditis elegans*. This model system serves as a valuable model for studying age-related degradation at the tissue and molecular levels. Furthermore, nematodes exhibit diminished late-life fitness associated with muscle and neuronal degeneration. In our study, we focused on understanding the intricate interplay between ADAPT-232 and the physiological processes that influence aging. Through a comprehensive analysis employing both phenotypic and stress assays, we have determined that ADAPT-232 is a promising supplement for maintaining metabolism and homeostasis throughout lifespan. The combined effects of the three adaptogenic plants in ADAPT-232 contribute significantly to the extension of the healthspan of *C. elegans* through modulation of stress-response mechanisms. Our findings highlight the potential of plant adaptogens, particularly ADAPT-232, as a promising avenue in the pursuit of interventions that promote healthy aging and increased longevity.

Keywords: adaptogens, healthspan, *Caenorhabditis elegans,* ageing, longevity

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S2.P96 *Harpagophytum procumbens* DC. ex Meisn. hairy roots extract alleviates inflammatory response in psoriasis dermatitis

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Psoriasis is an inflammatory skin pathology affecting 2-3% of the worlds' population. The disease manifests itself with characteristic skin lesions and is associated with multiple comorbidities and significant negative impact on patients' quality of life (Griffiths et al., 2021). Plant-derived bioactive compounds are promising as adjuncts to the conventional pharmacotherapy (Koycheva et al., 2021a, 2021b). We have investigated the anti-inflammatory and anti-psoriatic potential of the extract of transformed hairy root culture of *Harpagophytum procumbens* DC. ex Meisn. (HPE-HRs) and its secondary metabolite verbascoside on imiquimod-induced psoriasiform model in mice. The *in vitro* effects of HPE-HRs and verbascoside on the expression of the mouse chemokine receptors CXCR3, TGFβRI, as well as the production of the cytokines IFN-γ, IL-17F were analyzed by flow cytometry in skin- and spleen-derived T-lymphocytes, respectively. Concentration-dependent decrease was observed in the percentage of immune cells expressing CXCR3 and TGFβRI receptors and no induction of the inflammatory cytokines secretion upon HPE-HRs or VER treatment. Topical application of a cream containing HPE-HRs (1.25, 2.5 and 5%) on the shaved backs of C57BL/6 mice was done over the period of imiquimod-induced psoriasiform dermatitis in mice. The 5% HPE-HRs cream ameliorates psoriasis-like skin manifestations by an alleviation of epidermal hyperplasia, reduction of skin thickness and scale formation compared to the control groups. Further, gene expression analysis by RT-qPCR was performed to assess the changes in critical signalling pathways related to psoriasis development. The results suggest that HPE-HRs may serve as a promising therapeutic option in psoriasis management.

Keywords: psoriasis, *Harpagophytum procumbens*, CXCR3, TGFβRI, epidermal hyperplasia

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S2.P97 Caloric restriction mimicking through NRF-2-mediated mechanisms by plant-derived bioactive leads

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Obesity is a multifactorial disease associated with metabolic dysregulations, which could lead to multimorbidity affecting both the quality of life and the lifespan (Savova et al., 2023; Vasileva et al., 2020). Caloric restriction initiates the adaptive metabolic strategies that produce stress resilience and the nuclear factor erythroid 2-related factor 2 (NRF2) is known as a master regulator in the integration of these signaling pathways (Spadaro et al., 2022). Several natural products commonly used as dietary supplements (resveratrol, spermidine or rapamycin) have been defined as caloric restriction mimetics (CRM) due to the overlapping molecular mechanisms involved in their biological activity and caloric restriction-mediated healthspan (Madeo et al., 2019). For instance, curcumin is among the most potent natural NRF2 activators that has been proposed as a CRM. As an advancement in our continuing search for potent anti-obesogenic compounds from plants we have directed our focus toward the potential of natural NRF2 activators to promote improved metabolic homeostasis. We have evaluated the changes in skn-1 (the orthologue of NRF2) transcriptional activity in *Caenorhabditis elegans* induced upon high glucose feeding and the potential of curcumin to modulate this process. The confocal GFP-based fluorescent detection revealed a 2-fold increase in skn-1/NRF2 upon curcumin (100 μM) supplementation that was further supported by the mRNA expression analysis. The obtained findings reinforced the idea that NRF2 serves as a hormetic mediator that not only provides integration of detoxification processes but also could account as a target to alleviate the severity of age-related illnesses including obesity and metabolic dysregulations.

Keywords: caloric restriction, obesity, aging, bioactive compounds, NRF-2

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S2.P98 *SKN1/NRF2*-dependent modulation of nutrient sensing network in *Caenorhabditis elegans* upon curcumin treatment

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Metabolic dysregulations that root from obesity affect the nutrient sensing signaling pathways and mitochondrial homeostasis. The nuclear factor erythroid 2-related factor 2 (NRF-2) acts as a master regulator in cellular stress response and is suggested to be involved in obesity, diabetes, and metabolic syndrome (Vasileva et al. 2020). Curcumin and some of its derivatives are recognized as natural activators of NRF-2 and exhibit anti-inflammatory, antioxidant, chemopreventive, anti- nociceptive, anti-proliferative, and anti-parasitic properties (Shin et al., 2020). In the context of obesity and metabolic disorders, curcumin has been reported to inhibit adipogenesis, regulate lipid metabolism, stimulate energy expenditure, and benefit gut microbiota (Sampath et al., 2021). The current investigation provides further insights into the molecular mechanism of the beneficial effect of curcumin in modulating glucose-induced lipid accumulation in *Caenorhabditis elegans*. Moreover, we applied orlistat as an anti-obesogenic control, which is an approved drug for obesity therapy. Our preliminary experiments in *C. elegans* obesity model suggest that a combination of orlistat (12 μM) and curcumin (100 μM) significantly lowers accumulated lipids by 57.6% compared to vehicle group). Regarding the molecular mechanisms of curcumin as an NRF2- modulating caloric restriction mimetic agent, our results demonstrate that curcumin elevates the expression of *skn-1*, *sek-1* and *nhr-49*, which are important and evolutionary conserved genes involved in carbohydrate and lipid metabolism in *C. elegans*. Additionally, modulation of mitochondrial dynamics, including mitochondrial potential, was evaluated. Based on these results, we conclude that curcumin exerts its favorable biological activity through modulation of complex nutrient sensing signaling pathways.

Keywords: NRF-2, *Caenorhabditis elegans*, Curcumin, Caloric restriction mimetics, Obesity

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S2.P99 The phenylethanoid glycoside forsythoside B as a promising candidate for photoprotective dermatological products

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Complex internal and extrinsic variables accelerate skin aging. The intrinsic aging leading to maturation processes is driven by epigenetic changes, circadian rhythm disruption, and senescence that impair skin texture. Premature skin aging caused by external factors, the main of which is chronic sun exposure, also known as photoaging, leads to irreversible photodamage and reduces skin capacity to self-renew (Stoykova et al., 2024; Zhang et al., 2023). Products that include sunscreen compounds and interfere with metabolism can be harmful and frequently cause serious cutaneous adverse effects such as irritations and allergy (Dhaliwal et al., 2019; Yamada et al., 2020). Over the past two decades, the scientific community has become increasingly interested in the "green pharmacy", where natural ingredients are evaluated for potential use as cosmetic agents (Dhaliwal et al., 2019). The aim of our research is to determine the impact of ultraviolet radiation (UVA/UVB) on photoaged human keratinocytes and to prevent photodamage by application of plant-derived bioactive compounds. Forsythoside B (FORB), found in *Verbascum xanthophoeniceum* Griseb, was previously reported to have an anti-inflammatory effect in primary human epidermal keratinocytes (Georgiev et al., 2012). We determined the cytotoxic and photoprotective potential of FORB which is the primary aspect in personal care product. Identified as a secondary factor of photodamage, the high levels of reactive oxygen species (ROS) induced by the UV irradiation were measured by confocal-fluorescence microscopy. The obtained data exposed that FORB significantly inhibited the increased levels of ROS induced by UVA/UVB in HaCaT cells, which may be related to its photoprotective activity.

Keywords: Forsythoside B, keratinocytes, photoprotection, photoaging

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S2.P100 Exploring the therapeutic potential of *Punica granatum* leaves extract in enhancing healthspan and lifespan in *Caenorhabditis elegans*

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In recent decades, substantial efforts have been devoted to enhancing both healthspan and lifespan encompassing pharmacological and non-pharmacological interventions (Todorova et al., 2024a). Moreover, the exploration of natural products that modulate the molecular network associated with lifespan holds significant promise in the pursuit of longevity and disease-free years (Todorova et al., 2024b). *Punica granatum* L., commonly known as pomegranate, has served as a nutritional source and a traditional remedy for centuries. Abundant in bioactive compounds, predominantly flavonols and flavones, pomegranate leaves have been renowned for their anti-inflammatory and anti-diabetic properties (Abo-Saif et al., 2023; Nasir et al., 2024). However, there remains a gap in comprehending their role in modulating healthspan and longevity. Hence, our study has delved into the capacity of a crude extract from pomegranate leaves to mitigate the aging process using *Caenorhabditis elegans* model system. Our investigation revealed that the extract, in doses 50, 100 and 200 µg/mL, enhances resistance to stressors, including heat and oxidative stress. Supplementation with 200 µg/mL extract significantly increases survival upon heat stress to up to 85% and 78% on day 5 and day 10 of the nematodes' lifespan, respectively, compared to the control group. Moreover, the extract improves locomotion, as the mean bending rate upon 200 µg/mL was increased by up to 106% (day 5) and 110% (day 10), indicating a positive impact on neuronal and muscular function. Treatment of *C. elegans* with the highest concentration resulted in a mean lifespan of 24.8 days, which is 1.62-fold change in comparison to the control animals. In conclusion, our findings underscore the potential of *P. granatum* leaves extract in promoting healthspan and longevity, and its positive impact at both the molecular and functional levels.

Keywords: Longevity, Healthspan, *Caenorhabditis elegans, Punica granatum,* Pomegranate

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S2.P101 Baicalein, a major component of the root of *Oroxylum indicum* (Bignoniaceae) vasodilates isolated pulmonary arteries through endothelium-dependent and -independent mechanisms

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Recently, we demonstrated that *Oroxylum indicum* (L.) Kurz (Bignoniaceae) root extract (OIRE) and its major constituent baicalein display phosphodiesterase-5 inhibitory activities (unpublished data), suggesting their potential use for treating pulmonary artery hypertension (PAH). The present study investigated the vasorelaxant effects of OIRE and baicalein on isolated pulmonary arteries (PA) compared to a conduit vessel from the systemic circulation (aorta). OIRE was prepared by maceration with 95% ethanol and baicalein was purchased from Sigma. Vascular reactivity was explored on isolated aorta and PA from Wistar rats. After pre-constriction with phenylephrine, vessels were exposed to cumulative concentrations of OIRE or baicalein. To unravel the role of endothelium-dependent and -independent pathways in PA relaxation, the same experiments were performed in the presence of specific inhibitors. OIRE and baicalein both induced a vasorelaxation on PA, greater than in the aorta (E_{max}=85±5% vs 25±4%, $p<0.001$ for OIRE, and E_{max}= 86±10% vs 32±7% for baicalein $p<0.001$). The EC_{50} values in PA and aorta were 0.035 and 0.018 µg/mL for baicalein, and 7.13 and 0.59 µg/mL for OIRE. Mechanistically, the effects of OIRE on PA relied on eNOS and COX activation, and the production of endothelium-derived hyperpolarizing factors. Baicalein-induced vasorelaxation involved the same endothelial pathways as OIRE but also endothelium-independent mechanisms such as α_1-adrenergic receptor antagonism, K_V^+ channels blockade, and a decrease in intracellular Ca^{2+} release. The present study identified new pharmacological properties of OIRE and baicalein as PA vasodilators. In combination with their phosphodiesterase-5 inhibitory activities, our data encourage the investigation of their effects on PAH.

Keywords: *Oroxylum indicum,* baicalein, vasorelaxant effect, pathways

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S2.P102 Chemical profile and cytotoxic activity of methanol extracts of eight *Geranium* L. species

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The aim of this study was to determine the chemical composition and cytotoxic activity of methanol extracts from underground and aerial parts of eight *Geranium* species (*G. macrorrhizum* L., *G. robertianum* L., *G. palustre* Torn, *G. sanguineum* L., *G. columbinum* L., *G. pyrenaicum* Burm. and *G. lucidum* L.). These polyphenol-rich extracts have previously been shown to have significant antioxidant and antimicrobial potential (Ilić et al., 2021). A total of seven phenolic acids (ellagic, gallic, galloylquinic, galloylshikimic, protocatechuic, chlorogenic and brevifolin carboxylic acid), 12 flavonoids (quercetin, quercetin-galloyl-hexoside, two pentosides of quercetin, quercetin-deoxyhexoside, hyperoside, isoquercitrin, kaempferol, astragalin, astragalin-2′′- *O*-gallate, kaempferol-methylether and kaempferol-dimethylether) and 13 tannins (geraniin and its isomer, corilagin, two anomers of tellimagrandin I and three isomeric compounds, tellimagrandin II, hebulagic acid and 4 galloyglucoses) were identified and quantified by LC-MS analysis. The *in vitro* cytotoxic activity of extracts, geraniin and ellagic acid, was evaluated in the MTT assay against human prostate (PC-3), colon (HT-29), cervical (HeLa) and melanoma (Hs 294T) cancer cell lines. All tested extracts induced a reduction of viability against HeLa cancer cells, and the most effective were the extracts of the underground parts of *G. sanguineum*, *G. pyrenaicum*, *G. columbinum* and *G. macrorrhizum* (IC_{50} = 2.52-8.94 μg/mL). The extracts showed no significant cytotoxicity against other cells, with the exception of the extract from the aerial parts of *G. lucidum* against HT-29 cells (IC_{50} = 22.54 μg/mL). Geraniin was also most effective against HeLa cells (IC_{50} = 12.88 μg/mL), with moderate activity against other cells (IC_{50} = 44.41-80.94 μg/mL), while ellagic acid showed weaker activity (IC_{50} = 65.62-221.51 μg/mL).

Keywords: *Geranium* species, LC-MS profile, cytotoxicity

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S2.P103 The composition of the essential oil of *Pimpinella serbica* and its relationship to other *Pimpinella* species from the central part of Balkan Peninsula

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Pimpinella serbica (Vis.) Drude (Apiaceae), is an endemic species of the Balkan Peninsula, formerly referred to as *Pancicia serbica* Vis. and considered a monotypic genus (Nikolić, 1973), while in the current taxonomic classification it is included in the genus *Pimpinella* L. (Euro+Med 2014). A number of plants in this genus are used for their medicinal properties, which are partly due to the presence of essential oils with various pharmacological/biological effects (Nasir and Yabalak, 2021). In this study we investigated the chemical composition of essential oils from different organs of *P. serbica* and their relationship with the previously analyzed essential oils of *P. tragium*, *P. saxifraga* (Slavkovska et al., 2022) and *P. alpina*, using cluster analysis. Phenylpropanoids dominated in the essential oils of *P. serbica*, dillapiole in the aerial parts (50.7-63.0%) and nothoapiole in the roots (79.0%) and fruits (73.4%). Although a high amount of nothoapiole (24.0%) was present in one sample of the aerial parts. Phenylpropanoids of pseudoisoeugenol type and trinorsesquiterpenes, which are considered chemical markers of *Pimpinella* species (Tabanca et al., 2005), were not present in the *P. serbica* essential oils. Cluster analysis also revealed the separation of *P. serbica* essential oils from the essential oils of other *Pimpinella* species. These results indicate that the chemical profile of the essential oils of *P. serbica* does not support its classification in the genus *Pimpinella*, and suggest the possibility of using the essential oil composition as a chemotaxonomic marker in clarifying the status of this endemic species.

Keywords: *Pimpinella serbica,* essential oil composition, chemotaxonomy

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S2.P104 Different extracts of three *Veronica* species: the study of their antioxidant activity

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The genus *Veronica* L. is extremely variable in its morphology and its species are very well adapted to different living conditions (Barreira et al., 2014). There are a larger number of studies on various phenolic compounds of *Veronica* species and their antioxidant activities (Barreira et al., 2014; Živković et al., 2017; Sharifi-Rad et al., 2016). The *Veronica* species included in this study were *V. polita* Fr., *V. persica* Poir. and *V. anagallis-aquatica* L. For each selected species, three extracts with phenolic compounds were isolated from aerial parts of the plants: aqueous, methanolic and ethanolic. All extracts were tested for antioxidant activity using two methods: DPPH (2,2-diphenyl- 1-picrylhydrazyl) and ORAC (oxygen radical absorbance capacity). In addition, standards for compounds that were detected in the highest amount in all species were tested for their antioxidant activity: apigenin, *p*-hydroxybenzoic acid, caffeic acid and vanillic acid. Caffeic acid showed the highest antioxidant activity in both methods studied with an IC50 value for DPPH activity of 1.99 μg/mL. Among the plant extracts, methanolic or ethanolic extracts generally showed higher activity than water extracts in both methods, which was to be expected, as organic solutions extract more phenolic compounds. Nevertheless, this was not the case for all species, so this will be discussed through relation of antioxidant activity and the amount of each compound in the phenolic composition. The methanolic extract of *V. polita* showed the highest antioxidant activity in the ORAC method. In the DPPH method, the methanolic extract of *V. anagallis-aquatica* and ethanolic extract of *V. persica* showed the highest activity.

Keywords: *Veronica* species, *antioxidant activity, polyphenols, DPPH, ORAC*

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S2.P105 Anti-inflammatory potential of chamomile flowers (*Matricariae flos*) extract in topical applications

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Chamomile flowers *(Matricariae flos*) are one of the oldest and most well-documented plant materials in folk medicine from the Asteraceae family. It is a popular and widely used therapeutic plant traditionally used as an anti-inflammatory and wound-healing topical remedy for skin diseases (EMA, 2015; Srivastava, 2010). Moreover, chamomile flowers are an active ingredient in many popular ointments, creams, and cosmetics (Melnyk, 2022). The objective of the research was to conduct *in vitro* studies of 70% ethanolic chamomile flowers extract's effects on the inflammatory response of skin cells. Immortalized human keratinocytes (HaCat) and primary normal human fibroblasts (NHDF) were chosen as cell lines. The inflammation induced by tumor necrosis factor α (TNF-α)/interferon γ (IFN-γ), and lipoteichoic acid (LTA) accordingly. The secretions of interleukin 6 (IL-6) and 8 (IL-8) were determined using enzyme-linked immunosorbent assay. Additionally, the chemical composition of the extract was characterized by HPLC-DAD-MS. The experimental findings revealed a notable reduction in the release of IL-6 and IL-8 upon exposure to the extract in NHDF cells. Investigations conducted on HaCat cells indicated a significant decrease in the secretion of IL-6 following treatment with the extract. Meanwhile, no statistically significant variances were observed in the IL-8 secretion levels among the experimental groups. The obtained results confirmed the anti-inflammatory potential of chamomile flowers in topical therapy of skin inflammations.

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Keywords: *Matricariae flos,* Chamomile flowers, anti-inflammatory, skin diseases, interleukins

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S2.P106 A comparative study on biological activities of different extracts of *Scrophularia xanthoglossa* Boiss

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Scrophularia L. genus (Scrophulariaceae) is represented by 76 species in Türkiye, including 37 endemics. Some *Scrophularia* species were used in folk medicine for the treatment of various diseases including skin diseases, eczema and cancer (Lall and Mill, 1978; Özdemir and Alpınar, 2015; Chaibeddra et al., 2020). In the study, the extracts of *S. xanthoglossa* were prepared from ethyl acetate, ethanol, ethanol/water (70%), and water. We focused on the *in-vitro* enzyme inhibition (α-glucosidase, α-amylase, acetylcholinesterase (AChE)/butyrylcholinesterase (BChEAChE), 5- lipoxygenase (5-LOX) and tyrosinase (TYR) and non-enzyme antioxidant activities (TPC, TFC, $DPPH^{+}$ and $ABTS^{\cdot+}$ radical scavenging assays, CUPRAC, FRAP and iron-chelating activity assays) of the extracts. The total contents of phenolics and flavonoids in the extracts varied from 18.68 to 24.22 mg GAE/g and from 2.12 to 15.43 mg RE/g, respectively. The total antioxidant capacity was also determined by the phosphomolybdenum assay (0.77-2.11 mmol TE/g). According to the results of enzyme inhibition activities of AChE (2.48 mg GALAE/g), BChE (9.27 mg GALAE/g), tyrosinase (46.73±0.31 mg KAE/g) and a-amylase (0.67 mmol ACAE/g) enzyme inhibition activities, ethyl acetate extract was found to be the most effective extract. This study was the first to investigate the biological activities of *S. xanthoglossa* in Türkiye. The extracts obtained with organic solvents showed high biological effects. Furthermore, the results suggest that *S. xanthoglossa* could serve as a potential source of bioactive compounds.

Keywords: *Scrophularia xanthoglossa*, Scrophulariaceae, antioxidant activity, enzyme inhibition

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S2.P107 Andrographolide as a potential anti-inflammatory molecule in prostate cancer

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Prostate cancer is the most frequently diagnosed non-cutaneous cancer and the second cause of cancer-related deaths in American men (Siegel et al., 2020). An emerging area of cancer therapeutics is the combination of standard-of-care treatment with natural products (Chhabra et al., 2018). Andrographolide, a labdane diterpenoid and main bioactive component of *Andrographis paniculata*, has shown therapeutic potential. Our laboratory demonstrated that andrographolide inhibits cell growth, tumor growth and angiogenesis in prostate cancer. The objective of this study is to understand the role of andrographolide on inflammation in prostate cancer. Tumors from a mouse xenograft model injected with 22Rv1 cells and treated with andrographolide (10 mg/kg and 25 mg/kg), were used for microarray analysis. Ingenuity Pathway Analysis (IPA) revealed a total of 674 and 218 genes differentially expressed in tumors treated with andrographolide (10 mg/kg and 25 mg/kg), respectively, when compared to their control. Results showed alterations of inflammatory response genes such as ATM, DHFR, HIF-1-α, CDKAL1, NOX1, C5, CCL4, COG6, and TTC3. To further study the inflammatory response of andrographolide in prostate cancer, we used an array to measure changes in cytokine expression in PC3 and 22Rv1 prostate cancer cells. IGF-I, IL-1 β, IL-4, MCP-2, I-309, IL-1α, IL-5, MDC, PDGF BB, SDF-1, and Angiogenin were differentially expressed in PC3 cells treated with andrographolide (25 mg/kg). Our findings reveal that andrographolide modulates the tumor microenvironment contributing to its possible mechanism of action and anticarcinogenic effect. Understanding these biological pathways is essential to determine pharmacological targets of andrographolide in prostate cancer.

Keywords: prostate cancer, inflammation, microarray analysis, andrographolide, mouse studies

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S2.P108 The impact on proinflammatory cytokines secretion by human neutrophils and keratinocytes by extracts from *Arctii lappae fol, Serpylli hba* and *Millefolii hba* – raw materials traditionally used in Poland in skin disorders

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In Poland, a wide range of plant materials are traditionally recognized as medicines for skin disorders (Kaczmarczyk-Sedlak et al., 2020; Kuźnicka et al., 1987). Among them, we have chosen thirteen to the first step of our project. All plant materials were harvested from a Warsaw University of Life Sciences cultivated field. We have determined the effect of aqueous and 70% (*v/v*) ethanolic extracts on the activity of enzymes (hyaluronidase and lipoxygenase) and their antioxidant activity in cell-free systems (Mainka et al., 2021). Based on the previous results to the second step of our research, six extracts from three plant materials were selected: *Arctii lappae fol.* (All_H_2O, All_EtOH), *Serpylli hba* (Th_H_2O, Th_EtOH) and *Millefolii hba* (K_H2O, K_EtOH). The impact on ROS secretion by neutrophils stimulated with f-MLP, viability of cells (NRU test) and cytokines secretion by neutrophils and keratinocytes were analyzed. The studies were performed using chemiluminescence, colorimetry and ELISA methods, respectively. In the case of neutrophils stimulated with lipopolysaccharide TNF-α, IL-1β and IL-8 secretion, while on keratinocytes stimulated with UVB, IL-6 and IL-8 secretion were determined. As positive controls, dexamethasone and urolithin B were used. Tested extracts showed no or slight cytotoxicity on used cells. All extracts except All_H2O inhibited ROS secretion. In neutrophil tests, all extracts showed stimulatory activity on TNF-α and IL-8 secretion, while moderate impact on IL-1β secretion. In tests on keratinocytes all extracts inhibited secretion of IL-6 and IL-8, in the concentration of 100 μg*mL^{-1} the most active were All_H_2O (65.81±10.95%) and Th_H_2O (63.17±8.17%), respectively.

Keywords: *Achillea millefolium, Arctium lappa, Thymus serpyllum,* neutrophils, keratinocytes

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S2.P109 Enhancing the anticancer activity of oleandrin from *Nerium oleander* L. (Apocynaceae) through novel formulations

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Nerium oleander Linn., also known as oleander, is a medicinal plant with potent bioactive compounds currently being explored for their potential therapeutic applications. Recent research focuses on its potential in cancer therapy due to the presence of a specific group of secondary metabolites called cardiotonic glycosides. Among these, oleandrin, the cardioactive compound, has emerged as a promising candidate due to its anticancer activity and ability to induce apoptosis in tumor cells. However, its narrow therapeutic window poses a significant challenge for clinical use. Therefore, developing new formulations with improved bioavailability and reduced systemic toxicity is crucial for advancing oleandrin's potential in cancer treatment. This project aims to address the bioavailability limitations of oleandrin and pave the way for its introduction into clinical therapy. In this context, *N. oleander* leaves were collected from Avcilar/Istanbul, dried at 50 C, and ground into fine powder. Oleandrin was isolated using a modified method adapted from Ryer et al. (1948). Further purifications were performed via chromatographic methods, and the structure of the compound was elucidated by comparing our NMR data with the relevant literature (Karakoyun et al., 2021; Bedir et al., 2021). Oleandrin yield of the process was approximately 0.3% (w/w). Currently, we are developing and evaluating novel formulations designed to enhance the bioavailability and selectivity of oleandrin toward cancer cells, minimizing potential side effects. Developing highly bioavailable and selective formulations would allow for lower dosages and reduced side effects, improving patient outcomes and quality of life.

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Keywords: oleandrin, cardiac glycoside, anticancer, secondary metabolite, *Nerium oleander*

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S2.P110 Oral acute toxicity and preliminary phytochemical characterization of *Hyptis albida*

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Hyptis albida is a plant commonly used in remedies for the treatment of gastrointestinal disorders, skin infections, rheumatism, cramps, and muscle aches. (Sánchez *et al.* 2013) In the present work, different extraction methods were used with the purpose of carrying out the preliminary phytochemical characterization of Hyptis albida. A triterpene was purified: ursolic acid (Fig. 1). As a safety test we evaluate the acute oral toxicity, the LD50 of the hydroalcoholic extract of Hyptis albida was established following the OECD 423 guideline, starting with a dose of 300 mg/kg and continuing with a dose of 2000 mg/kg where no deaths or toxic symptoms of the animals were observed for both doses, which is why this extract belongs into category 5 or unclassified according to the Globally Harmonized System.

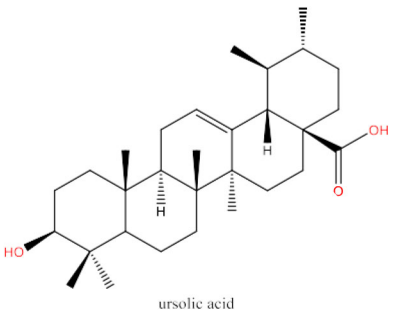

Fig. 1. Structure of ursolic acid

Keywords: oral toxicity, Hyptis albida, ursolic acid, OECD 423, Globally Harmonized System

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S2.P111 *Ex vivo* assessment of potential *Eucalyptus cinerea* antihypertensive

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Eucalyptus cinerea, a member of the Myrtaceae family, is primarily recognized for its ornamental and extensive traditional medicinal (MT) use due to its anti-inflammatory and antioxidant properties, particularly its essential oil and its applications in respiratory diseases such as relaxing activity (Surbhi et al., 2023) Despite its widespread use in MT there remains a lack of evidence regarding its potential as an antihypertensive agent linked to relaxing activity. In this study, our aim was to assess the broader pharmacological potential of leaves of *E. cinerea* hydroalcoholic extracts by evaluating their vasorelaxant activity using a standardized protocol in rat aortic tissue. Ethical considerations, following the principles of the 3Rs, were strictly observed throughout the experiment (Fig. 1).

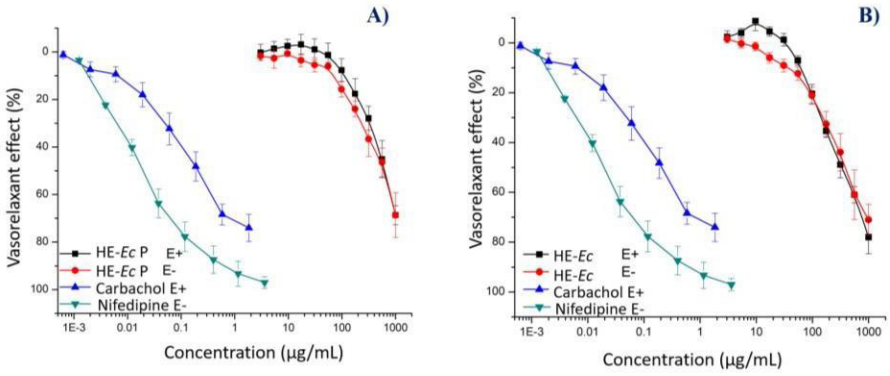

Fig. 1. Concentration-response curves of the relaxing effect induced by hydroalcoholic (HE-*Ec)* and precipitate extract (HE-*Ec* P) on isolated rat aortic, the effect was partially endothelium-dependent.

A), HE-*Ec* P (Emax 70%, E+; Emax 81% E-) **B)** HE-*Ec* (Emax 80% E+; Emax 82%E-)

Our results reveal a partially endothelium-dependent vasorelaxant effect, indicating the presence of bioactive compounds in the extract that may interact with various endothelial pathways, including the prostacyclin pathway, endothelial M3-type muscarinic receptor agonists, nitric oxide synthase, or endothelium-derived factors (Sanchez et al., 2020).

Keywords: *Eucalyptus cinerea,* antihypertensive, relaxing effect

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S2.P112 The anti-asthmatic effect of artichoke in BALB/c mouse model

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Asthma is a chronic inflammatory disease of the respiratory tract, with symptoms such as wheezing, difficulty breathing, and coughing. Artichoke (*Cynara cardunculus* var. *scolymus L*) is a dicotyledonous plant native to the Mediterranean coast. Artichoke has traditionally been widely used for digestive diseases, hyperlipidemia, liver disease, and antioxidant research. However, its efficacy as a therapeutic agent for asthma is not well known. In this study, we investigated anti- asthmatic effect of artichoke using the animal model. Asthma was induced in BALB/C mice with a mixture of ovalbumin and aluminum hydroxide, and then artichoke was oral administered. The number of inflammatory cells was reduced in the bronchoalveolar lavage fluid (BALF) from mice treated with artichoke compared to non-treated mice. The level of serum IgE also reduced in mice treated with artichoke. Using Fluorescence Activated Cell Sorting (FACS) analysis, it was confirmed that the number of immune cells was reduced in BALF isolated from mice treated with artichoke. The level of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β was reduced in BALF from mice treated with artichoke and the level of IL-4, IL-5 and IL-13, a specific cytokine in asthma, was also reduced in BALF from mice treated with artichoke. Our findings suggest that artichoke has anti-asthmatic activity and can be applied to the development of treatments in atopic diseases.

Keywords: asthma, artichoke, BALF, animal model

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S2.P113 Antimicrobial activity and chemical composition of *Taraxacum aleppicum* Dahlst. (Asteraceae)

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Taraxacum aleppicum belongs to the genus *Taraxacum* which is a member of the Asteraceae family. The genus *Taraxacum* is found in warmer places of the Northern Hemisphere and it is represented in Turkiye as 54 taxa under nine sections (Uysal et al., 2016). *T. aleppicum* is a member of the Scariosa section which is characterized by a reddish, brown or straw-coloured achene and is used for eye diseases in animals (Gürdal, 2015, Selvi et al., 2023). As a part of our current study, *T. aleppicum* was collected from Sakarya in Marmara region, Turkiye. Air-dried roots and aerial parts were separated and extracted with ethanol. Ethanol extracts of roots and aerial parts were partitioned with petroleum ether, ethyl acetate and *n*-butanol, respectively. The chemical composition of root and aerial part fractions was elucidated using LC-HRMS. The main components of the root EtOAc fraction are chichoric acid, sinapinic acid and chlorogenic acid, respectively. The EtOAc fraction of the aerial part contain mainly chichoric acid, chlorogenic acid and luteolin-7-rutinoside. The main component of BuOH fractions of both root and aerial parts was found to be chichoric acid. The antimicrobial activity of both root and aerial parts fractions was examined against standard strains using the minimum inhibitory concentration (MIC) method (CLSI, 2000 & 2006).

Table 1. Antimicrobial activities of the fractions (µg/mL). PE: Petroleum ether, EtOAc: Ethyl acetate, BuOH: Butanol, ap. fr.: aerial part fraction, ro. fr.: root fraction, *S.a.: Staphylococcus aureus, S.e.: Staphylococcus epidermidis, K.p.: Klebsiella pneumonia, E.c.: Escherichia coli, P.a.: Pseudomonas aeruginosa, C.a.: Candida albicans, C.t.: Candida tropicalis, C.p.: Candida parapsilosis,* ATTC: American Type Culture Collection

Strains Samples	S.a. ATCC 29213	S.e. ATCC 12228	K.p. ATCC 4352	E.c. ATCC 25922	P.a. ATCC 27853	C.a. ATCC 10231	C.t. ATCC 750	C.p. ATCC 22019
PE ap. fr.	625	72.8	1250	625	625	312.5	625	625
EtOAc ap. fr.	625	156.25	1250	625	625	312.5	625	625
BuOH ap. fr.	625	312.5	625	1250	625	312.5	625	625
PE ro. fr.	625	625	625	1250	625	312.5	312.5	625
EtOAc ro. fr.	1250	625	625	625	625	312.5	625	625
BuOH ro. fr.	625	625	625	1250	625	625	312.5	625

According to the results (Table 1), while root fractions of *T. aleppicum* were found to be moderately effective against all standard strains, fractions of aerial parts were found to be very effective against *Staphylococcus epidermidis* ATCC 12228.

Keywords: *Taraxacum aleppicum,* antimicrobial, LC-HRMS

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S2.P114 The hydroethanolic extract from *Calendula officinalis* L. herba demonstrated potent anti- inflammatory properties *in vitro*

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Calendula officinalis L. is traditionally indicated for the symptomatic treatment of minor inflammations of the skin and as an aid in healing of minor wounds that are physiologically accompanied by local inflammation, including activation of the NF-kB pathway and release of pro- inflammatory mediators such as leukotrienes and prostaglandins. Here we aimed to evaluate the anti-inflammatory efficacy of an hydroethanolic extract from *Calendula officinalis* L. herba. Dry extracts from *Calendula officinalis* L. were prepared using liquid extracts from fresh aerial parts of the flowering Calendula plant (mother tincture *Calendula officinalis* 2a Ø (GHP)). NF-kB activation was analysed in a reporter assay with the human Jurkat cell line. Analysis of inhibition of the proinflammatory and pain-related enzymes 5-LO and COX-2 as part of the arachidonic acid pathway was performed using recombinant human enzymes. Calendula herba concentration- dependently inhibited NF-kB activation (IC_{50}: 74.9 µg/mL). In a cell-free enzyme inhibition assay, Calendula herba concentration-dependently inhibited both, 5-LO and COX-2 enzyme activity (IC_{50}: 39.6 µg/mL and 29.4 µg/mL). In this experimental *in vitro* approach the complex mixture of active compounds contained in the Weleda Calendula herba extract demonstrated potent anti- inflammatory and pain-related activity (Fig. 1). Additional studies are performed to further characterise therapeutic benefits of Calendula herba.

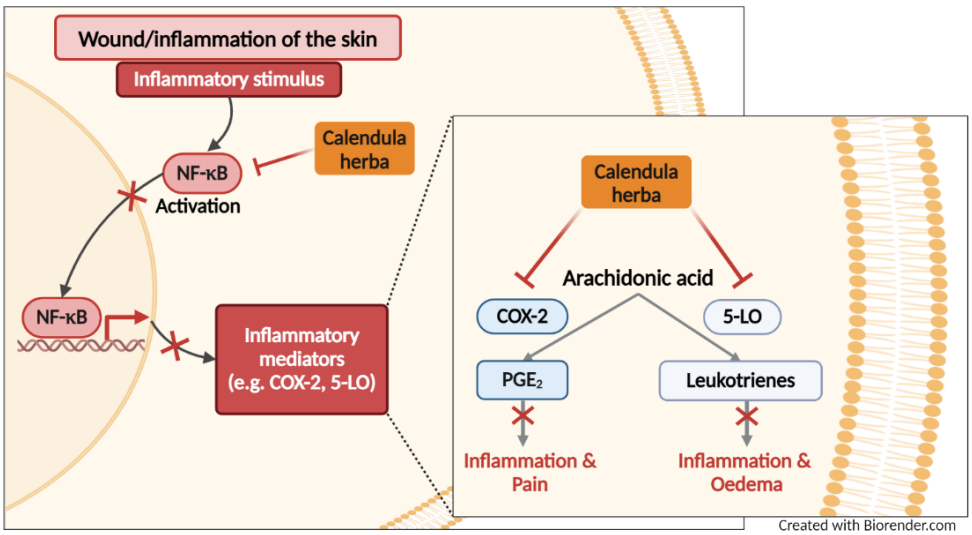

Fig.1. Summary of anti-inflammatory properties of the hydroethanolic Calendula herba extract.

Keywords: *Calendula officinalis*, inflammation, NF-kB, cyclooxygenase-2, 5-lipogygenase

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S2.P115 A study on the antiproliferative activity of the triterpenoid constituents in *Erica erigena*

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Erica erigena (Ericaceae) grows abundantly in specific regions around the west coast of Ireland, particularly in counties Galway and Mayo (Nelson, 2019). In our studies to date we have found that the aerial part of this plant is a rich source of numerous pentacyclic triterpenes based around the ursane, lupane and oleanane series. An ethyl acetate extract of the powdered leaves and flowers were prepared and loaded onto a flash column. Using a gradient mobile phase system, based around different ratios of hexane: EtOAc, specific bands were collected and analysed by GCMS to identify and quantify the triterpenoids present in the isolated fractions. Using the MTT assay, antiproliferative activity of each fraction was determined using the human prostate cell line, PC3.

Across the entire study, fractions were collected where various ratios of the following terpenoids were identified: α-amyrin, β-amyrin, lupeol, oleanolic acid, micromeric acid and ursolic acid. The minor constituents present included α-amyrenone, β-amyrenone, lupenone, erythrodiol, uvaol, betulin, ursolic aldehyde, β-sitosterol as well as coumaroyl triterpenes. The most active fractions were those that contained different ratios of ursolic:oleanolic:micromeric acids followed by a lupeol rich fraction. Further studies are planned for the evaluation of the active triterpenoid fractions against a panel of tumour cell lines.

Keywords: *Erica erigena,* Ericaceae, GC-MS, antiproliferative, human prostate cell line

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S2.P116 Cyclic peptides derived from sunflower trypsin inhibitor 1 (SFTI-1) for autoantibodies neutralization in rheumatoid arthritis

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Bioactive peptides from plants can be used beyond their endogenous functions as defense and hormones. Sunflower trypsin inhibitor I (SFTI-1) is a 14 residues long peptide with a unique bicyclic structure, formed by the cyclic backbone and one disulfide bond. The native peptide is a potent trypsin inhibitor, but numerous examples have shown that this peptide can be modified to display an array of different pharmacological effects. (Luckett et al. 1999, Franke et al. 2018, De Veer et al. 2021) Drawing inspiration from the inherent stability of SFTI-1 found in sunflower seeds, we have employed SFTI-1 as a scaffold to detect and isolate autoantibodies. Previously, we successfully utilized SFTI-1 to display citrullinated peptide epitopes from autoantigens, resulting in grafted peptides with strong binding affinity for anti-citrullinated protein antibodies (ACPA), hallmark of rheumatoid Arthritis (RA).(Gunasekera et al. 2018). To further investigate grafted peptides as potential ACPA scavengers and their potential use in diagnosis and treatment of RA. Five citrullinated peptide antigen epitopes were chosen to replace the trypsin-inhibitory loop of SFTI-1 while preserving its secondary loop. Peptides synthesized via Fmoc-SPPS were then tested against ACPA-positive patient plasma in a ELISA-based ACPA neutralization assay, to evaluate the scavenging potential of grafted peptides. Additionally, the grafted peptides were employed in affinity purification to isolate sub-specific antibodies from ACPA positive individuals. The isolated ACPA subpopulation displayed partial specificity, meanwhile structural and functional studies are still ongoing.

Keywords: scaffold peptide, SFTI-1, rheumatic arthritis

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S2.P117 Detailed LC-MS analysis and evaluation of cytotoxic effects of various extracts obtained from aerial parts of *Glaucium acutidentatum* Hausskn. & Bornm

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Among Glaucium spp., *Glaucium acutidentatum* Hausskn. & Bornm. (GA) is one of the less investigated species endemic to Turkey. Only two papers report its significant anticancer activity (Kocanci et al., 2017; Hamamcioglu et al., 2018). The present study aims to evaluate the cytotoxicity of methanolic and aqueous extracts of *G. acutidentatum* obtained using different extraction techniques. Extracts prepared by maceration, homogenizer assisted extraction (HAE) and infusion were qualitatively analysed using LC/ESI-QToF/MS-MS technique to examine in detail their phytochemical profile. The cytotoxic effects of all GA extracts, were evaluated on non- cancerous (VERO) and cancer-derived (AGS - human gastric adenocarcinoma), (FaDu - human hypopharyngeal squamous cell carcinoma) and (RKO - human colon cancer) cell lines using MTT assay. According to the LC-MS results, three types of alkaloids were identified in all samples as the predominant compounds. Second class of polyphenols noted solely in methanolic GA extracts were glycosides of flavonoids. The *in vitro* studies showed that methanolic GA extracts showed lower cytotoxicity towards non-cancerous cells than aqueous extracts. Application of HAE method resulted in higher toxicity than maceration in terms of both extracts. All GA extracts showed selective cytotoxicity towards FaDu cancer cells (selectivity index between 1.62 and 9.04). The maceration-derived aqueous GA extract revealed the lowest CC50 value (24.98 µg/mL) against FaDu cells, which was significantly ($p<0.0001$) lower than against non-cancerous cells. In conclusion, these results indicate that an appropriately selected extraction method provides GA extract with potential cytotoxic activity against certain types of cancer cells.

Keywords: *Glaucium acutidentatum*, cytotoxic effect, alkaloids

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S2.P118 *In vivo* evaluation of the anti-inflammatory activity of the marine pigment echinochrome A on hairless mice skin

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Skin inflammation is the most common symptom observed in almost all dermatological diseases. Exposure to UV irradiation causes acute and chronic effects on skin, such as inflammation, aging and cancer. The UV-induced reaction depends on the intensity of solar radiation and the time of exposure, with acute exposure leading to skin damage, such as edema, erythema and itching. UV radiation generates reactive oxygen species resulting in oxidative stress and increase of inflammatory mediators. Thus, compounds with radical scavenging properties could prevent or attenuate the adverse effects of UV radiation on the skin. Inflamed skin is usually treated by topically applied products, such as creams, gels and lotions, with their main disadvantage being the non-controlled application. Topical patches could be a promising alternative since they can offer controlled release administration. Echinochrome A (7-ethyl-2,3,5,6,8-pentahydroxy-1,4-naphthoquinone, EchA) is the most abundant pigment found in various sea urchin species. In the form of its sodium salts it is the active ingredient of the antioxidant drug Histochrome® used for the treatment of various cardiovascular diseases (Kim et al., 2021). In this study, in order to compare its anti-inflammatory activity in different pharmaceutical formulations, EchA was incorporated either in hydrophilic ointments or in electrospun micro/nanofibrous patches and applied on the UV- inflamed skin of SKH-1 hairless mice. Clinical evaluation, photo-documentation and histopathological analyses were performed and biophysical parameters, eg. erythema, hydration, transepidermal water loss and sebum production, were assessed. Both formulations exhibited significant efficacy but in different doses, ie. 0.1% for the ointment and 1% for the patches.

Keywords: marine natural products, echinochrome A, sea urchins, anti-inflammatory activity, SKH- 1 hairless mice

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S2.P119 Anti-inflammatory effect of *Glaucium flavum* extracts

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Glaucium flavum Crantz (yellow hornpoppy, Papaveraceae), distinguished by its vibrant yellow blossoms, is commonly cultivated for ornamental purposes. Yet, its value extends far beyond mere landscape aesthetics. This plant is a rich source of specialized metabolites, offering significant potential in medicine and pharmacy. Consequently, extracts from *G. flavum* obtained from the *in vitro* culture-based biotechnological approach have been explored for their anti-inflammatory and cytotoxic properties. Shoots were cultured on the MH3 medium, supplemented with kinetin (KIN) at 2.5, 4.5, and 7µM, and indoleacetic acid at 0.5 µM. Additionally, extracts from flowers, roots, and stems of plants cultivated in Kraków and Lublin were also used for comparison. The secretion of IL-1, IL-8, and TNF-α as well as cytotoxic effect were examined using ethanol and methanol extracts [1, 5, 10 µg/mL] on a human neutrophils model. The tested extracts did not show cytotoxicity towards neutrophil cells. The secretion-inhibiting effect was concentration dependent but differed between extracted organs and the particular cytokine. For TNF-α, the secretion was most efficiently inhibited by the extract from stems harvested in Lublin, at a concentration of 1 µg/ml. For IL-1 and IL-8, the highest activity was recorded for 1 µg/mL extracts from *in vitro* shoots cultured on 7 µM KIN MH3 medium and non-supplemented 1/2 MH3 medium, respectively. Additional research is required to elucidate the phytochemical composition of these extracts, thereby pinpointing the specific group of compounds responsible for these observed effects.

Keywords: *Glaucium flavum, in vitro* cultures, anti-inflammatory effect

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S2.P120 Alpha-glucosidase inhibitory profile of sesquiterpenes lactones from *Centaurea* species

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Centaurea is a genus of flowering plants widely distributed in the Mediterranean region, belonging to Asteraceae family. Many of its species have been used for a long time in traditional medicine as herbal remedies for various purposes, it has been reported that the organic extracts of many *Centaurea* species displayed a high inhibitory activity on α-glucosidase (Fattaheian-Dehkordi et al., 2021), but the bioactive compounds responsible for this activity have not been identified yet. Alpha- glucosidase is a carbohydrate-hydrolase located in the brush border cells of the intestine, which acts on the *O*-glucosidic α (1→4) linkage. The enzyme breaks down starch and glucose oligosaccharides into single α-glucose monosaccharides. Its inhibition reduces the digestion rate of carbohydrates, which are not broken down into glucose molecules, reducing glucose levels in the blood. Therefore, the alpha-glucosidase inhibitors represent a class of drugs used to control the glycemic level in non- insulin-dependent diabetes mellitus (type 2), particularly postprandial hyperglycemia. Thus, in order to deepen the potential use of *Centaurea* species to treat metabolic syndrome and identify the bioactive compounds, a screening of various sesquiterpenes lactones isolated from the chloroform extract of different *Centaurea* species aerial parts, including *C. drabifolia*, *C. kotschyi* and *C. sicula* has been carried out. As first step, the inhibitory activity of 19 sesquiterpene lactones against α- glucosidase (from *Saccharomyces cerevisiae*) was evaluated by a spectrophotometric assay. The results obtained showed how some compounds exhibited IC_{50} values in a range from 39.8 to 224.8 μg/mL. The sesquiterpene melitensin resulted the most active compound with an IC_{50} of 39.8 μg/mL, comparable to that observed for acarbose (IC_{50}= 27.9 μg/mL), the antidiabetic compound used as reference compound. Compounds rhizantholide A, kandavanolide, kotschyol A and 11-epi- 11,13-dihydroartemisiifolin showed IC_{50} values lower than 50 μg/mL. Successively, molecular docking experiments were carried out to assess the possibility for selected compounds to form complexes with the α-glucosidase active site. To be consistent with the biological assays performed, they were carried out on the investigated compounds using the 3D protein model of α-glucosidase from *S. cerevisiae*, generated through homology modeling experiments. The analysis of the output poses highlighted the establishment of a network of interactions with residues belonging to the binding site of the enzyme, and specifically with those involved in the catalytic activity, i.e., D214, E276, D349.

Keywords: *Centaurea,* metabolic syndrome, sesquiterpene lactones, α-glucosidase, Asteraceae

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S2.P121 Phytochemical profiling and *in vitro* biological activities on *Myrtus communis* L.

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Myrtus communis L. (myrtle) is a medicinal plant belonging to the Myrtaceae family. It is an evergreen plant present in most of the Mediterranean countries as wild or cultivated specie. In this study *in vitro* antioxidant and enzyme inhibition tests, as well as cell viability experiments were performed on extracts and fractions. Additionally, the neurorestorative effects were investigated *in vitro* to drive the bioactivity-guided fractionation method (BAGF). Phytochemical analyses were performed on this species. The leaves from wild samples were collected during the flowering period. Using the BAGF method, the antioxidant/antiradical activities of the extracts, and fractions were determined by ABTS, CUPRAC, FRAP, DPPH, and NO tests. Moreover, tyrosinase enzyme inhibition, and cytotoxic activity experiments were carried out. The α-synuclein aggregation model developed in the SH-SY5Y cell line was employed. Phytochemical analyses were performed on 80% EtOH macerate. The structures of the isolated compounds were established by NMR and LC- MS. The phytochemical analysis reported the presence of gallomyrtucommulone C, myrcitrin, quercitrin, and ursolic acid, corosolic acid, asiatic acid. All fractions showed antioxidant activity. The infusion was more efficient in reduce the cell viability in the MTT assay. As a result of the tyrosinase enzyme inhibition test, the 80% EtOH macerate was found to be more active than infusion. Further studies on the identified compounds are still ongoing to investigate the pharmacological activities since focusing on the development of natural products.

Keywords: *Myrtus communis*, phytochemistry, BAGF, phenolic compounds, α-synuclein aggregation

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S2.P122 Dietary molecules from *Glycyrrhiza foetida* and their modulation on metabolic syndrome pathways

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Glycyrrhiza is a genus of edible plants, commonly known as licorice, with a long use in folk medicine for their adaptogenic, anti-inflammatory, antioxidant effects, estrogenic activity and hepatoprotective properties. Among the less investigated species of this genus, *G. foetida* seems to be a new promising tool to treat metabolic syndrome (MetS). In fact, this plant has been pointed out as a source of amorfrutins, acetate-deriving polyphenols that explicate a potent insulin-sensitizer effect as PPARγ agonists that are quite rare in this genus (Weidner et al., 2012). The minor members of this class of compounds have not been in-depth characterized, thus, after a preliminary LC-UV- MS^2 dereplication step, we have carried out a NMR-based phytochemical screening of *G. foetida* aerial parts that led us to the characterization of 29 compounds, mainly polyphenols, including 11 previously undescribed amorfrutins and an unprecedented flavanone. The amorfrutins were tested on PPARγ and PPARα and, besides amorfrutin A, the most active compound on PPARγ, we have identified the new amorfrutin H as a selective PPARα agonist and the unprecedented amorfrutin E as a dual PPARγ/α agonist (Serino et al., 2023). Moreover, all the compounds were tested on other two MetS dysfunctional pathways, highlighting amorfrutin M and decarboxyamorfrutin A as mitochondrial stimulators, while amorfrutin 2 as a glucose uptake promoter. Thus, *G. foetida* is rich source of many bioactive ingredients that, through to the concomitant modulation of different pathways, can ameliorate MetS, emerging as a new potential functional food.

Keywords: *Glycyrrhyiza foetida,* metabolic syndrome, amorfrutins, mitochondrial activity, GLUT

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S2.P123 Smoking hot – the story of the "pepper bark tree" *Warburgia salutaris*

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In southern Africa, the aromatic, peppery-tasting bark of *Warburgia salutaris* is widely known for its antimicrobial activity especially as treatment for upper respiratory tract infections. Historically, smoke inhalation or smoking of the bark or root alone (or with another plant such as *Cannabis sativa)* has been used in traditional medicine for the treatment of upper respiratory tract infections (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; van Wyk et al., 1997). The bioactive compounds are concentrated in the bark and therefore this plant-part is most often used for infusions, decoctions, and to a lesser extent for smoke therapy in traditional medicine. Recently, in South Africa, a study among the Vhavenda people in Limpopo province, reported the use of *W. salutaris* bark as smoke therapy (11%) (Ramarumo et al., 2019). Furthermore in a recent study, the drimane sequiterpenes in the smoke fraction of *Warburgia salutaris* bark was reported for the first time (Leonard, 2021). In this study, a smoke recovery system was used to capture these compounds. The sesquiterpenes were isolated using preparative high-performance liquid chromatography (HPLC) and using nuclear magnetic resonance (NMR) were identified as cinnamolide-3β-acetate, ugandensidial, bemadienolide, and cinnamolide. All these sesquiterpenes have been shown to have anti-infective properties and thus this could offer another therapeutic route of administration as well as assisting in conserving the plant as less material is needed for this therapeutic route of administration.

Keywords: Traditional medicine, Sesquiterpenes, Smoke therapy, *Warburgia salutaris*

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S2.P124 The effect of cannabidiol, cannabidiol and cannabigerol on the motoric activity of the rat proximal colon – *ex vivo* study

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The study was aimed at evaluating the effect of cannabidiol (CBD), cannabidiol (CBN) and cannabigerol (CBG) on the motoric activity of the gastrointestinal tract. The experiments were carried out on rat proximal colon preparations incubated in modified Krebs-Henseleit solution (37°C, pH 7.35-7.45) under isometric conditions in an organ bath set under a load of 0.01 N. The strips were treated with solutions of cannabinoids (in the range of 0.08 – 800 μg/mL) and subsequently with acetylcholine (ACh, 1 μM), without flushing. The impact of cannabinoids on contractility of the colon preparations was registered. The spontaneous activity and ACh-reactivity of colon muscles in the presence of solvent (0.5% DMSO) was the control.

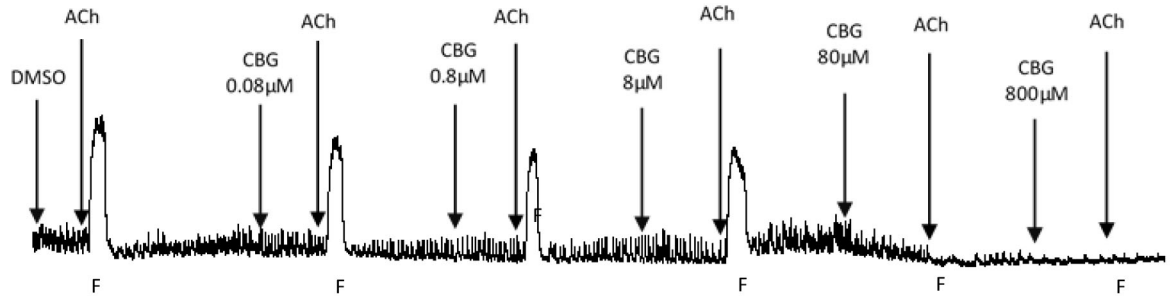

Fig. 1. Example recording of a rat proximal colon strip's reactivity to acetylcholine (ACh) in the presence of DMSO (0.5%) and cannabidiol solutions (CBD). F – flushing

Incubation of proximal colon preparations in the presence of cannabinoid solutions caused significant myorelaxation. The obtained results indicate that all cannabinoids dose-dependently affected the spontaneous activity and reactivity of the colon strips, and that the effect differed depending on the type of cannabinoid. The reaction to ACh when cannabinoids solutions were introduced preceding ACh application was reduced. It was demonstrated that the reduction of contractions was strongest in the case of CBG solutions and amounts to 22.1±21.6% and 7.9±9.1% when the preparations were treated with 80 and 800 μM of cannabinoid, respectively. The incubation of tissue slices in CBN solutions caused the least changes; in the case of reactivity to ACh, the significant reaction was observed when colon preparations was incubated in the maximum concentration and it amounts to 40.9±31.4% of control reaction to ACh. The obtained results are a valuable contribution to the area of research on the use of cannabinoids in supportive therapy of gastrointestinal diseases.

Keywords: cannabinoids, rat, colon, *ex vivo*

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S2.P125 Coumarin polypharmacology and elucidation of an underlying molecular mechanism of action

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Coumarins, widely present in herbal drugs, spices, and food, are recognized for their diverse pharmacological properties, including anti-inflammatory, anticoagulant, anticancer activities, and effects on central nervous system disorders (Skalicka-Woźniak et al.). In vivo, they exert unepected biphasic pharmacological effects (Kowalczyk et al., Skiba et al.). Our investigation utilizing Nuclear Magnetic Resonance (NMR) reveals the thiophilic nature of the unsubstituted 3,4-double bond in coumarins. Through the creation of clickable coumarin probes (CLICOPs), we demonstrate for the first time that various coumarins form covalent bonds with specific proteins in living cells, murine tissue homogenates, and human blood. Employing a range of different CLIOPs enabled competition with natural coumarins, facilitating the identification and visualization of previously unknown covalent coumarin-protein interactions on SDS-PAGE using chemoproteomics. Notably, attempts to label selected proteins identified in chemoproteomics using purified proteins did not yield covalent reactions. However, the addition of macromolecular crowders, such as Ficoll-400, promoted the thiophilic reaction *in vitro*. Our findings indicate that the presence of artificial or cellular macromolecular crowders is essential for coumarins to react. Further insights revealed that coumarins can elicit no, allosteric, or orthosteric pharmacological effects on specific proteins via this covalent mechanism, underscoring their hitherto underestimated polypharmacological potential and raising questions about their toxicity. Additionally, our study challenges conventional *in vitro* approaches by demonstrating that natural coumarins may not react in certain conditions but covalently interact in a complex cellular environment. This complexity underscores the polypharmacology of coumarins and carries significant toxicopharmacological implications, providing a novel molecular mechanism underlying their diverse effects.

Keywords: Coumarins, mechanism of action, macromolecular crowding, polypharmacology

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S2.P126 Deciphering the targets of trypanocidal chalcones in *Trypanosoma cruzi*

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The protozoan parasite *Trypanosoma cruzi (T. cruzi)* is the causative agent of Chagas disease (CD) which affects millions of primarily indigenous people in Latin America (Bern et al., 2011; Salm and Gertsch, 2019). Infection with *T. cruzi* is usually life-long, and up to 30% of individuals develop chronic Chagas disease, with symptoms that include cardiomyopathy and/or digestive mega syndromes. Treatment of *T. cruzi* infection with the nitroheterocyclic drugs benznidazole and nifurtimox, introduced in the 1970s, is suboptimal. This highlights the urgent need for improved drugs. Based on an extensive screening of 775 extracts of botanical drugs used in Bolivia in the context of CD and botanical drugs from unrelated indications from the Mediterranean De Materia Medica (Salm et al. 2021), we investigate the natural product class chalcones (α, β-unsaturated ketones) (Fig. 1B) which are often present in plants together with flavonoids and showed low micromolar antichagasic effects.

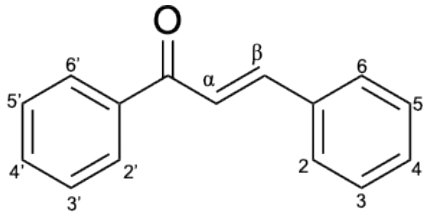

Fig. 1 Chemical structure of the chalcone scaffold

It was recently shown that chalcones mediate covalent interactions with specific enzymes in *Leishmania* spp. (Escrivani et al., 2021). To identify the molecular mechanism of action of trypanocidal chalcones in *T. cruzi*, we employed a chemoproteomics strategy by generating clickable chalcone probes. Through chemical modification, we successfully improved the selective toxicity of chalcones towards *T. cruzi* and established a strategy to identify their targets. Intriguingly, the peroxidase enzymes targeted by chalcones in *T. cruzi* also appeared to have a role in benznidazole and nifurtimox resistance. By employing targeted and untargeted LC-MS/MS based metabolomics we aim to decipher the role of these enzymes in *T. cruzi* biology. Overall, chalcones are an interesting new trypanocidal class of natural products and clickable probes may help to study the biology of peroxidases in this parasite.

Keywords: *T. cruzi,* chalcone, chemoproteomics, antichagasic effects

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S2.P127 Innovative methods for analysing flavonolignan complex from *Silybum marianum* L.

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Silymarin is a mixture of flavonolignans that is mostly present in the fruits of the milk thistle plant (*Silybum marianum* (L.) Gaertn., Asteraceae). Research on *S. marianum* has shown that it has antiatherosclerotic, antidiabetic, hepatoprotective, antihypertensive properties. At least 1.5% w/w of silymarin in dried seeds is required for the European Pharmacopoeia test, which entails a drawn-out and complicated sample preparation process and comprehensive HPLC analysis. As a result, the goal of this study was to ascertain the amount of silymarin contained in milk thistle fruits and extracts from both wild and cultivated plants, in accordance with the Ph. Eur. 11.0 monograph, as well as by utilizing quick sample preparation and quantification techniques to accelerate the analysis. The silymarin concentration of milk thistle fruit samples, which were collected over several years, ranged within a range of 0.3% to 1.8% w/w, according to the acquired HPLC data. The Ph. Eur. sample preparation was substituted with a very effective one-hour subsequent ultrasonic extraction. The fruits and extracts were also examined using spectroscopic methods. A partial least-squares (PLS) model for quantification was constructed utilizing the obtained spectra, with HPLC as a reference technique. The chemometric analysis based on the data obtained from the FTIR and Raman spectra points out satisfactory statistical indicators for employment of these methods for quantitative determination of silymarin.

Keywords: Milk thistle, extraction, spectroscopic methods, chemometric analysis

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S2.P128 Phytochemical analysis of *Hyssopus officinalis* L. cultivated in western Turkey

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H. officinalis L. belonging to the family Lamiaceae, possesses antioxidant, antimicrobial and several other pharmacological activities. The essential oil of hyssop is widely used in food, pharmaceutical and cosmetic industries throughout the world (Tahir et al., 2018). In the present work we performed phytochemical analysis on the methanolic extract (yield 9.55 %) and essential oil (yield 0.53%) of *H. officinalis* which was cultivated in western Turkey. A reversed-phase HPLC- DAD method has been used and validated for the determination of rutin and apigenin in the methanolic extract from *H. officinalis*. The content of rutin and apigenin were determined as 3552.913±249.324 μg/g and 12.383±1.102 μg/g extract, respectively. In previous studies, apigenin was detected as the major component (Fathiazad et al., 2011), while rutin was not detected or was detected in very low amounts in *Hyssopus* extracts (Tahir, 2018; Proestos, 2005). The chemical constituents of essential oil obtained by steam distillation from the aerial parts of *H. officinalis* were analyzed by GC-MS. It was determined that hyssop essential oil contained isopinocamphone (25.0%), pinocamphone (15.6%) and β-pinene (8.5%) as major components. According to the previous studies, so far three types of *Hyssopus* oils have been encountered, namely; monoterpene ketone-type, 1,8-cineole type and methyeugenol-type. In the present study, the essential oil found to be rich in monoterpene ketones and among these, the content of isopinocamphone was higher than the content of the same compound found in the oils previously reported from Turkey (Kürkçüoğlu et al., 2016), except in the oil of *Hyssopus* cultivated in southeastern Turkey, with 57.27% (Kızıl, 2010).

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Keywords: *Hyssopus officinalis,* essential oil, rutin, apigenin

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S2.P129 Enhancing wound healing: sericin and *Chelidonium majus* L. as potential dressings

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Wound healing, a complex physiological process orchestrating intricate cellular and molecular events, seeks to restore tissue integrity. The burgeoning interest in leveraging the therapeutic potential of natural substances for advanced wound dressings is a recent phenomenon. Notably, Sericin, a silk-derived protein, and *Chelidonium majus* L. (C. majus), a botanical agent, have emerged as compelling candidates, providing a unique combination of natural elements that may revolutionize conventional wound care approaches. Sericin, renowned for its diverse properties, displays unique properties that accelerate the wound healing process. Simultaneously, C. majus, with its diverse pharmacological compounds, shows promise in reducing inflammation and promoting tissue regeneration. As the demand for innovative wound care solutions increases, understanding the therapeutic potential of natural products becomes imperative. This review synthesizes current knowledge on Sericin and C. majus, envisioning their future roles in advancing wound management strategies. The exploration of these natural substances as constituents of wound dressings provides a promising avenue for the development of sustainable, effective, and biocompatible materials that could significantly impact the field of wound healing.

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Keywords: tissue repair, biomaterial, medical device, silk, greater celandine

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S2.P130 Evaluating the efficacy of topical application of *Genista tridentata* (L.) (*Fabaceae*) leaf extract on wound healing in experimental diabetes

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Diabetic wounds are a serious complication of diabetes along with peripheral neuropathy, macrovascular and microvascular disease, impaired angiogenesis, and chronic inflammation (Pawar et al., 2021). In this work methanolic leaf extracts from *Genista tridentate* (L.), known for their antioxidant and anti-inflammatory properties (Laranjeira et al., 2023), were investigated *in vitro* and *in vivo* for their antioxidant activity, fibroblasts cytotoxicity, and their potential in promoting wound healing in experimental diabetes. The *G. tridentata* extract exhibited strong antioxidant activity both in *in vitro* and in the TBHP test while the MTT test (Oliveira et al., 2018) in fibroblasts showed no toxicity. Using a STZ-NA-induced diabetes model (Diab) (Masiello et al., 1998) in association with an excision wound model (Nasir et al., 2016), 21 rats were divided into three groups (n=7 each; daily treatment for 10 days): (i) Diab+Gt, topically treatment with *G. tridentata* incorporated base- cream; (ii) Diab+Veh, topically treatment with a base-cream; and (iii) Diab, no topical treatment. Skin histopathological analysis demonstrated improvements of several wound healing parameters, namely in wound contraction, decreased granulocytic tissue area, and increased neo-epidermis thickness after treatment with the base-cream with *Genista tridentata* extract. Our data shows the topical application of a methanolic extract from *Genista tridentata* leaves improves wound healing in diabetic rats, highlighting its potential use in the pharmaceutical industry.

Keywords: *Genista tridentata, Diabetes mellitus*, medicinal Plants, wound-healing

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S2.P131 *Lepidium meyenii* – isolation of imidazole alkaloids by centrifugal partition chromatography

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Lepidium meyenii (maca) is one of the plants used by Peruvians for over two thousand years as an edible plant (Da Silva Leitão Peres et al., 2020). Most studies indicate its positive effect on fertility, but it has also been shown to have neuroprotective, dermatological, antimicrobial and anticancer effects (Ulloa del Carpio et al., 2024). Lepidilines are a unique and characteristic group of compounds from the 'Peruvian ginseng'. These four imidazole alkaloids that have been isolated by the authors are currently available only as synthesized compounds, which are very expensive. Thus the aim of this study was to use centrifugal partition chromatography to purify all four imidazole alkaloids (Fig. 1) from the total water-methanol extract of *Lepidium meyenii* tubers and, if possible, to separate them from one other. This liquid-liquid separation technique offers low solvent consumption, high selectivity, and repeatability, but also up-scaling potential, which makes the elaborated purification protocols valuable in terms of their potential industrial applications. Due to a common parent structure shared by all lepidilines and only slight differences in the R_1 and R_2 substituents, the selection of a biphasic liquid system (BLS) was a challenge. To achieve the best results in the preparation of promising partition coefficients, we lowered the pH of the BLS, and after establishing appropriate separation conditions, i.e., elution mode, rotation speed, and flow of the mobile phase, we managed to separate lepidilines from the total extract.

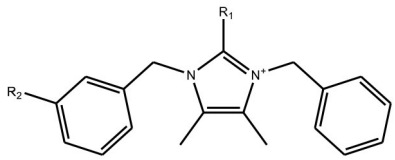

Fig. 1. Parent structure of imidazole alkaloids (R_1: –H; $–CH_3$, R_2: –H; $–OCH_3$)

Keywords: *Lepidium meyenii,* brassicaceae, lepidilines, isolation, centrifugal partition chromatography

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S2.P132 Connecting targets of the standardized herbal combination STW 5-II to gastrointestinal disorders

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STW5-II, a standardized combination of six plants, is indicated in Germany for the treatment of Functional Dyspepsia (FD) and the Irritable Bowel Syndrome (IBS). Both are complex and heterogeneous disorders with multiple pathomechanisms. We predicted molecular targets of STW5- II in FD and IBS by constructing compound-target networks between compounds supposed to contribute to the biological activity of STW5-II and relevant targets for FD and IBS. These targets were identified by searching the GEO database for comparative gene expression (GE)-profiles from colon biopsies of IBS or FD patients and healthy controls. The predicted targets were validated by wet lab experiments with NCM460 colon cells treated with STW5-II and its components. Deep sequencing and RT-PCR of these samples confirmed the predicted regulations of AKT1, NOS3, HSP90AA, TRPV and SMAD2 by STW 5-II (Ulrich-Merzenich et al. 2021, Shcherbakova et al. 2022). In a second step gene ontology and reactome pathways were analyzed and indicated significant upregulations of cell motility (GO:2000145), epithelium development (GO:0060429) and lipid metabolism (R-HSA-556833) by STW 5-II, whereas ATP metabolic processes (GO:004634) were downregulated. We extended the comparative GE-analysis of NCM460 colon cells treated with STW5-II to colon biopsies of IBS patients during a 7-day red meat diet. 37 genes were differentially regulated. E.g.Tropomyosin 1, which is considered a novel marker for cancer of the gastrointestinal tract, was decreased. We can provide further evidence that STW5-II may not only addresses disease-specific but also food-related pathomechanisms.

Keywords: plant combination, synergy, compound-target network, IBS, FD

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S2.P133 The anti-inflammatory and skin barrier function recovery effects of *Schisandra chinensis* in mice with atopic dermatitis

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The fruit of *Schisandra chinensis* (Turcz.) Baill. is widely used medicinally to treat coughs, asthma, exhaustion, eczema, and pruritus in Northeast Asian countries, including Korea, China, and Japan (Kim et al., 1997). This study was designed to investigate the effects of *S. chinensis* on dermatitis and skin barrier dysfunction in mice with calcipotriol (MC903) induced atopic dermatitis (AD) (Moosbrugger-Martinz et al., 2017). In this experiment, the inhibitory effects of an ethanolic extract of *S. chinensis* (EESC) on skin lesions, water content, water holding capacity (WHC), histopathological abnormalities, and inflammatory cytokine and chemokine levels were evaluated in mice with AD induced by MC903. In our results, topical EESC ameliorated skin lesions, reduced skin water content and MC903-induced WHC increases. EESC also prevented MC903-induced histopathological abnormalities such as epidermal disruption, hyperkeratosis, spongiotic changes, and immune cell infiltration in inflamed tissues. Also, topical EESC reduced MC903-induced levels of pro-inflammatory cytokines and chemokines, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-4, IL-6; IL-8, monocyte chemotactic protein (MCP)-1, and thymic stromal lymphopoietin (TSLP). Furthermore, unlike dexamethasone, EESC did not reduce spleen/body weight ratios. In conclusion, these results suggest that *S. chinensis* can be used as an alternative to external corticosteroids and that its anti-inflammatory and skin barrier dysfunction-restoring effects are related to downregulations of pro-inflammatory cytokines and chemokines, such as TNF-α, IL-4, IL-6, IL-8, and TSLP.

Keywords: *Schisandra chinensis*, herbal medicine, inflammation, atopic dermatitis, eczema

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S2.P134 Comparison of amount of tannins in Ericaceae plants using different water extraction methods

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Tannins constitute a rich group of phenolic compounds in plants and are soluble in water. However, there is no single tannins extraction with water method suitable for all tannins accumulating plants. Therefore, to extract higher content of tannins from different raw materials, it is necessary to choose a suitable extraction with water method for each species separately (Chuo et. Al., 2022).

The aim was to compare content of tannins and phenolic compounds in *Vaccinium vitis-idaea*, *Vaccinium myrtillus*, *Arctostaphylos uva-ursi*, *Calluna vulgaris*, *Empetrum nigrum, Andromeda polifolia*, and *Rhododendron tomentosum* leaves, using hot water extraction, ultrasonic extraction as well as maceration with water. Folin-Ciocalteu method was used to determine tannins and phenolics expressed as tannic acid equivalents (TAE). Tannin content was calculated by subtracting of phenolics remaining after binding of tannins to polyvinyl polypyrrolidone from the total phenolics content.

Hot water was the most effective for extraction of phenolics. For example, hot water extracted four times more phenolic compounds from *A. uva-ursi* than maceration (35.30 ± 0.96 and 8.07 ± 0.46 % (TAE), respectively). Meanwhile, there was no single tannins extraction method suitable for all studied species: extraction with hot water was the most efficiently for *R. tomentosum*, *E. nigrum*, *A. polifolia* and *V. myrtillus* (10.80 ± 1.97, 3.94 ± 0.04, 6.81 ± 0.58, and 5.63 ± 0.54 % (TAE), respectively), ultrasonic extraction – for *A. uva-ursi* and *V. vitis-idaea* (6.54 ± 1.72 and 4.83 ± 0.72 % (TAE), respectively), and maceration – for *C. vulgaris* (3.02 ± 0.36 % (TAE)).

Keywords: Ericaceae, tannins, water, extraction methods

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S2.P135 Biosynthetic relationship and self-assembly nanoparticles of royleanone isolated from Plectranthus species

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The *Plectranthus* genus belongs to the *Lamiaceae* family and consists of around 300 species distributed in Africa, Asia, and Australia. *Plectranthus* spp. has been reported to be rich in diterpenoids, such as different types of royleanones **1** (Fig. 1). These royleanones are known for their different biological activities including antimicrobial, antioxidant, general toxicity, and cytotoxicity activities (Ntungwe et al., 2013). Therefore, this work aimed to isolate large amounts of cytotoxic royleanones from acetone extracts of *plectranthus* species through an ultrasound-assisted extraction method. These royleanones were tested for their cytotoxicity and employed as a starting material for the synthesis of self-assembled squalene and oleic acid nanoparticles. The obtained compound was characterized by spectroscopic methods, mainly NMR techniques. Additionally, the characterization of the nanoparticles (size, zeta potential, and polydispersity using DLS and SEM), the ability to release the drug unit, and the preliminary evaluation of their cytotoxicity in different cell lines were also tested. Due to the similarity in the chemical structure of these royleanones were set to study their biosynthetic relationship using Fukui indexes. This work established that there is a biosynthetic relationship between the isolated abietane diterpenoids and that the synthesized nanoparticles could act as a strategy to vehiculate the drug.

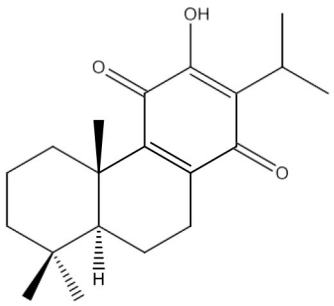

Fig. 1. Royleanone **1** structure

Acknowledgments: This work has run within the research project PTDC/QUI-QFI/1880/2020. The CQC-IMS is supported by the Fundação para a Ciência e a Tecnologia (FCT), through projects UI0313B/QUI/2020 (DOI: 10.54499/UIDB/00313/2020), UI0313P/QUI/2020 (DOI: 10.54499/UIDP/00313/2020) and LA/P/0056/2020.

Keywords: royleanones, self-assembly nanoparticles, biosynthetic relationship

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S2.P136 Yamogenin – a steroidal plant metabolite triggers extrinsic and intrinsic pathway of apoptosis in gastric cancer cells

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Steroidal saponins are high-molecular weight compounds widely distributed in plants, among which *Agavaceae*, *Liliaceae*, and *Dioscoreaceae* are the main source of these steroids. The compounds have many pharmacological and biological properties, e.g. antimicrobial, anti-inflammatory, antitumor, fungicidal, and insecticidal activity (Hostettmann and Marston, 1995; Qin et al., 2012; Stefanowicz-Hajduk et al., 2022). Yamogenin is one of the spirostane saponins, a stereoisomer of diosgenin and occurs in *Trigonella foenum-graecum*, *Asparagus officinalis*, and *Dioscorea collettii*. In this study, we evaluated cytotoxic activity of yamogenin *in vitro* on gastric cancer AGS cells and determined the role of selected cellular factors in cell death. The viability of the cells was estimated with MTT assay. The cell cycle arrest, mitochondrial membrane depolarization, the level of oxidative stress, and the activity of initiator and executioner caspase-8 and -9 was estimated with flow cytometry and luminometry, respectively. Genes expression analysis at the mRNA level was conducted with Real-Time PCR. The obtained results indicate that yamogenin has cytotoxic activity in AGS cells with an IC_{50} value of 18.50 ± 1.24 μg/mL and strongly inhibits the cell cycle in the sub-G1 phase. The compound also significantly decreases mitochondrial potential, increases reactive oxygen species (ROS) production, and strongly activates caspase-8 and -9, what indicates that the receptor and mitochondrial way of apoptosis are involved in cell death.

Keywords: cytotoxicity, saponins, receptor and mitochondrial pathway of apoptosis, flow cytometry

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S2.P137 Phytochemical analysis and neuroprotective activity of extracts from *Paliurus spina-christi* (Rhamnaceae)

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Paliurus spina-christi Mill., the only species of the genus *Paliurus* Mill. growing in Turkey (Davis, 1967), is used traditionally for various medical purposes such as a diuretic, and against kidney stones and diabetes (Sargın et al., 2013). The plant has recently attracted attention due to its phenolic content and promising biological activities (Takım, 2021). In the present study, the total phenolic, total flavonoid and rutin content, a common major compound found in the plant, were determined in the leaf and fruit extracts. In addition, cell viability was evaluated using an MTS/PMS assay in a cellular model of oxidative stress following the exposure to extracts at various concentrations (1-100 µg/mL). Methanolic extracts were prepared using an ultrasonic bath (3 times) and evaporated to dryness. In the leaf extract (yield 25.55%), total phenols and total flavonoids were determined as 102.974 ± 4.511 mg GAE/g and 68.677 ± 3.016 mg QE/g, whereas in the fruit extract (yield 10.57%), total phenolic and flavonoid contents were found to be 62.051 ± 3.307 mg GAE/g and 46.872 ± 1.124 mg QE/g, respectively. A reversed phase HPLC-DAD method was employed and validated for the quantitative determination of rutin. The amount of rutin was detected as 2961.167 ± 88.355 µg/g extract in the leaves (extract yield 21.94%) and 3593.833 ± 247.718 µg/g extract in the fruits (extract yield 9.26%). Neuroprotection by the extracts in the presence of hydrogen peroxide was highlighted by a significant reduction of cellular loss at concentration less than 10 µg/mL ($p<0.05$).

Keywords: *Paliurus spina-christi,* rutin, HPLC-DAD, neuroprotective activity

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S2.P138 Bioactive molecules separation from *Acmella oleracea* (Jambu) from Amazon Florest

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Acmella oleracea (L). R. K. Jansen (Asteraceae), popularly known as Jambu, is consumed in aqueous preparations as food in Latin America, more precisely in the north of Brazil. This research focused on the chemical compounds released into the water that can justify its anaesthetic or anti- proliferative medicinal action on tumour cell lines such as spilanthol **1** (Fig. 1), its main constituent (Pereira, 2023; Williams, 2013).

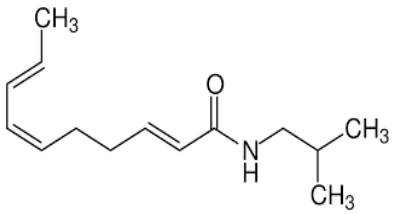

Fig. 1. Structure of spilanthol **1**

The initial purpose of this study was to devise the best solvent system to perform the separation of the ethyl acetate and butanol fractions extracted from an infusion prepared with Jambu aerial parts for subsequent use in HSCCC and HPLC. Aerial parts of *Acmella oleracea* were collected at Fazendinha district (S 0°02′30.40′′/W 5106′37.5′′), Macapá, Brazil. An infusion was prepared and extracted with ethyl acetate and butanol. After testing four different mobile phases with a combination of ethyl-acetate, acetic acid, formic acid, butanol and water at different proportions, the (butanol 3: acetic acid 1: water 4 was chosen to analyse the compounds in both extracts. Similarly, we could choose the best solvent system separating the extracts on HSCCC. For both cases, after testing four possibilities, the best results were achieved with ethyl acetate: butanol: water 4,5:0,5:5. One run using the reverse phase mode allowed for separating different compounds, and spilanthol was obtained in the final test tubes. It was then purified using Sephadex LH-20. We are currently trying to purify the minor compounds present in the sample, which are believed to be flavonoids.

Keywords: *Acmella oleracea*, HSCCC, spilanthol, flavonoids

Acknowledgements: FAPEAP, UNIFAP, Trinity College Dublin.

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S2.P139 The protective impact of *Aronia melanocarpa* L. berries extract against cadmium-induced changes in the concentrations of calciotropic hormones in the serum

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The results of our studies, conducted in an experimental rat model of current environmental human exposure to cadmium, allowed us to hypothesize that *Aronia melanocarpa* L. (Michx.) Elliott berries (chokeberry) products may be an effective strategy in protection against health outcomes caused by this toxic metal, including skeleton damage (Brzóska et al., 2015; Mężyńska et al., 2019; Ruczaj et al., 2024; Smereczański et al., 2023). However, the protective use of chokeberry products in people exposed to cadmium requires further research. The present study aimed to evaluate, in our experimental model (1 and 5 mg Cd/kg of diet, 3–24 months), the impact of low-level and moderate intoxication with cadmium, on the concentrations of calcium and calciotropic hormones (1,25- dihydroxyvitamin D – 1,25$(OH)_2$D, parathormone – PTH, and calcitonin – CT) in the serum and whether the supplementation with a 0.1% extract from *A. melanocarpa* berries can influence these parameters. Calcium was determined by atomic absorption spectrometry, whereas calciotropic hormones were assayed with the use of commercial kits. The exposure to cadmium, dose and duration dependently, decreased the serum concentrations of 1,25$(OH)_2$D and CT and increased PTH concentration. Calcium concentration was unchanged except for its decrease after the 3-month exposure to 5 mg Cd/kg of diet. The co-administration of chokeberry extract prevented all cadmium-induced changes in the concentrations of calciotropic hormones. In conclusion, the supplementation with *A. melanocarpa* berries extract during exposure to cadmium allows for the maintenance of proper levels of calciotropic hormones in the serum but it does not influence the concentration of calcium.

Keywords: *Aronia melanocarpa* berries, cadmium, calcium, calciotropic hormones, protection

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S2.P140 LC-MS/MS analyses of triterpenes and polyphenols from less-polar fractions of *Rosa rugosa* leaf extract and their biological activity

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Plants from the rose family (Rosaceae) have high edible and medicinal value and a long history of therapeutical application. Our previous research showed that rose leaves contain considerable amounts of free and bound phenolic acids and flavonoid aglycones (Olech et al., 2020a). In this research, we investigated less-polar rose components. Therefore, air-dried crushed leaves of rugosa rose (*Rosa rugosa* Thunb.) were exhaustively extracted with portions of 80% and 95% ethanol to obtain ethanolic extract (RREt). RREt was then fractionated using liquid-liquid extraction, which yielded several fractions with different polarity levels. Among them, water insoluble (non-polar) and chloroform fractions were obtained with a relatively high yield (13.3 and 1.7%, respectively).

The content of pentacyclic triterpenes was determined with the newly developed method using liquid chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry (LC- APCI-MS/MS). It revealed the presence of pomolic, fupenzic, maslinic, corosolic, betulinic, ursolic, and oleanolic acids as well as uvaol, lupeol, β-amyrin, and α-amyrin. The highest contents of these compounds were found in the water insoluble fraction. The identification and quantification of phenolic acids and flavonoids was carried out using an improved LC-ESI-MS/MS-MRM method (Olech et al., 2020b). The fractions were found to contain twelve phenolic acids (including three caffeoylquinic acids), eleven flavonoid glycosides, and seven flavonoid aglycones. Moreover, enzyme inhibitory and antioxidant activities were determined in the samples in *in vitro* assays. The oxygen radical absorbance capacity varied from 445.12 to 644.63 μg Trolox/mg. The ability to inhibit α-glucosidase, α-amylase, and pro-inflammatory enzymes (lipoxygenase, hyaluronidase) increased with the sample polarity.

Keywords: rosaceae, enzyme inhibitory activity, anti-inflammatory activity, tandem mass spectrometry

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S2.P141 Volatiles, triterpenes, phytosterols and anti-inflammatory activity of *Rhododendron luteum* Sweet (Ericaceae) supercritical CO_2 extracts

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Terpenes are a diverse group of plant secondary metabolites, including monoterpenes, diterpenes, sesquiterpenes, triterpenes, and saponins. This group is also diverse in terms of biological activity. They have been proven to have anti-inflammatory, anti-diabetic, anti-proliferative, and antiviral activities (Castellano et al. 2013; Vázquez et al. 2011; Xiao et al. 2018). The present study aimed to determine the terpene profile of supercritical CO_2 extracts of *Rhododendron luteum* leaf (SCE-RLL) and analyze their anti-inflammatory and antioxidant activity. Extracts were subjected to LC-APCI- MS/MS analysis, revealing the occurrence of some pentacyclic terpenic acids and phytosterols. Using the HS-SPME-GC-FID-MS method allowed the detection of more than 100 volatile compounds in one of the extracts, mainly limonene, eugenol, β-phenylethanol and β-caryophyllene. Next, all samples were subjected to biological studies using ELISA spectroscopic methods. The performed analyses have shown extracts ability to inhibit enzymes involved in inflammation: lipoxygenase, hyaluronidase, and xanthine oxidase. Moreover, they presented high antioxidant potential in the ORAC test. This is the first study of SCE-RLL composition and biological activity. Based on the results, *R. luteum* Sweet can be a valuable source of pentacyclic terpenes, phytosterols, and other phytochemicals with anti-inflammatory and antioxidant activity.

Keywords: *Rhododendron luteum* Sweet, LC-MS, GC-MS, enzyme inhibition

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S2.P142 Antioxidant and anti-inflammatory activity of the *Berberis vulgaris* L. bark extract

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Berberis vulgaris L. (Berberidaceae) is a medicinal plant with over 300 years of tradition in folk medicine. *Barberry vulgaris* L. is valuable in Chinese folk medicine, where it is called "Sankezhen", in Ayurvedic medicine, and has also been used in traditional medicine in Europe in recent centuries (Och et al., 2021). Despite its well-established traditional use, scientific research reveals little about the biological activity of barberry. Modern research has confirmed the anti- inflammatory effect of barberry, but mainly in the field of fruit extracts (Li et al. 2016; Majeed et al. 2016). *Berberis vulgaris* L. is able to accumulate alkaloids in the bark and its pharmacological effect is probably related to its alkaloid composition. However, the broader chemical composition of its bark is still unexplored. The aim of this study was to evaluate the chemical profile of methanol extract from barberry bark and to analyze its antioxidant and anti-inflammatory effects. The extracts obtained with the ASE (accelerated solvent extraction) method were subjected to LC-MS/MS analysis, which showed the presence, among other compounds, of high-level Eleutheroside E – well-known for its anti-inflammatory activity (Och et al. 2023). The *in vitro* biological activity assays demonstrated the ability of the extract to inhibit the enzyme lipoxygenase involved in the inflammatory process and showed high antioxidant potential in the DPPH, ABTS, and ORAC tests. The results suggest that the bark of *Berberis vulgaris* L. can be a valuable source of phytochemicals with anti-inflammatory and antioxidant activity.

Keywords: *Berberis vulgaris* L, antioxidant activity, anti-inflammatory activity

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S2.P143 The use of herbal medicines for gynecological ailments: patient-reported outcomes

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Gynecological ailments have a negative impact on quality of life and productivity. Standard treatment is associated with poor tolerability and other issues related to public health. Herbal Medicines (HMs) are used traditionally for the treatment of gynecological complaints for centuries and constitute an excellent addition to current treatment options. HMs are well tolerated and therapeutically efficacious as evidenced by various clinical studies. Aim of this study was to expand this knowledge with real world evidence from patient reported outcomes. We analyzed a data sample taken from the pharmaco-epidemiological database PhytoVIS regarding therapeutic benefit and side effects of HMs used for the treatment of menstrual and menopausal ailments as well as uncomplicated urinary tract infections. We found that more than 80% of the patients in the sample assessed the overall therapeutic benefit of HMs for the treatment of gynecological complaints as very positive. Over 90% of the patients using HMs perceived no or no significant side effects. Treatment habits differed depending on the type of complaint. In this context, the majority of women with menstrual or menopausal ailments preferred to treat for time period of 1 month or longer, while those affected by uncomplicated urinary tract infections reduced the application of HMs to the length of their symptoms. Interestingly women with even strong symptoms relied on the therapeutic benefit of HMs. Real-world outcome data are an important supplement to clinical data. Our results reveal the excellent benefit-risk ratio of HMs and help to implement them into novel therapeutic strategies.

Keywords: gynecological ailments, herbal medicines, real-world data, pharmaco-epidemiological database, therapeutic efficacy

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S2.P144 An analysis of real-world data regarding therapeutic efficacy and adverse effects of herbal medicines in mental health issues

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Mental health issues (MHI) are commonly treated with psychotropic drugs which can cause central nervous, anticholinergic, and cardiovascular adverse effects (AE) besides compliance issues (Falkai et al., 2021). Herbal medicines (HM) could offer an alternative due to their therapeutic benefits and favorable tolerability (Salm et al., 2023). Nonetheless, they may also provoke AEs including drug interactions. In a data set of patients with MHIs taken from the pharmaco-epidemiological database PhytoVIS, we investigated the therapeutic benefits of HMs (mainly derived from *Valeriana officinalis* L., *Lavandula angustifolia* Mill., *Ginkgo biloba* L. and *Hypericum perforatum* L.) as well as the occurrence of AEs. The data set consisted of Patient-Reported Outcomes reflecting the real world of everyday medical care. Around 53% of the patients applied only HMs, while 45% took at least one additional medication. Over 90% perceived a therapeutic benefit from HMs while only few had unchanged or worsened symptoms. Around 87% did not perceive any AEs and only less than 3% reported significant AEs. Regarding AEs there was no statistically significant difference between patients who took HMs and those who took concomitant medication (Mann-Whitney U- Test: p=0.084). Likewise, there was no significant difference between those two groups regarding the severity of AEs (Mann-Whitney U-Test: p=0.088). Our results indicate that HMs have a favorable benefit-risk-ratio and a low potential for AEs. The integrated analysis of Real-World outcome data in context of clinical data will be a milestone to implement HMs into novel therapy strategies - not only for MHIs.https://bpspubs.onlinelibrary.wiley.com/doi/10.1111/bcp.13490

Keywords: herbal medicine, adverse effects, interactions, phytovis, real-world data, patient-reported outcomes

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S2.P145 Antiproliferative activity of homolupane triterpenoids

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From our previous studies, we searched for new bioactive triterpenoids and their derivatives based on the lupane scaffold, homolupanes. Source of these lupane-type triterpenes comes from outer part of birch bark (*Betula* species) (Hayek et al., 1989). Antiproliferative activity *in vitro* of these compounds was investigated in four human cancer cell lines and compared to healthy human BJ fibroblasts. In cervical carcinoma HeLa cells, three derivatives were the most promising with lower micromolar IC_{50}s and no toxicity to fibroblasts, thus showing a high therapeutic index. In addition, induction of apoptosis was detected in HeLa cells after 24 h treatment. This new series is more interesting than the previous lupane and homolupane triterpenes and saponins, due to their nontoxicity towards healthy human cells and stronger cytotoxicity to various cancer cell lines. This approach increases their potential as natural products-based anticancer agents. In addition, using other bioassays we proved also more biological properties, such as antiangiogenic or anti-inflammatory activities.

Keywords: lupanes, antiproliferative activity, apoptosis, cancer cells, fibroblasts

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S2.P146 Anticancer and antioxidant properties of extracts from *Rubus caesius* L. (Rosaceae)

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The European dewberry (*Rubus caesius* L., Rosaceae) is a medicinal shrub known and used for its pro-health properties in many parts of Europe and Asia. Extracts obtained from the leaves and stems of the European dewberry are a rich source of biologically active compounds with antioxidant, antiaging and antibacterial properties (Hering et al., 2022; Grochowski et al., 2016; Velickovic et al., 2015). Strong or long-lasting oxidative stress is often associated with the development of cancers. The European dewberry is a source of polyphenolic compounds with high ability to capture free radicals. In this poster authors present the results of anticancer and antioxidant properties of extracts obtained from young leaves and stems of the European dewberry. The anticancer properties of the European dewberry extracts (water and 50% ethanol extracts from stems and leaves) were tested *in vitro* on two cell lines - gastric adenocarcinoma (AGS) and human normal fibroblasts. The antioxidant activity of the extracts was estimated with DPPH, ABTS, FRAP and molibden reduction assays. The results indicated that the most active against gastric adenocarcinoma cells was ethanol extract obtained from leaves with IC_{50} = 11.72 µg/mL. Fibroblasts used as a control cells were not sensitive to the European dewberry extracts with the exception of water extract obtained from leaves, which reduced cell viability to about 60% at concentration of 400 µg/mL. The antioxidant test indicated, high ability of obtained extracts to reduce oxidative stress in similar way to standard- ascorbic acid.

Keywords: European dewberry, *Rubus caesius,* Rosaceae, fibroblasts, gastric cancer, antioxidant

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S2.P147 Broad antiplatelet activity of the flavones zapotin, chrysin, and tropoflavin in human platelet - rich plasma

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Flavonoids identified in plants, have been shown to exert cardioprotective properties that can be attributed, at least partially, to their antiplatelet (anti-aggregation) activity (Guerrero et al., 2005). However, whether their potential is (platelet) response- and agonist-specific is still only partially established. Three flavones: zapotin (5,6,2',6'-tetramethoxyflavone), chrysin (5,7- dihydroxyflavone), and tropoflavin (7,8-dihydroxyflavone), were selected for evaluation of their antiplatelet properties in the presence of different platelet agonists, i.e., collagen, adenosine diphosphate (ADP), and the thromboxane A_2 receptor agonist U46619 in platelet-rich plasma (Strawa et al., 2022). Flow cytometry measurements (Gołaszewska et al., 2024) towards platelet secretion (quantified as P-selectin exposure), aggregation (measured as fibrinogen binding) and pro-coagulant response (evaluated as phosphatidylserine (PS) exposure) were performed in the presence of a 200 μM concentration of each flavone. It was established that zapotin reduced collagen-evoked platelet secretion and PS exposure by 35% and 52%, chrysin by 47% and 42%, and tropoflavin by 38% and 60%, respectively. ADP-induced secretion and aggregation was diminished by zapotin by 23% and 46%, by chrysin by 5% and 36%, and by tropoflavin by 37% and 52%, respectively. U46619-triggered secretion and aggregation was inhibited by zapotin by 58% and 60%, by chrysin by 20% and 27%, and by tropoflavin by 27% and 31%, respectively. Our preliminary results point to flavones as broad-range inhibitors of platelet responses triggered by different principal platelet agonists in a close-to-native platelet environment (platelet-rich plasma). Further prospective study comprising structure-activity relationship examination and revealing the specific mechanism of the observed antiplatelet effects is expected.

Keywords: flavones, blood platelets, flow cytometry, pro-coagulant response, P-selectin

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S2.P148 Anti-aging, anti-neurodegenerative and healthspan-promoting compounds from *Dendrobium officinale* Kamura et Migo (Orchidaceae)

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Aging is a degenerative process associated with a gradual decline in cellular components and functions, ultimately leading to death. The quest for longevity has led to the exploration of various medicinal substances for their potential anti-aging properties. This research delves into the anti- aging potential of polysaccharides extracted from *Dendrobium officinale* (DOP). The study investigated its effect on lifespan extension, stress resistance, healthspan improvement, among other parameters, in the *Caenorhabditis elegans* model. *D. officinale*, a widely used medicinal herb in Asia, has been reported to possess various medicinal properties, including anti-aging property. However, due to high demand and scarcity, alternative cultivation methods are being explored. In this study, DOP grown in three different environments (tree, greenhouse, and rock) were utilized. The results showed that at 1000 µg/mL, DOP from the greenhouse extended the mean lifespan by 14% and the maximum lifespan by 25%. DOP from all three sources enhanced resistance to H_2O_2- induced stress at 2000 µg/mL, with only rock-grown DOP exhibiting resistance to thermal stress. All DOP sources enhanced Endoplasmic Reticulum stress response, and decreased α-synuclein aggregation. However, only greenhouse-grown DOP delayed β-amyloid-induced paralysis. In conclusion, DOP exhibited promising anti-aging activity, and these findings provide valuable insights into its health benefits, adds to the growing list of potential anti-aging interventions, and suggests best practices for cultivating *D. officinale* for maximum medicinal applications.

Keywords: anti-aging, anti-neurodegenerative, *Dendrobium officinale* polysaccharides

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S2.P149 Effects of STW 3-VI (St. John's wort) on the paracrine signaling between neurons and microglia under pro-inflammatory conditions *in vitro*

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Neurological insults and chronic stress are essential factors for inducing depression, affecting microglia function, so leading to enhanced release of pro-inflammatory cytokines, which causes a reduction of synaptic plasticity in mental disorders. Extracts of *Hypericum perforatum*, like STW 3- VI (SJW), showed protective, neurotrophic, and anti-inflammatory properties and are used in mild to moderate depressive episodes (Jungke et al., 2011). Aim was to determine the effects of SJW on the paracrine signalling under non- and pro-inflammatory conditions between neurons and microglia co-cultured *in vitro*. Co-cultured mouse SIM-A9 microglia and hippocampal HT-22 neurons were treated with different concentrations of SJW, hyperoside (HS), escitalopram (EC), nerve growth factor (NGF) as a positive control or ibuprofen (Ibu) as an anti-inflammatory control, with or without lipopolysaccharides (LPS). BDNF and TNF-α release were quantified by ELISA and neurite outgrowth by a staining kit. Treatment with SJW (250ng/ml) or HS (9 ng/mL) significantly stimulated the neurite formation by 3.8-fold and 2.3-fold, respectively, compared to negative control, similar to EC, Ibu or NGF. After LPS (250ng/ml) activation, neither SJW, HS, EC Ibu nor NGF induced neurite formation compared to LPS control. However, SJW, HS, EC, Ibu or NGF reduced the release of TNF-α for 20-30%. Microglia regulates neurite formation after treatment with SJW or HS, but not under pro-inflammatory conditions. SJW and HS have anti- inflammatory properties and positive effects on neuroplasticity, which might contribute to the clinically proven anti-depressant activity of STW 3-VI.

Keywords: *Hypericum perforatum L.,* hypericaceae, anti-depressant, neuroplasticity, microglia, paracrine signalling

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S2.P150 Gut microbiota - a new aspect for understanding the antidepressant effects of *Hypericum perforatum*?

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Hypericum perforatum L. is widely used for mild to moderate depressive disorders. Despite numerous clinical studies and meta-analyses, the mechanism of action and the responsible constituents are not fully elucidated. With the overall aim to investigate the role of gut microbiota for the activity of herbal preparations in mental health, a hydroethanolic extract of *H. perforatum* (STW 3-VI) was subjected to *in-vitro* upper gastrointestinal tract digestion (Brodkorb et al., 2019), and subsequently incubated *ex-vivo* with fecal samples from ten healthy donors (24h, anoxic, 37°C) (Pérez-Burillo et al., 2021). DNA extraction, followed by 16S rRNA gene sequencing, was used to evaluate the shift in microbial community composition during the incubation. Differential abundance analysis was performed using the combination of CLR abundances as input and Wilcoxon for dependent samples/Mann-Whitney U test for independent samples. The analysis showed that 2 phyla, 7 families, 9 genera and 21 OTUs were significantly modulated by treatment with *H. perforatum*. Many of the significantly changing taxa have been associated with depression. For example, *H. perforatum* treatment enhanced the Firmicutes/Bacteroidetes ratio, and decreased the levels of *Odoribacter* ($p= 0.0007$) and Erysipelotrichaceae_UCG-003 ($p= 0.034$) (opposite effects have been observed for individuals with depression (Barandouzi et al., 2020)). Provided that they also occur *in-vivo*, these changes in the microbial community composition suggest a potential additional non-CNS mechanism of action for the antidepressant effects of *H. perforatum*.

Conflicts of interest: The investigations and MEG have been funded by, HAK and OK are fully employed by Steigerwald Arzneimittelwerk, Bayer Consumer Health.

Keywords: *Hypericum perforatum*, gut microbiota, differential analysis, depression

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S2.P151 The cytotoxic effect of chrysosplenetin, a natural product from *Artemisia annua*, investigated with different human cancer cell lines, as well as *in vitro* CYP_{450} enzymatic activity in human liver microsomes

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The polymethoxyflavone Chrysosplenetin [5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3′,6,7-trimethoxychromen-4-one] (Fig 1) is one of the many chemical compounds found in the aerial parts of the *Artemisia annua* plant (Ferreira et al., 2010). *A. annua* is best known as the source plant for artemisinin, the sesquiterpene precursor for a range of first-line antimalarial drugs (Tu, 2011; Woerdenbag et al., 1990). Our interest is in the polymethoxyflavones which are abundantly present in this medicinal crop plant, and which may find application as drugs in their own right. The cytotoxic effect of chrysosplenetin was tested against the triple negative breast cell line (MDA-MB- 468), a tumorigenic cell line which expresses CYP1, and the normal breast cell line (MCF10A) which is devoid of CYP1 expression.

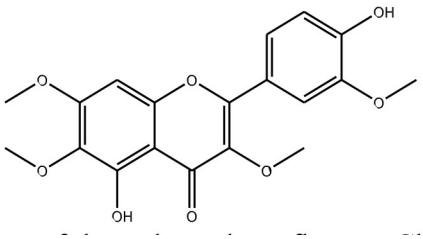

Fig. 1. Structure of the polymethoxyflavone Chrysosplenetin

The antiproliferative effect of chrysosplenetin was investigated by MTT assay, and it showed greater toxicity in the CYP1-expressing breast cell line (MDA-MB-468) with a significantly lower IC50 of 0.4 µM as opposed to 20 µM in the normal MCF10A cells. Additionally, the biotransformation of chrysosplenetin by CYP1 microsomes was investigated using High performance Liquid Chromatography (HPLC-DAD) and mass spectrometry (LC-MS). Two metabolite peaks were observed with retention times at 2.8 and 3.3 minutes, both presenting with an average mass of 361.3g in positive ionisation mode. We therefore propose that the polymethoxyflavone Chrysosplenetin (Fig. 1) may act as a prodrug that is selectively activated in hormone-dependent cancer cells.

Keywords: *Artemisia annua,* chrysosplenetin, cytotoxicity, CYP1 expression, biotransformation

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S2.P152 Bioprospecting for plant extracts with protective effects on endothelial function

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Vascular endothelium is a metabolically active organ which releases substances involved in vasodilation, vasoconstriction, coagulation, fibrinolysis, inflammatory and immunological processes, thus regulating vascular homeostasis. Any alteration in vascular homeostasis leads to endothelial dysfunction which is involved in atherosclerosis, coronary artery disease and hypertension. Plant extracts and pure phytochemicals may protect endothelial function and prevent against various cardiovascular diseases (Bartáková et al., 2021). Extracts from *Crataegus pentagyna* Waldst. et Kit. ex Willd., *Cornus mas* L., *Sorbus aucuparia* L. and *Viburnum opulus* L. were investigated regarding potential vasorelaxant and antiplatelet activities. *V. opulus* fruit and *C. pentagyna* leaf extracts induced strong endothelium-dependent vasorelaxation effects (EC_{50} = 6.31 and 1.91 µg/mL, respectively). The mechanism of action was found to be related to stimulation of endothelial nitric oxide synthase and suppression of arginase. Moreover, the extracts showed a decrease in vasorelaxation following endothelium removal. When investigating the antiplatelet effects, the same extracts proved to be the most active. *C. pentagyna* leaf extract significantly inhibited platelet aggregation (IC_{50} = 0.109 mg/mL) induced by ristocetin (1 mg/mL), an inductor of platelet agglutination in the presence of von Willebrand factor. *V. opulus* fruit extract showed an inhibitory effect (IC_{50} = 0.368 mg/mL) on platelet aggregation induced by convulxin (7 ng/mL), a potent activator of collagen receptor GP VI. *V. opulus* and *C. pentagyna* extracts are promising for the development of herbal preparations with potential benefits in cardiovascular diseases related to endothelial dysfunction.

Keywords: extracts, vascular endothelium, vasorelaxation, platelet aggregation

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S2.P153 A purified fraction from 70% hydroethanol extract of whole herb of *Platostoma africanum* P. Beauv. (Lamiaceae) inhibited formation of β-haematin formation

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Compounds inhibiting formation of β-haematin are potential antiplasmodial substances especially when they are natural products from medicinal plants (Li et al., 2019). The inhibitors of β-haematin formation form an important category of partner drugs of ACTs, the WHO-recommended antimalarial regimens. New sources of inhibitors, however are needed because of widespread malaria parasite resistance (WHO, 2023). The whole herb of *Platostoma africanum* P. Beauv. is used in ethnomedicine in South-Western Nigeria to treat malaria. A bioactivity-guided fractionation of the 70% ethanol extract of the whole herb of the plant for inhibition of β-haematin formation has been presented. The 70% ethanol extract of the whole herb prepared by maceration was initially fractionated to give *n*-hexane (NH), diethyl ether (DE), ethyl acetate (EA), *n*-butanol (NB) and aqueous fractions (AQ). The crude extract (25 mg/mL) and fractions (20 mg/mL) were tested for inhibition of β-haematin formation (Vargas et al., 2011), expressed as $I_{analysis}$ using quinine hemi- succinate as reference drug. Values of $I_{analysis}$ considered were positive net absorbance computations and maximal inhibition corresponded to initial absorbance of haematin present. The *n*-butanol and aqueous fractions were fractionated (reversed-phase column chromatography, Sephadex) and analysed (TLC, LC/DAD). The order of inhibition of β-haematin formation was DE (0.046) < NH (0.056) < EA (0.062) < AQ (0.068) < NB (0.083). A purified isolate (10 mg/mL) from AQ showed $I_{analysis}$ of 0.097 mg/mL lower than quinine hemi-succinate ($I_{analysis}$, 0.971). *Platostoma africanum* showed inhibition of β-haematin formation and an isolate from the aqueous fraction is potential antiplasmodial drug lead.

Keywords: *Platostoma africanum,* inhibition of β-haematin formation, antimalarial

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S2.P154 Studies of St. Johns's Wort (*Hypericum perforatum* L.) dry extracts: no hints on mutagenicity

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The genotoxic safety of preparations of *Hypericum perforatum* L., herba have already been assessed and reviewed over the last years (EMA, 2016), given that these preparations are used in the treatment of e.g. mild to moderate depressive episodes (EMA, 2016). One traditional form is the comminuted or powdered herbal substance alone. The way to test their genotoxic potential is the so called "bracketing and matrixing concept", where extracts covering the whole range of polarities are tested, to obtain a representative sample of all components in the drug in these three extracts as a whole. Additional data on the genotoxic potential are therefore desirable to assess the therapeutic safety of these HMPs, using the Ames test according to the genotoxicity guideline of the Herbal Medicinal Product Committee HMPC of the European regulatory agency EMA. Three dry extracts of *Hypericum perforatum*, representing the whole spectrum of polarities of the extraction solvents (water – 50% ethanol (v/v) – n heptane) were tested in the Ames test, according to the OECD [5] and HMPC guidances. The extracts showed no mutagenic effect, even not in the highest concentrations according to the OECD guidance. The results of the tested dry extracts can be extrapolated by a "bracketing and matrixing concept" for other dry extracts of *Hypericum perforatum* in the tested polarity range of the extraction solvents. The data support the therapeutic safety of the extracts and the drug powder and add the assessment in the HMPC monograph of Hypericum perforatum (EMA, 2016).

Keywords: mutagenicity, ames test, genotoxic safety, St. John´s wort, depression

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S2.P155 Shortcutting iridals to irones transformation in orris root: unleashing the potential of microwave drying for rapid irone production

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Orris root is the *Iris germanica* L. rhizomes known as German iris, is famous for producing valuable irone compounds which are famous in perfume and food industry. Irones compounds normally derived from iridals, traditionally requiring several years of storage for slow oxidation transformation (Roger, et al., 2010). This study explores a shortcut to expedite irone compound production, significantly reducing the time required in German iris rhizomes. In fact, six drying methods (sun, shade, infrared, microwave, oven, and oven vacuum) were employed and compared for both whole and sliced rhizomes. Essential oil extraction was performed using a Clevenger type apparatus on the dried samples, followed by gas chromatography (GC) and gas chromatography- mass spectrometry analysis. The analysis of compounds showed that 35 compounds were identified in different drying methods, of which 8 compounds were found to be the main compounds (above 5%) (Ghasemi, et al., 2023). The results demonstrate that drying methods play a crucial role in irone compound production. cis-α-Irone and γ-Irone, flourished under microwave drying, constituting 71.86% and 18.24% in whole rhizomes (totally 90.1% irones), and 40.91% and 41.44% in sliced rhizomes (totally 82.35% irones), respectively. In contrast, the control fresh sample exhibited a minimal iron content of 0.20%. Other drying techniques could not reach to the microwave drying results. In fact, Microwave drying emerged as a game-changer, serving as a shortcut to rapidly synthesize irone compounds in orris root, by bypassing the traditional years-long oxidation process of iridals. In conclusion, these findings underscore the transformative potential of microwave drying, revolutionizing the traditional timeline for Irone synthesis in German iris rhizomes. By providing a time-saving alternative to the lengthy oxidation process of iridals, microwave drying streamlines fragrance development processes, enabling rapid and enhanced production of captivating scents and fragrances.

Keywords: *Iris germanica*, microwave drying, irone compounds, essential oil, fragrance development

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S2.P156 Didesmethylrocaglamide cytotoxic activity in high grade serous ovarian cancer in preclinical *in vitro* and *in vivo* models

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Didesmethylrocaglamide (DDR) (**1**) is a naturally occurring derivative of rocaglamide with potent antitumor activity isolated from *Aglaia* plant species (Fig. 1) (Chang et al., 2020). DDR displays nanomolar cytotoxic activity in high grade serous ovarian cancer (HGSOC) cell lines including OVCAR8. HGSOC is the most lethal gynecological cause of death in women and requires new treatments to help tackle chemoresistance (Feng et al., 2021). Rocaglamides are known to function as translation inhibitors and trigger apoptosis in other types of solid tumors. We found that racemic **1** induced cytotoxicity in ovarian cancer cell lines as early as 24 hours and activated caspase-3 indicating pro-apoptotic activity. In addition, **1** was cytotoxic for the PEO4 and MCF7ADR cell lines that are resistant to cisplatin and paclitaxel, respectively. In addition, we evaluated **1** in OVCAR8 xenografts and observed a reduction in tumor burden. We hypothesized that combinatorial treatments of **1** with autophagy inhibitors like phyllanthusmin-34 would enhance cell death by blocking translation and the ability to recycle amino acids for protein production through autophagy (Salvi et al., 2022). The data obtained indicates that blocking autophagy or DNA repair in combination with **1** increased cell death in the sensitive and resistant models. Its effective cytotoxicity in drug sensitive and resistant cell models suggests **1** has potential as a new therapeutic strategy against HGSOC.

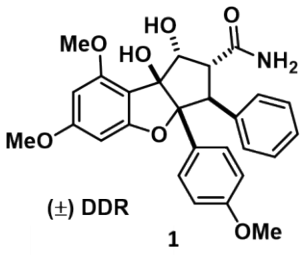

Fig. 1. Structure of (±)-didesmethylrocaglamide (**1**)

Keywords: didesmethylrocaglamide, ovarian cancer, HGSOC, cytotoxicity, chemoresistance

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S2.P157 The herbal preparation, STW 5, protects against changes in the microbiome in experimental functional dyspepsia

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The herbal preparation, STW 5, consists of nine hydro-alcoholic (31 % alcohol) extracts of *Iberis amara, Matricaria recutita, Carum carvi, Melissa officinalis, Mentha piperita, Glycyrrhiza glabra, Silybum marianum, Angelica archangelica* and *Chelidonium majus.* It has been shown to be clinically effective in irritable bowel syndrome (IBS) and functional dyspepsia (FD), but the involvement of the intestinal microbiota has not been adequately studied. The aim of the present study was to examine whether changes in gut microbiota induced in an experimental model of functional dyspepsia could be prevented by STW 5. FD was induced in rats by subjecting them to neonatal maternal separation followed by subjecting them to restraint stress for 90 minutes/day for one week. During that week, one group (8 animals) was treated with the liquid form of STW 5 (5 ml/Kg) and one with its vehicle (31% alcohol) at the same dose level. Animals were sacrificed 24 h after the last drug administration. Fecal samples were taken from the cecum. Genomic DNA was isolated and changes in selected bacterial phyla and genera were assessed using quantitative Real Time-PCR (qPCR). The main phyla studied under the influence of stress in the model were the Bacteroidetes, Firmicutes, Fusibacterium and Actinobacteria. FD led to a 50% drop in the *Lactobacilli* population but to a two-fold rise in *Proteobacteria* abundance but did not affect significantly the changes in *Bacteroidetes*. Furthermore, the *Methanobrevibacter* population was raised more than two-fold while the genomic DNA concentration in *Bifidobacterium* dropped from ca. 3 ng/g feces to nearly nil. In general, STW 5 tended to ameliorate and protect against the changes induced by the stress model. The results lend further support to the use of STW5 in FD and IBS by providing evidence that it acts at least in part through influencing beneficially the changes in intestinal microbiota induced by stress. Furthermore, the findings shed more light on mechanisms involved in potential usefulness of STW 5 in gastro-intestinal disorders.

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S2.P158 Modulatory effects of *Glycyrrhiza glabra* L. on endocrinological shift in ovariectomy-induced menopausal mice: Network pharmacology guided *in-vivo* study

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Menopause is a major endocrinological shift leading to hot flushes, anxiety, mood swings, and increased vulnerability for cognitive impairment, hypertension, and obesity in women. In this condition, an abrupt decline in estrogen and serotonin is directly associated with increased expression of MAO-A. Hormonal replacement therapy in post-menopausal conditions has limitations, including increased risk for estrogen-sensitive cancers and cardiovascular diseases. A more promising therapeutic approach lies in the use of medicinal plants with estrogenic as well as serotonergic activity. The present study investigated the neuromodulatory effects of LC-MS characterized *Glycyrrhiza glabra* extract (GGE) against ovariectomy-induced (OVX) menopausal mice through *in-vivo* study. Four experimental groups of mice were formed: sham (0.9% saline, oral), OVX only (0.9% saline, oral), OVX+E2 (30 µg/ kg b.w., s.c.) and OVX+GGE (150 mg/kg b.w.). After 6 weeks of dosing, mice underwent behavioural analysis for memory, anxiety, depression, hot flushes and body weight. The expression analysis of targeted proteins was done through immunoblotting and immunohistochemistry. Increase in rectal temperature, body weight, anxiety and loss of spatial memory was observed in OVX only group, suggesting severity of menopause. Further, in the hippocampus and hypothalamus region of OVX only group, elevated MAO-A and decreased TPH-2 as well as ERβ expression was observed. However, in OVX+E2 and OVX+GGE groups we found a significant reduction in MAO-A expression while increased expression of TPH-2 and ERβ. GGE also enhanced the blood serum levels of estrogen and serotonin. Altogether, GGE is effective against the endocrinological shift generated through surgical menopause *via* modulating vasomotor symptoms.

Keywords: post-menopausal symptoms, ovariectomy, *Glycyrrhiza glabra*, estrogen, anxiety

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S2.P159 *Ziziphus nummularia* crude and fractionated extracts attenuate the malignant phenotype of human triple negative breast cancer cells

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Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and has limited therapeutic options. Effects of Ziziphus nummularia against TNBC have not been investigated yet. An ethanolic extract of *Ziziphus nummularia* (Burm.f.) Wight & Arn. (ZNE) was prepared and chromatographically fractionated. Phytochemical composition of ZNE and its chromatographically isolated fraction (F6) was identified both qualitatively by spectrophotometric assays and analytically by HPLC-PDA-MS/MS. Effects of ZNE and F6 on the viability of several cancerous cell lines were tested by MTT assay. The anti-cancerous potential of ZNE and F6 was tested *in vitro* in MDA-MB-231 cells, a TNBC cell line. ZNE and F6 radical scavenging capacity was tested using DPPH assay, and their effects on reactive oxygen species (ROS) generation *in vitro* in cells by DCFDA staining. Propidium iodide-based FACS analysis was used for cell cycle analysis. Scratch wound healing and trans-well migration chamber assays were used to assess MDA-MB-231 cell migration and invasion. Western blotting analysis was used to analyse changes in the levels of cell cycle, apoptosis and autophagy proteins. Findings showed that ZNE and F6 reduced the viability of several cancerous cell lines including MDA-MB-231 cells. F6 decreased MDAMB-231 viability more than crude ZNE or F6. ZNE and F6 are rich in phytochemicals and HPLC-PDA-MS/MS analysis identified several metabolites that were previously reported to have anti-cancerous effects. Both ZNE and F6 showed potent antioxidant capacity in the DPPH assay, but promoted reactive oxygen species (ROS) production in MDA-MB-231 cells; an effect which was blunted by the antioxidant N-acetyl cysteine (NAC). NAC also blunted ZNE- and F6-induced reduction in TNBC cell viability. We also demonstrated that ZNE and F6 induced an arrest of the cell cycle, and triggered apoptosis- and autophagy-mediated cell death. ZNE and F6 inhibited metastasis-related cellular processes by modifying cell migration, invasion, and adhesion. Collectively, our findings reveal that *Z. nummularia* is rich in metabolites that can attenuate the malignant phenotype of TNBC and may provide novel approaches for the discovery of new drug leads for treatment of TNBC and other cancers.

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S2.P160 Anti-inflammatory properties of jaboticaba peel bioactive compounds during *in vitro* gastrointestinal and colonic digestion

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Food rich in polyphenols are correlated to health benefits in literature, especially for their anti- inflammatory potential. The bioactivities of these compounds are directly correlated to their bioavailability during digestion. Jaboticaba, aka. "Brazilian berry", is a small fruit with dark purple peel, normally discarded, which is rich in anthocyanins, tannins and phenolic acids. Despite the extensive research on jaboticaba peel (JP) health benefits, the bioavailability of its bioactive compounds is not always considered. Therefore, the objective of this work was to evaluate the influence of the digestion of JP in a Simulator of Human Intestinal Microbial Ecosystem on its phenolic composition, antioxidant capacity and anti-inflammatory effects in RAW 264.7 macrophages. Antioxidant capacity analysis presented a slight divergence, except for JP extract superiority to stomach and duodenum values. However, FRAP presented lower antioxidant results for colonic samples than JP extract, while colonic samples results for ORAC overcame JP extract. As expected, anthocyanins are degraded in stomach, and in duodenum in a greater extent, with a small recovery on colonic samples, followed by a significant decay after 7 hours of colonic fermentation. JP extract (500 µg/mL) effectively reduced NF-κB, TNF-α and MIP-2 levels by 94,2%, 78,6% and 94,2%, respectively. Most of the samples have shown valuable TNF- α modulation, with the lowest inhibition at 10%. However, NF-κB and MIP-2 could only be modulated to a certain extent after JP digestion, being only stomach and duodenum samples effective regarding NF-κB, whereas reductive effects could be observed in MIP-2 until 8 hours of digestion.

Keywords: *Plinia cauliflora, in vitro* digestion, macrophages, NF-Kb pathway, antioxidant capacity

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S2.P161 Improving sleep quality and daytime function with a synergistic combination of tryptophan, magnesium, *Lactuca*, and *Melissa*: A prospective pilot study in adults with sleep disturbances

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Insufficient sleep is a widespread issue, with existing treatments often leading to side effects (1). This monocentric, single-arm, open-label study assessed a novel granulate formulation containing fresh *Lactuca sativa*, *Melissa officinalis*, Tryptophan, and Magnesium in adults with sleep disturbances. Conducted in Germany in 2023, 50 participants consumed the formula nightly for 14 days, with outcomes measured through diaries, questionnaires, cognitive tests, wearables, saliva samples, and in 10 cases, polysomnography (PSG). Descriptive statistics were used to analyze outcomes. The results showed significant improvements: nightly awakenings reduced by 31% ($p<0.001$) and early morning awakenings by 16% ($p\leq0.003$). PSG confirmed a 28% increase in overall deep sleep ($p>0.05$) and a 70% increase in deep sleep phase N4 ($p=0.042$), while REM sleep decreased by 18% ($p>0.05$). The Apnea/Hypopnea Index scores also dropped by 26% ($p>0.05$), suggesting a lower apnea/hypopnea risk. The primary outcome, a 14% improvement in the SF-B/R Sleep Quality Index ($p=0.003$), highlighted enhanced sleep quality overall, especially in individuals with high anxiety, who saw a 37% improvement ($p\leq0.001$). Further exploratory analysis using wearables, PSG, and saliva bioanalysis yielded inconsistent results. Furthermore, the study reported a 22-28% increase in restedness ($p\leq0.001$), up to a 29% reduction in psychological tension ($p\leq0.01$), and improved daytime performance, including a 13% decrease in daytime sleepiness and a 23% improvement in mood ($p\leq0.014$). Cognitive function improved by 13% ($p\leq0.001$), tested by objective computerized test batteries (COMPASS). Adherence to the intervention was high, with no serious adverse events reported. In conclusion, the tested food supplement formulation safely improves sleep quality, deep sleep, and psychological well-being, enhancing daytime mental function and mood.

Keywords: *Lactuca sativa,* clinical trial, food supplement, deep sleep improvement (PSG), cognitive improvements (COMPASS)

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S2.P162 Harnessing gut microbiota through immunomodulation by LM-1 attenuates HDM-induced asthma

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The chronic recurrent exacerbated nature of asthma and the side effects of long-term use of conventional medicine for asthma lead patients to seek out novel therapies. In addition to Th2- dominated immune response, the gut microbiome may represent a key target for the management of allergic asthma. Liu-Jun-Zi-Tang (LJZT; decoction containing *Panax ginseng, Atractylodes macrocephala, Poria cocos, Pinellia ternate, Glycyrrhiza uralensis, Citri reticulatae, Zingiber officinale,* and *Zizyphus jujuba*) and Ma-Xing-Gan-Shi-Tang (MXGST; decoction containing *Ephedra sinica, Prunus armeniaca, Glycyrrhiza uralensis, and Gypsum Fibrosum*) are two frequently prescribed Chinese herbal granules for the treatment of pulmonary diseases in Taiwan. Here we investigate whether LJZT combined with MXGZT (abbreviated to LM-1) ameliorates asthma through the immunomodulation of the gut-lung axis. LM-1 (0.9 g/kg/day of MXGST + 1.8 g/kg/day of LJZT, oral for 5 days) attenuated pulmonary allergic airway inflammation by inhibiting Th2 cell, eosinophil and mucosubstances accumulation in asthma mice. LM-1 in combination with dexamethasone improved pulmonary function. LM-1 treatment altered gut microbiota, and faecal microbiota transplantation from LM-1-treated mice ameliorated asthma by regulating intestinal IFN-γ/IL-4 ratio (Fig. 1). Furthermore, LM-1 reduced $CD4^+IL\text{-}4^+$ cells in the PBMCs of patients with asthma. We conclude that LM-1 attenuates asthma by enhancing IFN-γ/IL-4 ratio through gut immunoregulation. LM-1 is a potential candidate for further clinical investigation.

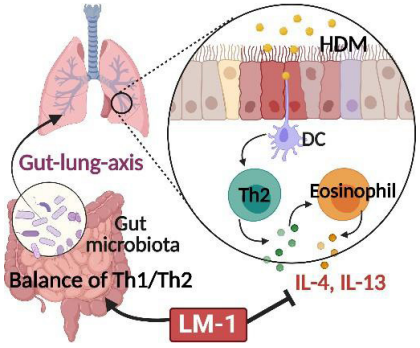

Fig. 1. Schematic of LM-1's effects on the immunomodulation of the gut-lung axis against asthma

Keywords: traditional Chinese medicine, microbiota, asthma, immunomodulation, fecal microbiota transplantation

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S2.P163 Ethnopharmacological study of the bark traditionally used for diabetes of *Croton guatemalensis* Lotsy: from the field to the action mechanisms

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The aim of this presentation is to address the ethnopharmacological study of the bark of *Croton guatemalensis* Lotsy (Euphorbiaceae). Commonly known as "copalchí", it was identified as one of the most prominent species traditionally used for the treatment of diabetes in a field study conducted among the Cakchiquels of Chimaltenango in Guatemala (Cruz and Andrade-Cetto, 2015). Posteriorly, the hypoglycemic and antihyperglycemic effects of the ethanol-water extract were confirmed, where its ability to counteract fasting hyperglycemia was highlighted due to its "glibenclamide-like" effect and where the inhibition of α–glucosidase activity was ruled out as a potential effect to manage postprandial hyperglycemia (Andrade-Cetto et al., 2019). Subsequent phytochemical studies revealed the identification of eight compounds: five *ent*-clerodane diterpenoids and three flavonoids (Escandón-Rivera et al., 2022). Among them, junceic acid (**1**) stands out for being the most abundant (Fig. 1)

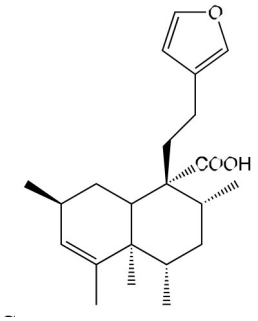

Fig. 1. Structure of junceic acid (**1**).

Later, it was confirmed the hypoglycemic effect of **1**, which was similar to that exerted by the extract. One of the major mechanisms presumably involved in the control of fasting hyperglycemia was the inhibition of hepatic glucose production. Notably, **1** inhibited the activity of the rate- limiting enzyme glucose-6-phosphatase more potently than the control. Moreover, both extract and **1** showed a similar capacity to reduce postprandial hyperglycemia. In the long term, the extract reduced fasting hyperinsulinemia and improved oral glucose intolerance by restoring the glucose- stimulated insulin secretion response and enhancing insulin signalling in the liver. In addition, it showed to decrease hepatic triglycerides and cholesterol.

Keywords: *Croton guatemalensis,* junceic acid, ethnopharmacology, diabetes, action mechanisms

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S2.P164 *Eryngium cymosum* F. Delaroche, a Mexican species traditionally used in the treatment of diabetes - ethnopharmacological insights

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Eryngium cymosum F.Delaroche (Apiaceae), commonly known as "piñuela", was identified as a recurring remedy against diabetes through a field study carried out in the Huejutla market located in Hidalgo, Mexico (Espinoza-Hernández et al., 2021). The aqueous extract elaborated from the aerial parts demonstrated to manage fasting and postprandial hyperglycemia, in which the inhibition of hepatic glucose production and the enhancement of insulin function were implicated. Specifically, it showed to inhibit the activity of two rate-limiting enzymes of liver glucose output (glucose-6- phosphatase and fructose-1,6-bisphosphatase) and protein tyrosine phosphatase 1B. In addition, the extract was considered safe in an acute toxicological test. Phytochemical analysis revealed the presence of phenolic acids, such as chlorogenic and rosmarinic acids, and flavonoids in the aqueous extract (Romo-Pérez et al., 2022). Interestingly, kaempferol-3-O-(2,6-di-O-trans-ρ-coumaryl)-β-D- glucopyranoside (1) (Fig. 1) showed to potently inhibit both glucose-6-phosphatase and fructose- 1,6-bisphosphatase, suggesting an important role in the inhibitory effect on hepatic glucose production.

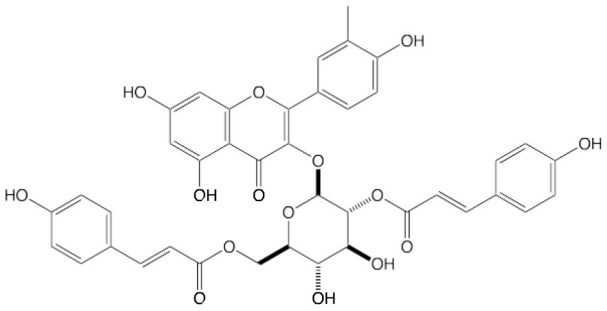

Fig. 1. Structure of kaempferol-3-*O*-(2,6-di-*O*-trans-ρ-coumaryl)-β-D-glucopyranoside (**1**).

When administrated chronically, the extract could reduce glycated hemoglobin, indicating a potential long-term glycemic control (Espinoza-Hernández and Andrade-Cetto, 2022). Moreover, it also enhanced insulin sensitivity by increasing the glucose disappearance rate and improved oral glucose intolerance by restoring the glucose-stimulated insulin secretion response. In addition, the extract decreased Akt hyperphosphorylation and promoted a better insulin signalling response in muscle.

Keywords: *Eryngium cymosum*, ethnopharmacology, diabetes, glycated hemoglobin, insulin sensitivity

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S2.P165 Onychocolone A produced by the fungus *Onychocola* sp. targets cancer stem cells and stops pancreatic cancer progression by inhibiting MEK2-dependent cell signaling

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Pancreatic cancer (PC) remains one of the deadliest cancers and it lacks targeted therapy. PC tumors are enriched with subpopulations of cancer stem cells (CSCs) that are resistant to antiproliferative drugs (Hermann et al., 2007), which raises a need to identify new chemotherapeutic agents. Onychocolones A-F **(#1-6)** are new anti-tumoral benzophenones compounds identified in a high throughput screening on MEDINA´s microbial Natural Product extract library. The compounds were purified by bioassay-guided isolation from an extract of the fungal strain *Onychocola* sp. CF- 107644 and their structures were established by spectroscopic methods. Compounds #**1**-**4** inhibited the growth of the pancreatic tumor cell lines MIA PaCa-2, BxPC-3, and PANC-1 with low- micromolar Median Effective Doses (ED_{50}). Compound #**1** (onychocolone A) showed pro-apoptotic effect on pancreatic CSCs and 3D spheroids, which are representative of tumor complexity (Langhans, 2021). The mechanism of action was linked to the inhibition of MEK onco-signaling pathway by protein expression assays. The efficacy of onychocolone A *in vivo* was confirmed in a heterotopic pancreatic xenograft mouse model generated by CSCs (Hernández-Camarero et al., 2019). ADME studies validated the drug-like properties of onychocolone A, including low probability of pharmacologic interaction, prediction of good absorption for oral administration, high protein binding, slow release, and a short half-life compatible with low toxicity. Altogether, the data suggest the potential of onychocolone A as a new small molecule for hit-to-lead development of a new treatment for PC.

Keywords: onychocolone, pancreatic cancer, cancer stem cells, 3D spheroids, MEK

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S2.P166 Human prolyl endopeptidase inhibitory activity of the peptide enriched extracts of *Momordica charantia* (Cucurbitaceae) fruits

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Prolyl endopeptidase (PREP) is a proline-specific serine protease that is expressed in several body tissues. In humans, PREP was shown to be involved in blood pressure regulation and central nervous system disorders. Plant peptides are known inhibitors of PREP, for instance the prototypic cyclic cystine-rich peptide psysol 2 from *Psychotria solitudinum* has inhibitory activity in the low micromolar range. *Momordica charantia* has been reported to contain cysteine-rich peptides that can inhibit trypsin, elastase and other serine proteases in nanomolar concentrations. This study will focus on the extraction of *M. charantia* cysteine-rich peptides for protease inhibition assays against human PREP. Four extraction methods for *M. charantia* fruits were applied in a comparative study, where the boiled water extraction proved superior. The crude extract was further purified using C_{18} solid phase extraction (SPE) to obtain peptide enriched samples by eluting peptides with stepwise increasing concentrations (5 – 45%) of acetonitrile. The presence of peptides was confirmed using MALDI-TOF/TOF-MS and HPLC-UV techniques. PREP inhibition of the peptide enriched fractions was studied using a fluorescent substrate-based *in vitro* assay. Fractions eluted between 15 – 30% showed considerable inhibitory potential (IC_{50} from 32 – 56 µg/mL) while the 20% fraction exhibited the most significant inhibition (IC_{50} = 32.4 ± 5.0 µg/mL). The peptide enriched extracts of *M. charantia* fruits demonstrated inhibitory activity against PREP. Next steps, we will isolate peptides from *M. charantia* by bioassay-guided isolation and characterize their potential for PREP inhibition, thus providing new leads for drug development in disease states where PREP is implicated.

Keywords: *Momordica charantia,* natural peptides, Cucurbitaceae, human prolyl endopeptidase, solid phase extraction

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S2.P167 Impact of phytocannabinoids THC, CBD and their acidic counterparts THCA and CBDA on TGF-β1-induced epithelial-mesenchymal transition in human conjunctival epithelial cells

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Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells lose their intercellular connections and acquire a fibroblast-like phenotype with an increased migratory capacity and extracellular matrix secretion (Thiery et al., 2009). EMT was suggested to be involved in the development of fibrosis in various tissues including the eye and TGF-β1 is known as its potent promoter (Rajić et al., 2020). Endogenous cannabinoids and cannabinoid receptors are found throughout the eye, indicating their potential significance in ocular physiology (Cairns et al., 2016). We assessed the effects of Δ9-tetrahydrocannabinol (THC), Cannabidiol (CBD), Δ9- tetrahydrocannabinolic acid (THCA), and Cannabidiolic acid (CBDA) on TGF-β1-induced EMT in cultured immortalized human conjunctival epithelial (HCjE) cells. Cells were exposed to TGF-β1 (10 ng/mL) for 72 hours with or without cannabinoids (2 to 10 μM). TGF-β1 induced an elongated spindle-shaped appearance in cells. Cells stimulated with TGF-β1 in presence of THC or CBD remarkably maintained cuboidal epithelial morphology, whereas THCA partially restored the epithelial phenotype of the cells and CBDA showed no effect. E-cadherin, a marker of epithelial phenotype, was assessed by immunocytochemistry. THC and CBD, but not THCA or CBDA, increased E-cadherin expression, which was downregulated by TGF-β1. A cell migration assay was conducted by introducing a cell free area in confluent cells and subsequently adding TGF-β1 ± cannabinoids. At 24 hours, TGF-β1 induced a complete wound closure. All cannabinoids decreased wound closure in a dose dependent manner, whereas CBD showed the strongest anti-migratory effect. In conclusion, THC and CBD effectively counteracted EMT-related alterations induced by TGF-β1.

Keywords: cannabinoids, epithelial-mesenchymal transition (EMT), TGF-β1, conjunctival fibrosis, human conjunctival epithelial cells (HCjE)

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S2.P168 Development of the immunopharmacognostic interventions on potentiating tumor recognition against prostate cancer

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The most challenge for treating prostate cancer (PCa) is the high incident rate (65~75%) of recurrence and/or bone metastasis. Although immunotherapies become a groundbreaking therapy for metastatic PCa, including antigen-specific dendritic cells (DC) vaccines[i.e. sipuleucel-T (Provenge)], the feature of PCa as the "cold" immune landscap still causes less efficacy or even unresponsiveness of immunotherapies. Here, we've identified a semi-purified fractionated extract (Phyto-X) with two chemically identified phytocompounds (Phyto-X-A and Phyto-X-B) from the medicinal plant (*Asteraceae*) confer a strong immunostimulatory activity among other phytoextracts in Taiwan endemic plants-based phytolibrary. We observed that Phyto-X-resultant tumor cell lysate (TCL) retarded the mOVA-RM1 tumor growth in conjunction with DC vaccine and T cell adoptive transfer through increasing DC maturation and CTL activity against tumor cells. Interestingly, we found that Phyto-X and its phytocompounds (Phyto-X-A and Phyto-X-B) have differential effects on tumor cells for induction of ATP release and HMGB1 (hallmarkers of immunogenic cell death (ICD)), autophagosome formation and autophagosomes-mediated DC activation. Besides, we found that Phyto-X can directly limit the expansion of tumor-associated bone marrow myeloid cells and M2 macrophage polarization, which could be through inhibition of PI3K-AKT-mTOR pathway. Most importantly, we found Phyto-X can synergize the effect of chemodrug-Docetaxol on induction of ICD, tumor-specific CTL activity and modulate key immune cells in the intratumoral microenvironment. To sum up, this study is to address the potential development of a full-defined phytoextracts (Phyto-X) as an immunopharmacognostic interventions through eliciting tumor recognition, modulating tumor microenvironment, and thus synergizing immunotherapies or/and chemotherapy.

Keywords: immunopharmacognostic interventions, immunogenic cell death, autophagosome, tumor recognition, dendritic cell

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S2.P169 Investigating the anti-inflammatory effects of a TCM formulation used for the prevention of rheumatoid arthritis

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Chinese medicinal formulations have a long tradition of use against rheumatoid arthritis (RA). RA is an autoimmune, inflammatory, and chronic disease that primarily affects the joints of 0.5%–1% of the population (Zeng et al. 2008). These formulations are based on combinations of typically 8- 10 plants, which are usually boiled and administered as a decoction or tea (Yeong-Deug et al. 2004). We have developed a natural products isolation methodology that can be employed to isolate potential anti-rheumatic natural products from a Chinese medicinal formulation using an example formulation called "Decoction A". These strategies involve a suite of biological assays against relevant anti-inflammatory pathways (e.g., Nf-kB and JAK/STAT pathways (Schwartz et al. 2017) and bioassay-guided purification. The natural products workflow is based on extraction in MeOH:CH_2Cl_2 (1:1), followed by separation on diol-bonded silica into seven fractions of increasing polarity. After activity testing of these fractions in cell reporter assays, actives are selected for subfractionation with a micro-scale HPLC workflow. Dozens of compounds with activity in various cell reporter assays have been identified. In conclusion, we have developed an efficient isolation workflow for the assessment of the anti-inflammatory activities of a TCM formulation. The functionality of the pipeline was validated through the identification of several compounds with previously reported anti-inflammatory activities.

Keywords: Traditional Chinese medicine, screening, anti-inflammatory, arthritis

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S2.P170 Biological potential of endophytes from liverwort *Marchantia polymorpha* L.

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The mutualistic coexistence between the host and endophyte is diverse and complex, including host growth regulation, and protection from microbial or herbivore attack. The latter is commonly associated with the production by endophytes of bioactive natural products. The endophytes of higher plants have been extensively studied, but there is a dearth of information on the biodiversity of endophytic microorganisms associated with liverworts and, more importantly, their bioactive metabolites (Stelmasiewicz et al., 2023a,b). This presentation will cover isolation of bacterial and fungal endophytes from liverwort *Marchantia polymorpha* L., analysis of the metabolites present in obtained extracts and their fractions, and evaluation of anticancer potential. The extracts were analysed using gas chromatography or liquid chromatography coupled to mass spectrometry (GC/MS and LC/ESI-QTOF-MS). The cytotoxicity and anticancer potential were assessed against noncancerous VERO cells and cancer cells—namely the HeLa, RKO, and FaDu cell lines. The most characteristic metabolites identified in the ethyl acetate extract and fractions were diketopiperazine derivatives. Among volatiles the most abundant were cyclo(L-phenylalanyl-L- prolyl), cyclo(L-leucyl-L-prolyl), and their stereoisomers (Fig. 1).

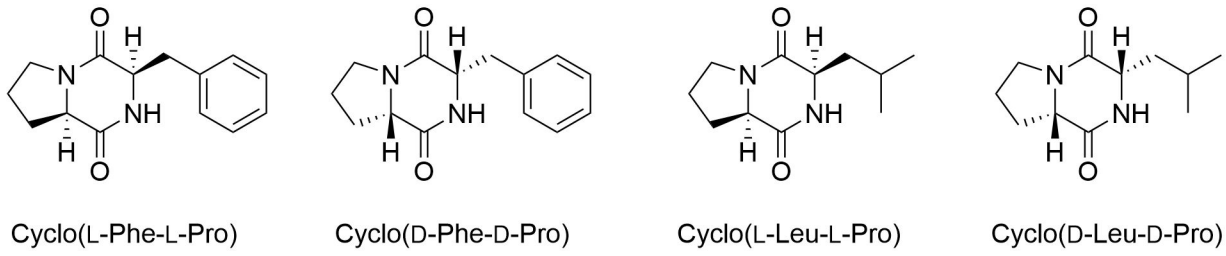

Fig. 1. Diketopiperazine derivatives identified in *M. polymorpha* endophytes

The endophyte extracts and isolated fractions showed a potential selective anticancer influence on all tested cancer cell lines. These results reinforce the potential of *Marchantia polymorpha* endophytes as a source of biologically active secondary metabolites.

Keywords: Liverwort endophytes, diketopiperazines, anticancer and antiviral activity

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Natural product research and the challenges of climate change – ecological and environmental solutions

S2.P171 Small molecule metallophores from bacteria, adaptation and isolation strategies

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Heavy metal pollution is a persistent environmental threat, but many of these elements are also essential to industry (Binnemans, 2013; Mathivanan, 2021; Reck, 2012). Numerous bioremediation- based strategies are being developed to mitigate heavy metal pollution (Mathivanan, 2021; Yaashikaa, 2022). In this presentation, we will present our laboratory's work to isolate metallophores from bacteria and evaluate their interaction with different heavy metals. As part of this work we have isolated cupriachelin siderophores from *Cupriavidus necator* B-4383 (Ahmed, 2003), and delftibactin siderophores from *Delftia lacustris* DSM 21246 (Ahmed and Boudreau, *in review*). To explore the novel chemical space of small molecule metallophores that bind to metals of interest we have also developed an enrichment workflow to isolate novel metallophore-producing strains from metal polluted environments. In these efforts, we relied on dual metal tolerance and Chrome Azurol S (CAS) siderophore production assays. Here we are hypothesizing that siderophores produced by these strains may have dual role in both iron acquisition and heavy metal tolerance, as has been observed before (Johnston, 2013; Johnstone and Nolan 2015). Initial hits from strains isolated from a former mining site in Montana include a *C. necator* strain which produces a putative taiwachelin homolog (Kreutzer and Nett, 2012), which supports that our approach can identify metallophore producing strains. Future aims for this project include identifying the chemistry that underpins the interaction of these metabolites with heavy metals beyond iron and efforts to understand the biological regulation of the biosynthetic pathways for these compounds under metal stress.

Keywords: siderophores, metallophores, metabolomics, bacteria, *Delftia*

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S2.P172 Exploring Tuscany onion (*Allium cepa* L.) heritage: unveiling ancient varieties through molecular network analysis and bioinformatic tools

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Preserving plant biodiversity and making sustainable use of genetic resources in agriculture is a topical issue with ecological, economic, and social repercussions (Ebert et al., 2020). *Allium cepa* L. (Amaryllidaceae) is worldwide studied as an important source of dietary phytochemicals (Tedesco et al., 2015). In Italy, some onion varieties, only cultivated by local farmers, are neglected and worth to be valorized. The aim of this work was to investigate for the first time the chemical composition of 10 indigenous Tuscany onion varieties, by comparing with the marketed "Tropea", "Fiorentina", and "Di Certaldo" onions. The chemical fingerprint was obtained by processing LC-qTOF-MS/MS data with MZmine3 (Schmid et al., 2023), generating molecular networks with MetGem (Olivon et al., 2018) and using compound annotation tools. Flavonoids such as kaempferol, (dihydro)quercetin and isorhamnetin derivatives, were detected in all the samples, thought in different relative ratio. "Di Certaldo" variety distinguished from the others for a molecular cluster of sulphur-containing compounds. "Acquaviva" and "Treschietto" displayed the presence of fatty acids and amino acids derivatives not detected in other samples. Combining the acquisition of large, high-resolution experimental datasets with powerful informatics tools, it was possible to identify the most known bioactive compounds distribution but also highlight the presence of molecular clusters specific to a few onion varieties, potentially correlated with the biological findings. As future work, the extracts will be investigated for their antiangiogenic activity by two *in vivo* models, chick chorioallantoic membrane/zebrafish embryos.

Keywords: *Allium cepa,* biodiversity, bioinformatics, chemical fingerprint, antiangiogenesis

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S2.P173 Extraction of *Cannabis sativa*: subcritical water vs. supercritical CO_2 and organic solvents

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The use of hemp (*Cannabis sativa*) has attracted great interest due to its versatile applications in the pharmaceutical, food and cosmetic industries. The plant is also increasingly investigated for use in sustainable extraction. By applying the 2^3 full factorial DoE approach, we investigated subcritical water extraction (Chakraborty et al. 2021) for obtaining hemp extracts (var. 'Futura 75') as an alternative to supercritical CO_2 extraction (Jokić et al. 2022), ultrasonic extraction (organic solvents, water), Soxhlet and high-pressure ethanol extraction. The extracts differed significantly in terms of composition and antioxidant activity. In the extraction with subcritical water, we confirmed statistically significant models for the yield, the antioxidant activity obtained in the DPPH and ABTS tests, and the content of polyphenolic compounds for the studied factors (temperature in all models, drug to solvent ratio and product temperature* drug to solvent ratio in the yield). The highest yield was obtained with subcritical water extraction (35.91%), and temperature and drug to solvent ratio were the main parameters affecting yield. Soxhlet extraction (36.06% CBG) and high-pressure ethanol extraction (17.39% CBGA) proved to be the most suitable for the extraction of cannabinoids. Subcritical water extraction yielded extracts with a comparably high antioxidant activity and polyphenol content as the extracts obtained with other methods. Temperature was a key parameter and showed a strong positive correlation with antioxidant activity and polyphenol content.

We conclude that extraction with subcritical water is a good choice for obtaining novel extracts from hemp, as the extracts show relatively high antioxidant activity and good yield.

Keywords: antioxidant activity, cannabinoids, *Cannabis sativa*, subcritical water extraction

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S2.P174 Response surface methodology guided RP-HPLC-PDA method optimization for the quantification of multi-analytes (six) in Indian kudzu and its greenness assessment

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In present study, response surface methodology (RSM) guided RP-HPLC-PDA method was established to maximize the bioactive metabolites *viz*., gallic acid, p-Coumaric acid, daidzein, biochanin A, genistein and puerarin content in Indian kudzu (*Pueraria tuberosa* DC.). Three independent variables i.e. extraction time (A), extraction temperature (B) and liquid to solid ratio (C) and central composite design (CCD) was used. The experimental design yields 20 runs and the optimum conditions for maximum yield of targeted metabolites were 25.16 minute of (A), 32.47 °C of (B) and 20 mL g^{-1} of (C). The yield of targeted metabolites *viz*, gallic acid, p-Coumaric acid, daidzein, biochanin A, genistein and puerarin were 0.166 ± 0.025, 0.0234 ± 0.008, 0.0249 ± 0.004, 0.015 ± 0.002, 0.0375 ± 0.006 and 0.476 ± 0.009 % (dry wt.) respectively and were insignificantly different from predicted (RSM model) values i.e., 0.168, 0.024, 0.024, 0.019, 0.036 and 0.465% respectively. The results demonstrate the RSM aided optimization of RP-HPLC conditions foe the highest yield of bioactive metabolites in *P. tuberosa*. The developed RP-HPLC method was also validated according to ICH guidelines (2022). The analytical method was linearly calibrated at 48- 240 ng run^{-1}, LOD and LOQ ranged from 7.88-14.89 and 23.88-45.13 ng among the six metabolites. The repeatability and reproducibility of method was, well within the specified limit of <2% and recovery study varies from 97.12-99.00 %. The greenness of analytical method was estimated using analytical eco scale (AES), NEMI, GAPI, AGREE and AGREEprep, and it was found environment friendly and energy efficient (Gałuszka et al., 2012; Keith et al., 2007; Pena-Pereira et al., 220; Płotka- Wasylka, 2018).

Keywords: central composite design (CCD), daidzein, *Pueraria tuberosa*, puerarin, response surface methodology (RSM)

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S2.P175 Development and validation of RP-HPLC method for estimation of anti-oomycete diterpenoids in *Larix decidua* extracts

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The study involved the development of reverse phase HPLC methods for quantifying two anti- oomycete diterpenoids, larixyl acetate (**1**), larixol (**2**) and the co-occurring inactive, epimanool (**3**) (Fig. 1) from the Larixyne® extract obtained from European Larch bark and Larch oleoresin. Larixyne® is effective in controlling grapevine downy mildew, *Plasmopara viticola,* that can cause 100% crop loss if left untreated (Mulholland et al.,2017 and Thuerig et al., 2017). Larixyne® offers potential to replace ecotoxicologically unfavourable copper fungicides that are reliably used to control downy mildew in vineyards. Currently, the viticulture industry is the fastest growing sector in UK agriculture and is producing award winning wines. This study, Green Laryxine is an Innovate UK funded project aiming to commercialise the use of Larixyne®. The reverse phase HPLC, involved chromatographic separation using Ascentis® Express F5 column (10 cm × 2.1 mm, 2.7 µm) with acetonitrile and water: 48:52% v/v as a mobile phase at a constant flow rate of about 0.3 mL/min. The development and validation were carried out at detection wavelength of 203 nm. We developed a robust RP-HPLC method, validated for linearity, precision, accuracy, specificity, and system suitability. The method demonstrated excellent linearity with correlation coefficient value r^2 of > 0.99 with linearity range 10-30 µg/mL for larixyl acetate (**1**), larixol (**2**) and epimanool (**3**). The LOD and LOQ were found to be lower, therefore the method is sensitive. The developed RP-HPLC method is an effective method for the quantification of larixyl acetate, larixol and minor constituents in Larch extracts.

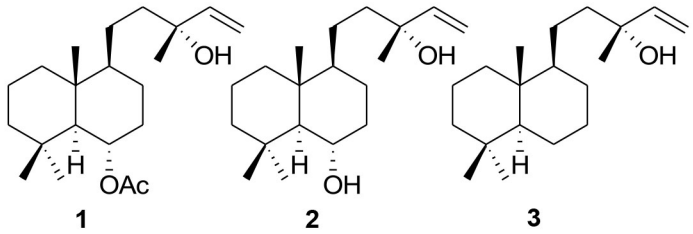

Fig. 1. Structures of larixyl acetate (**1**), larixol (**2**) and epimanool (**3**)

Keywords: *Plasmopara viticola*, diterpenoids, Larixyne, RP-HPLC

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S2.P176 Eutectic solvents as a sustainable solution for the recovery of high value free fatty acids from microalgal co-products

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Arthrospira platensis and *Porphyridium cruentum* are under intense study due to their high content of phycobiliproteins. The increasing demand for phycobiliproteins has led to an urgent need to develop a biorefinery approach to valorize non-polar metabolites of interest, like Free fatty acids (FFA) (Wils et al., 2021). Natural deep eutectic solvents (NaDES) are an environmentally friendly alternative to conventional solvents. Our laboratory has demonstrated the effectiveness of these solvents in extracting FFA from spirulina freeze-dried biomass (Wils et al, 2021). Based on these promising results, we expanded our research to include by-products of the aqueous extraction of phycobiliproteins (Fig. 1).

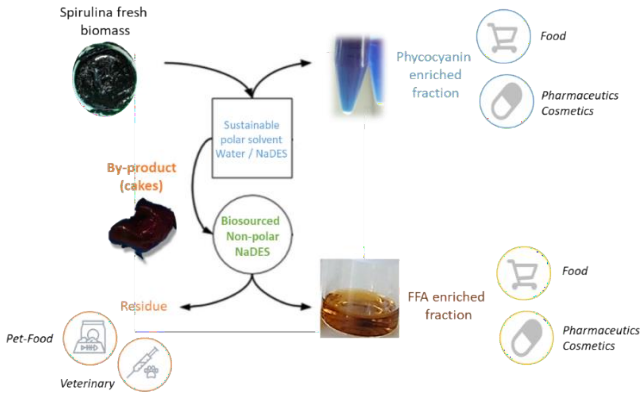

Fig. 1. General biorefinery approach for greener valorisation of microalgae cake

Wils et al. (2022) compared the extraction performances of a panel of non-polar NaDES. NaDES based on fatty acids were found to be the most promising for recovering FFAs from microalgae, with high selectivity towards saturated FFAs. The presence of polyols within the NaDES modulates the selectivity in favor of the polyunsaturated FFA. Microalgae cake has been shown to be very effective for FFA recovery. These findings provide a basis for designing a multi-stage microalgae biorefinery scheme using NaDES or sustainable solvents for the cosmetic or food industry.

Keywords: eutectic solvent, microalgae, lipids, green process

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S2.P177 EKO-YAM - investigating *Dioscorea* for a sustainable future

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The recently launched EKO-YAM project is presented. It focuses on the Chinese yam *Dioscorea polystachya*. Its tuber is used in Traditional Chinese Medicine given the potential against diabetes and cardiovascular diseases. The tuber is also an important foodstuff due to its nutritional value. That is why cultivation of yam is of interest. However, cultivation becomes increasingly labor-intensive, costly, and thus challenging. Therefore, this project aims at innovative cultivation methods (i.e., mobile raised beds, trench cultivation, mound beds, gutter cultivation). They should allow economical and sustainable production of Chinese yam and maintain or even improve the nutritional quality. These methods are intended to align with the organic regulations in Europe. The different cultivars are profiled regarding the pattern of proteins (using MALDI-TOF MS) as well as primary and secondary metabolites (HPLC-MS). The latter are mainly adduced as molecular markers to monitor breeding experiments and to secure quality assurance. In addition, conventional crossing trials are performed to obtain stronger varieties, e.g., with increased health-promoting effects. Generally, foods with such properties may have contributions to nourishment or health care of society. The EKO-YAM project purposes to promote the establishment of *Dioscorea polystachya* as a foodstuff and possibly as a phyto-pharmaceutical raw material, thereby contributing to the National Bioeconomy Strategy for a sustainable future.

Keywords: *Dioscorea polystachya,* Dioscoreaceae, cultivation, method development, secondary metabolites

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S2.P178 Wound healing and antimicrobial activity of European tree bark extracts

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Various types of wood bark have been identified to possess antimicrobial, antiviral, anti- inflammatory, and wound-healing properties (Emrich et al., 2022). This poster will present the research on wood bark extracts from *Fagus sylvatica*, *Prunus padus*, *Quercus robur*, *Pinus sylvestris*, *Alnus glutinosa* and *Betula pendula* in view of their wound healing and antimicrobial properties. The extracts were retrieved using the Soxhlet extraction method. The minimum inhibitory concentration (MIC) assay was used to determine the effectiveness of antimicrobial agents on *Cutibacterium acnes*, *Staphylococcus epidermidis*, MRSA-*Staphylococcus aureus* and *Escherichia coli, K. pneumoniae, P. aeruginosa*. To determine the wound healing capacity a scratch assay was performed on a human keratinocyte cell line. Wound closure was measured up to 72 hours in the presence or absence of bark extracts. All bark extracts, except *Prunus padus* show varying degree of bacterial growth inhibition (MIC range from 67 µg/mL to 1073 µg/mL) against gram positive bacteria whereas solely *Alnus glutinosa* demonstrated a partial inhibition against gram negative bacteria at a concentration of 1073 µg/mL. In terms of the wound healing capacity, all bark extracts lead to a significantly accelerated wound closure (timepoint and concentration dependent) compared to untreated cells (e.g. 100% wound closure at 38 hours with *Prunus padus* compared to 72 hours without extract). Their combined positive effect on wound-healing and antimicrobial activity has great potential for the treatment of various skin diseases (Häsler Gunnarsdottir et al., 2023). In addition, the sustainability element is tackled by utilizing bark, considered a byproduct in the forest industry.

Keywords: bark extracts, antimicrobial activity, wound healing capacity, sustainability

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S2.P179 An ethnobotanical survey investigating traditional remedies to treat infectious diseases in French Polynesia

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In French Polynesia, traditional medicine is still used and considered as a part of the Polynesian culture (Chassagne *et al.*, 2022, 2023; Girardi *et al.*, 2015). Anti-infectious remedies have been reported to treat several disorders, for all types of patients and in many islands, making them an important part of traditional Polynesian medicine. In this context, an ethnobotanical survey was implemented to identify remedies used for infectious diseases, inventory the plants, better understand the local representation of these therapies and provide efficacy and safety data on these uses. A field study was carried out from February to April 2024, on five islands from the Society archipelago (Tahiti, Moorea, Raiatea, Tahaa and Huahine) in French Polynesia. A declaration of research regarding access to biological resources and associated traditional knowledge was recorded according to the local regulations. A total of 83 persons was interviewed including experts in herbalism. This fieldwork led to the identification of 92 plants species for the treatment of 11 infectious diseases (e.g., sinusitis, abscess, urogenital infections). The majority of plants used are native or Polynesian introduced plants. Among them, *Cordia subcordata, Rorippa sarmentosa, Heliotropium arboreum, Coleus barbatus* and *Spondias dulcis* were the most cited and will be subjected to further biological and phytochemical analysis. This study is part of a larger project named "*Sustainable Traditional Medicine in French Polynesia*", funded by the French National Research Agency (ANR), and aiming to preserve, support, and valorise the traditional medical knowledge from French Polynesia.

Keywords: Pacific, traditional medicine, ethnobotany, medicinal plants, infectious diseases

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S2.P180 Water as a green solvent to extract polar and non-polar plant pigments

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Water is a green solvent, harmless to human health and the environment, and commonly used in food and cosmetic products (Chemat et al., 2019). Thus, water is a great solvent choice to obtain plant extracts that could be directly used as ingredients. Plant pigments are a heteroclite group of secondary metabolites reunited by their capacity to absorb light in the visible domain. They often display interesting activities, such as antioxidant or photoprotective (Brudzyńska et al., 2021). However, some have low polarity, making them challenging to extract using water. This work focused on designing efficient methods to extract pigments and active compounds with different polarities using water. For anthraquinones, medium-polarity compounds from madder (*Rubia tinctorum* L.), a pressurised water-based microwave-assisted extraction (PMAE) method was optimised. Microwaves generate much heat in less than a minute via ionic conduction and dipole rotation in the plant matrix and the solvent. Combined with the pressure, these conditions lower the dielectric constant of water and its polarity, enabling the eco-extraction of anthraquinones. For the carotenoids from achiote (*Bixa orellana* L.), which have low polarity, a bio-based co-solvent was added to develop a biphasic oil/water ultrasound-assisted extraction. Combining both solvents allowed the simultaneous extraction of polar and non-polar compounds. The antioxidant activity and pigment content of extracts from both plants were assessed using colorimetric assays and the extract characterisation was completed by UHPLC-HRMS/MS. Water-based extraction methods showed at least similar efficiency as conventional methods with organic solvents. The recovered extracts could be directly formulated into cosmetic products.

Keywords: Water, green extraction, cosmetics, plant pigments, antioxidants

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S2.P181 Long term experiments on applying a pulsed electric field (PEF) to living plant roots in order to extract flavonoids in *S. baicalensis* Georgi model

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Scutellaria baicalensis Georgi (Lamiaceae) has been used for centuries in Traditional Chinese Medicine and is nowadays listed in Chinese, British and European Pharmacopoeias (Wang *et al.,* 2018). It exhibits antioxidant, cytotoxic, anxiolytic and antimicrobial properties. Among many flavonoids produced in its roots, baicalin (**1**), wogonoside (**2**), baicalein (**3**) and wogonin (**4**) (Fig. 1) are the most abundant (Zhao *et al.,* 2019).

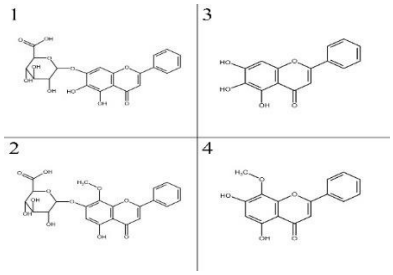

Fig. 2. Main flavonoids found in *S. baicalensis* root: baicalin (**1**), wogonoside (**2**), baicalein (**3**) and wogonin (**4**).

This research covers using Pulsed Electric Fields (PEF) to facilitate extraction of flavonoid compounds from living roots of aeroponically cultivated *S. baicalensis* plant model. Most plants survive this treatment, sometimes reaching size and content of root phytochemicals larger than control (Grzelka *et al.,* 2023). Presented work is a result of a two-year-long process of empirically adjusting PEF parameters and composition of Natural Eutectic Solvent (NES) mixture in order to optimize extraction yield. We found that the phytochemical content of extracts acquired in this refined process is quality-wise synonymous with that of control (80% MeOH extract from dried root material). The best extract was obtained using following PEF parameters: 33 fifty-microsecond-long impulses, 1 Hz frequency, 0.5 kV/cm field strength, delivered twice (5 min interval) and a NES mixture of choline chloride : xylose (1:2 + 30% water). It yielded the same amount of wogonin (**4**) as control (2.15 µg/mL), but with 60% survival rate of plants after 3 weeks of observation. This allows to extract from the same population again, which is much more time- and cost-efficient than cultivating a new one and can be applied to other plant models.

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Keywords: electroporation, extraction, *Scutellaria baicalensis*, NES, flavonoids

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S2.P182 Enhancing fire ant bait selectivity: Synthesis and evaluation of feeding deterrent activity of fire ant venom alkaloids and their analogs against invasive fire ants and native ants

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The red imported fire ant, *Solenopsis invicta*, has significantly impacted public health, agriculture, and ecosystems in the southern United States. Their painful stings pose a threat to humans, pets, livestock, and wildlife, causing allergic reactions and even fatalities in severe cases. These invasive ants aggressively compete with native species for food and territory, often displacing them and disrupting delicate ecological balances. Using bait is one of the most effective methods for controlling fire ants. Baits consist of insecticides mixed with attractive food substances, which ants take back to their colonies, effectively killing the entire colony. The low selectivity of fire ant bait has been the major drawback of this method. Prompted by the observation that fire ants feed their nestmates with their venom (Chen and Du, 2022), we evaluated the feeding deterrent activity of solenopsin A, a major constituent in the fire ant venom, against fire ants and two common native ants, little black ant (*Monomorium minimum*) and thief ant (*Solenopsis molesta*). Solenopsin A had a potent feeding deterrent activity against little black and thief ants but had no effect on fire ants. The addition of fire ant venom alkaloids and their analogs to bait represents a promising strategy for enhancing the selectivity of bait targeting this invasive pest

We prepared the fire ant venom alkaloids (**1**-**6**) and their analogs (**7**-**12**) and evaluated them for the feeding deterrent activity against little black, thief, and fire ants. Their activity and the structure- activity relationships will be presented

Keawords: red fire ant, *Solenopsis invicta*, venom alkaloids, feeding deterrent activity, bait

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S2.P183 Sucrosephenylpropanoid esters and isoflavonoids from *Belamcanda chinensis* (Iridaceae) roots and their potential anti-osteoclastogenic activity

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This presentation will cover the isolation, structure elucidation and biological evaluation of six new sucrosephenylpropanoid esters (**1**–**6**) and twenty-one known compounds (**7**–**27**) from the methanol extract of *Belamcanda chinensis* (L.) DC root (Iridaceae). *B. chinensis* was incorporated into the genus *Iris* as *Iris domestica* (L.) Goldblatt & Mabberley by APG (Missouri Botanical Garden) III (Wozniak et al., 2015). In some countries such as Korea, China, Japan, and Vietnam, this herb is used in traditional medicines to treat pharyngitis, coughing, bronchitis, and chronic tracheitis; its mode of action is by reducing pharyngeal swelling, heat-clearing, and detoxifying (Li et al., 2019). Repeated chromatography of the CH_2Cl_2 and EtOAc soluble fractions from the methanol extract of *Belamcanda chinensis* root yielded six new sucrosephenylpropanoid esters (**1**–**6**) and twenty-one known compounds (**7**–**27**). The structures of **1**–**6** were elucidated using diverse nuclear magnetic resonance (NMR) techniques and high-resolution mass spectrometry (HRMS) data analysis, together with chemical methods (Fig. 1).

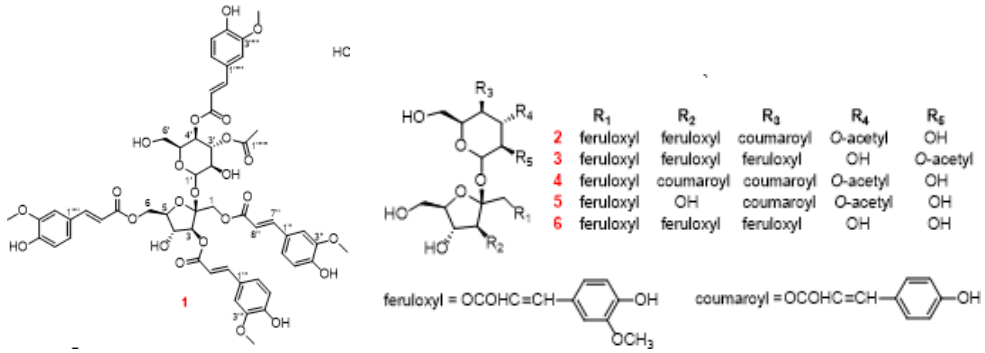

Fig. 1. Structure of sucrosephenylpropanoid esters **1**–**6**

All the twenty-seven isolated compounds were evaluated for their anti-osteoclastogenic activities. Preliminary screening results revealed that compounds **1** and **19** exhibited strong effects against RANKL-induced osteoclast formation in RAW264.7 cells. In addition, the treatment of mouse bone marrow macrophages (BMMs) with compounds **1** and **19** significantly decreased RANKL-induced TRAP-positive multinucleated osteoclast formation in a concentration-dependent manner without affecting cell viability. Further bioassay investigation showed that compounds **1** and **19** inhibited the expression of some osteoclast-specific marker genes and the transcription factor nuclear factor of activated T cells cytoplasmic 1 (NFATc1) in response to RANKL. To the best of our knowledge, this is the first investigation of anti-osteoclastogenic activity for compounds isolated from *B. chinensis*.

Keywords: *Belamcanda chinensis,* Iridaceae, sucrosephenylpropanoid esters, osteoclastogenesis, RANKL, NFATc1

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S2.P184 PTP1B inhibitory activity of serratene-type triterpenoids from *Lycopodium serratum* (Lycopodiaceae)

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This presentation will cover the isolation, structure elucidation and biological evaluation of two new serratene- type triterpenoids (**1** and **2**) and nine analogs [seven serratenes (**3**–**5**, **7**–**10**) and two serratanes (**6** and **11**)], together with one *Lycopodium* alkaloid (huperzine A, **12**) were isolated and characterized from *Lycopodium serratum* (Lycopodiaceae) (Fig. 1) (Ha et al., 2023). *Lycopodium* plants were reported to show interesting biological activities, such as cytotoxicity, cancer chemo-preventive activity, and the activities involving the treatment of AD such as acetylcholinesterase and butyrylcholinesterase inhibitory activities (Nguyen et al., 2017).

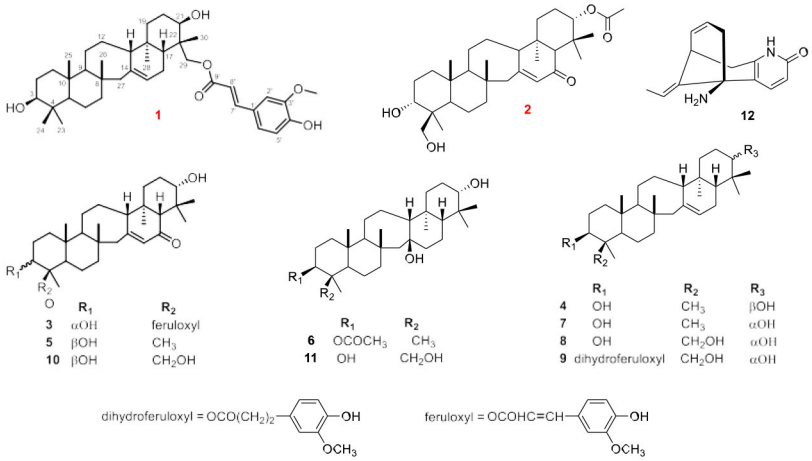

Fig. 1. Structure of compounds **1**-**12**

The structures of isolates from *L. serratum* were elucidated by the HRESI-MS, 1D and 2D NMR analysis, and comparison with NMR data in the literature. Among the isolates, triterpenoids (**4**–**6**) showed significant inhibitory effects on PTP1B (protein tyrosine phosphatase 1B) with IC_{50} values of 13.50, 17.65, and 16.64 μM, in comparison with that of the positive control, ursolic acid (IC_{50} = 5.98 μM) (Ha et al., 2023). While compounds **1**, **7**, and **8** exhibited moderate PTP1B inhibitory effects with IC_{50} values ranging from 25.30 to 51.56 μM. Enzyme kinetic studies revealed that all these most active compounds (**4**–**6**) exhibited the same competitive PTP1B inhibition type with K_i values of 7.9, 16.9, and 8.9 μM, respectively. In addition, the probable binding conformation of **4**–**6** within the PTP1B active site was studied through *in silico* molecular docking. The docking results demonstrated that these active triterpenoids could be docked stably into the catalytic site of the PTP1B enzyme with negative binding energies.

Keywords: *Lycopodium serratum*, Lycopodiaceae, serratene triterpenoids, PTP1B, molecular docking

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S2.P185 Introducing NaDES-based biphasic solvent families inspired by the Arizona family: a new approach for the sustainable use of centrifugal partition chromatography

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Centrifugal Partition Chromatography (CPC) has a great potential for developing new eco-friendly separation strategies. Being a support-free technique, solvent selection is crucial for CPC. Typically, natural compounds are separated using pre-established families of solvent systems that offer a wide range of polarity. Unfortunately, these families are still dominated by harmful organic solvents. The Arizona family, which is commonly used in separating natural compounds, contain toxic alkanes and alcohols. There are alternatives to these solvents, including Natural Deep Eutectic Solvents (NaDES). However, tailoring NaDES-based solvent systems can be a challenge. In this work, we introduce a new approach to build solvent systems by incorporating hydrophobic NaDES into the ARIZONA family. NaDES with low viscosity, such as terpene-based NaDES, are the most suitable for CPC (Fan et al., 2021), However, their number is limited. Hence, we conducted an extensive screening of terpene-based NaDES to compile a pool of potential candidates, that could be introduced into biphasic solvent systems Two NaDES were selected as starting points for the creation of two novel families, NADrizona 1 & 2, consisting of 23 biphasic solvent systems following the Arizona family model. The performance of these families was evaluated using various parameters, including volume ratios, settling times, stationary phase retention ratio (Sf), and polarity range predicted by the GUESS method (Friesen & Pauli, 2005). The GUESSmix was also used to evaluate separation capacities within CPC. Plant extract separation assays were used to validate the separation capacities of the NADrizona 1 & 2 families.

Keywords: CPC, green solvents, NaDES, arizona solvent systems

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S2.P186 Beyond the tuber: crafting high-value molecules from potato residues

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Steroidal glykoalkaloids (SGA) are prominent secondary metabolites in potatoes (*Solanum tuberosum* L.) and potato side-streams, like leaves, berries, and flowers (Roosen-Runge *et al.*, 1977). As potato tubers are the fourth most important food globally, the accumulation of these side products is significant (Zaheer *et al.*, 2016). The two major SGAs are α-solanine and α-chaconine, which have biological activity against different types of herbivores, act as natural pesticides and can therefore be used as a green alternative to traditional plant protection (Allen *et al.*, 1971). The aim of this project is to extract of the side-streams from potato production to obtain these compounds and generate new plant-based pesticides by a semi-synthetic (chemical and enzymatic) approach. The focus will be on the aglycon solanidine **1**, which can be obtained by cleaving the sugars (Fig. 1). A simple test- substrate strategy with cholesterol will be performed as a first proof of principle, since both compounds share a similar scaffold. Successful synthetic routes will then be adapted to synthesize derivatives of solanidine. Additionally, the total synthetic approach for solanidine will be performed (Zhang *et al.*, 2016) to compare both the synthetic and isolation-based route by means of economic and sustainability analyses.

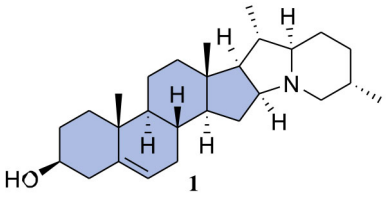

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Fig. 1: Structural motif of the aglycon solanidine.

Keywords: steroidal glycoalkaloids, plant-based pesticides, circular bioeconomy, *Solanum tuberosum* L., semi-synthesis

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S2.P187 Mulberry cell cultures: a promising source for health care

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Plants have long been exploited as a sustainable source of food and chemicals; however, with the increasing global world population and the climate change ending in losses of biodiversity and land, conventional agriculture unlikely can cope with the huge challenges head. These factors could be countered using new and ethically more justifiable technologies such as plant cellular agriculture, a biotechnological tool for the production of nutritious and healthy ingredients, as well as single bioactive compounds (Rischer et al., 2020; Gubser et al., 2021; Bapat et al., 2023). Given the importance of *Morus alba* L. in many traditional medicines due to its health benefits (Chen et al., 2021), the aim of this work was to establish *M. alba in vitro* cell cultures to study their phytochemistry and potential use for nutritional and healthy supplements. Different organs from *in vitro* grown plantlets were used as explant source, and different basal media were tested. The best callogenesis was obtained from stem explants cultured on MS basal medium supplemented with sucrose and 2,4-D. During the following subcultures, the calli resulted in a brownish, juicy, grainy biomass (Fig. 1).

Fig. 1. Calli of *Morus alba*

Considering that *in vitro* cell metabolism can change during the growth cycle, the phytochemical profile (HPLC-DAD), total phenol and flavonoid content and antioxidant activity (colorimetric assays) were analyzed on the juices obtained by squeezing the biomasses at: initial, mid and stationary phase of the growth cycle. The high content of phenols and flavonoids, as well as the antioxidant activity, showed an active metabolism of the culture.

Keywords: *Morus alba,* cell cultures, phenols, flavonoids, antioxidant

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S2.P188 Spatio temporal dynamics of favourable habitats for *Terminalia leiocarpa*, a traditional plant used in ethnoveterinary medicine in Benin (West Africa)

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This presentation will focus on *Terminalia leiocarpa* (DC.) Baill (Combretaceae), commonly known as *Anogeissus leiocarpa*. It a plant widely used in West Africa by livestock farming in ethnoveterinary medicine (Tchetan et al., 2022). The harvesting of its various organs, combined with the effects of climate change are leading to a decline in its natural populations (Houehanou *et al*., 2013). In order to contribute to the sustainable use of the plant, we analyzed the dynamics of *Terminalia leiocarpa*'s favourable habitats in Benin. Occurrence data were collected in the three climatic regions of Benin and combined with climatic and soil data and subjected to the maxent ecological niche modelling algorithm to assess the effect of future environmental conditions (RCP 4.5 and RCP 8.5 scenarios to 2055) on the potential distribution of the species in Benin. The results show that the areas currently favourable and very favourable for the presence of the species extend between the three climatic regions, but are much more localised in the Sudano-Guinean region. According to the climate projections, the current favourable areas will increase by 13.16% for RCP 4.5 and decrease by 9% for RCP 8.5. The currently very favourable areas will see a decrease of 9.23% for RCP 4.5 and an increase of 52.88% for RCP 8.5. Taken together, the currently favourable and very favourable areas will increase for both scenarios in all three climate regions. These results offer hope of saving this important species, whose natural populations have been reported to be declining.

Keywords: climate change, ecological niche modelling, sustainable management, *Terminalia leiocarpa*, Benin

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S2.P189 From the production of targeted metabolites to the complete valorization of *Coleus forskohlii* Briq. using aeroponic cultivation

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Accessing plant resources to extract compounds of interest can sometimes be difficult. To facilitate this access and limits environmental impact of transport, cultivation strategies can be developed. *Coleus forskohlii Briq.* a member of the Lamiaceae family (Ali Khan, 2012), used in Indian and Chinese traditional medicine and rich in diterpenoids, is widely cultivated for forskolin production. Indeed, this diterpenoid which presents anti-inflammatory activity (Suresh *et al.*, 2018) and activate adenylate cyclase (Seamon & Daly, 1981) is the major compound of interest, and it is found in the roots. In this regard, aeroponic cultivation of *C. forskohlii* had been developed in Region Centre Val de Loire (France) to facilitate access to the resources and more particularly to the roots. However, the phytochemical composition and the biological properties of a cultivated plant, that grows off-ground under another climate with less abiotic stress, when compared with the wild one, may vary. The first part of this study focused on optimizing the cultivation conditions to maximize forskolin production. Aeroponic cultivation has been realised for 18 weeks under various stresses. The quantification of forskolin using UHPLC-ELSD indicates that the use of LED lighting compared to the control 3-fold improve the total quantity of forskolin produced. Then a methodology employing non-targeted metabolomics approaches through UHPLC-HRMS/MS have been implemented to study the impact of this stress on other root metabolites. This methodology has been extended to the aerial part to explore potential co-valorization routes.

Keywords: *Coleus forskohlii* Briq., cultivation, aeroponic, forskolin, metabolomics

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S2.P190 *ViscY* NMR experiments combined with chiral discrimination for complex mixture analysis

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So far, there are only a few approaches for analyzing complex mixtures using liquid-state NMR spectroscopy. One of them is to take profit from spin diffusion under viscous conditions. Using viscous solvents slows the tumbling rate of small molecules so that the longitudinal cross-relaxation becomes very efficient and thus promotes spin diffusion over entire molecular spin networks (Lameiras, P. *et al.,* 2021). Consequently, resonances of every mixed molecule can be grouped according to their ability to share magnetization by spin diffusion. As a result, we can individualize each compound's NMR spectrum using a *ViscY* (*Visc*osity Enhancement Spectroscop*Y*) experiment (NOESY experiment under viscous conditions, Pedinielli *et al.*, 2020). Enantiomers, characterized by identical chemical shifts owing to their isochronous nuclei, present a challenge in distinguishing the individual component spectrum. The most common technique in NMR is to consider an additional chiral solvating agent (CSA) to induce the formation of diastereomer complexes. Therefore, they present different chemical shifts because of the differences in interaction between the CSA and both enantiomers (Wenzel, T. J., 2018). Our objective was to chirally discriminate and identify individual components of a racemic amino acid mixture made of histidine, tryptophan, threonine, methionine and asparagine by considering the combination of one CSA (Fig. 1) within two viscous solvent blends: DMSO-d_6/H_2O at 248 K and DMSO-d_6/H_3PO_4 at 288 K, at 500 MHz (^{1}H). Through a single 2D ^{1}H *ViscY* NOESY experiment, we successfully chirally discriminated between all amino acids and took advantage of spin diffusion to individualize all proton spectra.

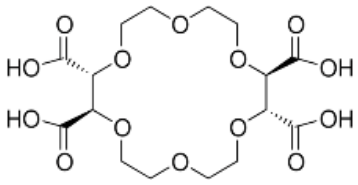

Fig. 1. Chemical structure of the CSA : (-)-(18-crown-6)-2,3,11,12-tetracarboxylic acid.

Keywords: NMR, viscous solvent, spin diffusion, chiral discrimination, chiral solvating agent

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S2.P191 A green approach for preparing alkaloid-rich extracts and fractions

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This work aimed to evaluate the performances of HCl and CH_2Cl_2 compared to citric acid (CA) and greener organic solvents (Prat et al., 2016) for preparing alkaloid-rich fractions. Plants were selected to constitute a diverse set of alkaloids: harmine (**1**), a β-carboline indole alkaloid from *Banisteriopsis caapi* (Spruce ex Griseb.) Morton, boldine (**2**), an aporphine alkaloid from leaves of *Peumus boldus* Molina, and mescaline (**3**), a phenylethylamine derivative from *Trichocereus macrogonus* var. *pachanoi* Britton & Rose (Fig. 1). Extractions were performed by dynamic maceration employing a classical acid-base extraction of alkaloids followed by liquid-liquid extractions (LLE). Each procedure was monitored by ultra-high-performance liquid chromatography coupled to a photodiode array detector and a mass spectrometer.

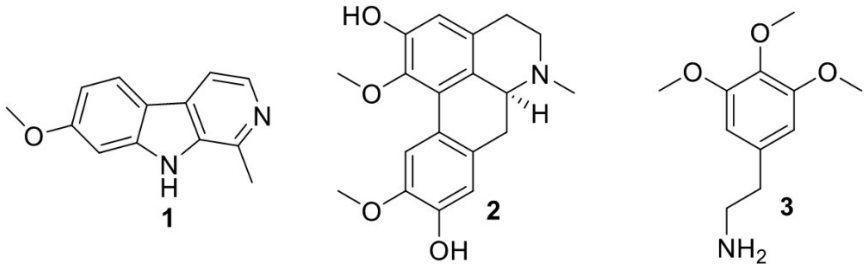

Fig. 1. Structure of alkaloids investigated in this work

The harmful HCl could be replaced with the greener CA (Wang et al., 2020) while keeping similar or better extraction efficiencies. EtOAc could substitute CH_2Cl_2 in at least one of the two LLE steps without reducing efficiencies. *tert*-amyl methyl ether (TAME), *n*-butyl acetate (BuOAc) or EtOAc led to gains in LLE performances when compared to CH_2Cl_2. The cases explored here refute the hypothesis that the hazardous CH_2Cl_2 should be the first option in this type of application. Based on prices currently charged by the main suppliers of chemicals used in this work (i.e., Sigma-Aldrich and Carl Roth), replacing CH_2Cl_2 with TAME, EtOAc, iPrOAc, BuOAc, or anisole would have little or no financial impact on the laboratory carrying out the investigation.

Keywords: green natural products chemistry, ecopharmacognosy, green chemistry

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S2.P192 Optimization of a green method for quantifying harmine in *Banisteriopsis caapi* stems used in the preparation of ayahuasca

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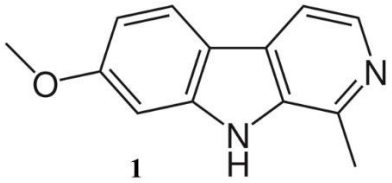

1

Fig. 1. Structure of harmine (**1**)

This work covers the optimization and validation of a green method for quantification of harmine (**1**) (Fig. 1) in stems of *Banisteriopsis caapi* (Malpighiaceae). Such stems are used in the preparation of a psychotropic drink called "ayahuasca", being **1** their major biomarker (Katchborian-Neto et al., 2020). A reference extract (Katchborian Neto et al., 2020) was initially used for the optimization of a UHPLC-PAD-ESI-MS condition. Five factors were inserted in a fractional factorial design (FFD). The initial % of EtOH, time of isocratic elution, gradient time, and injection volume were statistically significant. As the separation at the central point of this PFF led to the resolution of harmine ($R_s > 2$), this condition was adopted for subsequent experiments (i.e., 150 x 2.1 mm, 1.8 µm, ACQUITY Premier HSS T3 column; 40º C; Mobile phase of H_2O and EtOH both with 0.3% HCOOH; Flow rate of 0.27 mL/min; and injection volume of 1 µL). Then, the extraction process itself was subject to optimization. A new FFD evidenced three significant factors that were enclosed in a central composite design (CCD). The following optimal point was found and confirmed: plant/solvent ratio of 30 mg/mL, solvent at pH 1, for 75 min. The full procedure was subjected to validation until intermediate precision. All relative standard deviations for retention time and peak area of **1** were ≤ 10%, attesting to the quality of the new procedure for the intended purpose. A calibration curve was built, leading to 4.8 mg/g of harmine in stems of *B. caapi*.

Acknowledgments: This work was supported by The Sao Paulo Research Foundation (FAPESP, 2017/06216-6, 2018/01786-1).

Keywords: green analytical chemistry, ecopharmacognosy, Ayuasca

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S2.P193 Nutrient density and mineral content of radishes grown in black solider fly frass-enriched compost

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More sustainable food production methods are needed for the future, especially considering the challenges of climate change. *Hermetica illucens* (L.), also known as black solider fly (BSF), shows promise as a tool in the circular economy, as it can convert organic waste into protein-rich feedstocks (Lui et al., 2022). Moreover, a byproduct of BSF is frass, which is a mix of excrement and exoskeletons (rich in minerals, fats, and proteins) that could be an effective fertilizer for food crops (Lopes et al., 2022). Limited information is available about the impact frass has on nutrient density of foods grown in frass-enriched compost. This study evaluates the growth (weight and height), mineral content (Mg, Mn, Zn, Fe, Cu) and nutrient density (% BRIX) of radishes (*Raphanus sativus* cv. Diana) grown in a range of frass concentrations (0-25% frass). Significant growth was found with all frass concentrations ($P < 0.001$), with optimal growth at 10% frass. Leaf BRIX, and whole plant Fe decreased ($P < 0.001$) at all frass concentrations compared to the control. No significant mineral increases were observed except for Mn ($P < 0.001$) in the leaves and roots with all frass concentrations, and Zn ($P < 0.001$) in the roots with 25% frass. Although added frass increased the growth of the radishes, this was offset by decreases in most minerals and consistent with BRIX values. These results indicate more research is needed to evaluate how frass-enriched compost impacts the growth and nutrient density of food crops.

Keywords: frass, nutrients, radish, *Hermetica illucens*, fertilizer

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S2.P194 Myristoleic acid ameliorates the inhibition of rat dermal papilla cell growth by fine particulate matter via the activation of autophagy and Wnt/β-catenin pathways

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Air pollution, including fine particulate matter (PM), is recognized as a serious problem worldwide. PM has been reported to affect various organs of the human body, including cardiovascular, respiratory, and skin (Basith et al., 2022). However, its effect on alopecia remain unclear. In this study, we investigated the effects of PM on rat dermal papilla (rDP) cells and the protective effect of myristoleic acid against PM. PM suppressed the growth of rDP cells. To investigate the mechanism by which PM reduces rDP cells growth, we examined the effect of PM on signaling pathways. PM inhibited autophagy by increasing p-mTOR and p62 levels while decreasing the levels of beclin 1, Atg-3, -5, -7, and LC3. Furthermore, PM impairs the Wnt/β-catenin signaling pathway by reducing p-GSK3β and p-β-catenin. Myristoleic acid is an omega-5 monounsaturated fatty acid derived from the seeds of plants from the Myristicaceae family, which is already known to promote the growth of rDP cells. Myristoleic acid ameliorated the proliferation of rDP cells inhibited by PM through the activation of Wnt/β-catenin and autophagy. Our results provide insights into the mechanisms underlying the adverse effects of PM on hair follicles and suggest that myristoleic acid could restore of PM-induced hair follicle damage.

Funding: This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education(RS-2023-00246959 and RS-2023-00270936)]

Keywords: myristoleic acid, fine particulate matter, dermal papilla cells, autophagy, Wnt/β-catenin

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S2.P195 Identification of phenolic compounds in three *Veronica* species: *Veronica anagallis-aquatica* L., *Veronica persica* L. and *Veronica polita* L.

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Plants are known for the various biological activities which show, and for their specific chemical identity, which allows them to synthesize metabolites such as phenolic compounds, flavonoids, saponins, terpenes, coumarins, and alkaloids that possess protective properties against abiotic and biotic stresses (Cruz et al., 2023). Species of the genus *Veronica* are recognized for their traditional uses and the various biological activities they exhibit (Barreira et al., 2014). The genus *Veronica* (family Plantaginaceae) contains about 450 species, and so far only a few studies have confirmed that certain species possess biological activity such as antibacterial, antioxidant, anti-inflammatory and cytotoxic activities (Ignjatović et al., 2015). Therefore, the aim of this study was the extraction of phenolic compounds using different solvents (methanol, 80% ethanol, water) present in three *Veronica* species (*Veronica anagallis-aquatica* L., *V. persica* L. and *V. polita* L., and their identification and quantification using LC-MS/MS technique. The main phenolic compounds present in all three investigated *Veronica* species were: *p*-hydroxybenzoic acid, apigenin, vanillic acid and caffeic acid. Ethanol as a solvent proved to be better for the extraction of apigenin, while a higher amount of phenolic acids was found in the water extracts. It was extremely important to examine which solvent is the most optimal for the extraction of phenolic compounds and to see the differences in the chemical composition of the three species of the genus *Veronica* due to their influence on biological activity which will be tested.

Keywords: *Veronica* species, extraction, polyphenols, LC-MS/MS technique

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S2.P196 Turning the Tide: Harnessing the Invasive algae *Rugulopterix okamurae* for Commercial Applications

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The brown alga *Rugulopterix o*kamurae, originating from the Pacific near Japan and Korea, has spread invasively to the Strait of Gibraltar, impacting coastal areas in Spanish Mediterranean and southern Portugal. Commercial use of invasive algae for their bioactive compounds offers a cost- effective strategy for controlling their spread and creating beneficial products. This study explored the chemical properties of biomass from *R. okamurae* collected in the southern Portugal, focusing on its fatty acids methyl esters profile, proximate and total phenolics composition and minerals. Furthermore, polar (water) and apolar (*n*-hexane) extracts were obtained by overnight maceration and appraised for *in vitro* antioxidant activity by different radical and metal-based assays, and for inhibition of enzymes related to neurological disorders (acetylcholinesterase: AChE, butyrylcholinesterase: BuChE), skin hyperpigmentation (tyrosinase), obesity (lipase), diabetes (α- glucosidase and α-amylase) and skin ageing (elastase). Biomass had higher levels of protein, lipids, and total carbohydrates than other algae, such as Sargassum horneri, and moderate total phenolics. It had a prevalence of saturated fatty acids (SFA), especially methyl palmitate (C16:0M). The polar extract had higher *in vitro* antioxidant properties, especially ferric reducing properties. The extracts had reduced enzymatic inhibitory properties.

Funding: Work funded by Foundation for Science and Technology (FCT) through project UIDB/04326/2020, UIDP/04326/2020 and LA/P/0101/2020, and the operational programs CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PTALG-01-0145-FEDER-022121. FCT supported L.C. by the FCT Scientific Employment Stimulus (CEECIND/00425/2017) and M.J.R. by the FCT program contract (UIDP/04326/2020).

Keywords: marine invasive species, algal invasions, alien species, antioxidant properties

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S2.P197 Scale-up production of g-mangostin using Centrifugal Partition Chromatography

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g-mangostin, a polyprenylated xanthone isolated from the fruit pericarp of *Garcinia mangostana* (Clusiaceae), has demonstrated its value in agronomy as biocontrol agent acting on the UPR (Unfolded Protein Response) pathway of phytopathogenic fungi (Bataillé-Simoneau et al., 2023 ; Charpentier et al., 2023). To exploit this secondary metabolite industrially, we need to develop a large- scale purification method as green as possible. Since g-mangostin is most often isolated in the laboratory by Flash chromatography, a comparative study using Centrifugal Partition Chromatography (CPC) is currently being carried out on a 250 mL column (« lab scale ») with encouraging preliminary results (Fig.1). This development should enable us to carry out a scale-up on a 1 L column (« pilot scale ») before producing g-mangostin, at multi-kg scale, on a 5 L column (« Process scale »). The solvents used in all these steps are fully recycled. Finally, it should be noted that the initial extract of *G. mangostana* can be significantly enriched in g-mangostin by demethylation of the a-mangostin, the major compound.

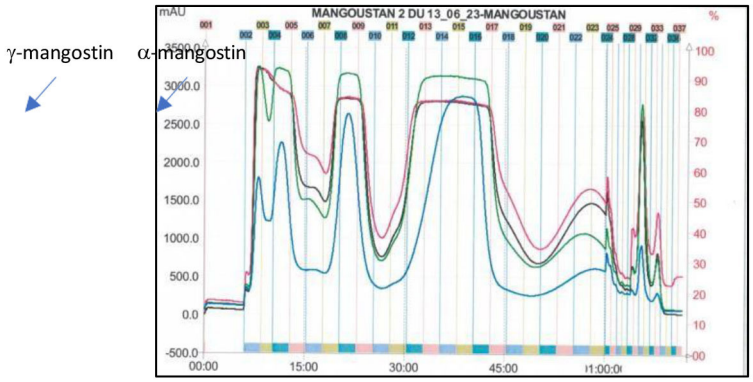

Fig.1: γ-mangostin purification by CPC (VERITY® CPC Lab System 250, Gilson)

Keywords: *Garcinia mangostana*, *g*-mangostin, flash chromatography, centrifugal Partition Chromatography

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S2.P198 *In vitro* callus culture of *Mespilus germanica* to improve the production of bioactive compounds from a forgotten fruit

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The common medlar is the fruit of *Mespilus germanica* L., (Rosaceae). Its consumption is today underestimated but anciently it was very successful; however, it seems that medlar is regaining an interest for its functional properties (Zołnierczyk et al.,2021). The *in vitro* culture of plant cells and tissues can be considered a valid and renewable source of material to obtain bioactive molecules without interferences of external phenomena (pollution, draught, seasonality, etc.) that could interfere in their production (da Silva Santos et al., 2022). Among the various *in vitro* cultures, callus one is interesting because it can be used as a starter for cell cultures on an industrial scale (Lystvan et al., 2018). This study aimed to develop a protocol to obtain production of callus biomass from medlar and subsequently, analyze and identify its composition. In detail, the ripe medlar pulp fruit was used as a source of explants for *in vitro* callus induction for the first time. Once the best conditions for biomass growth have been set, the metabolomic profile of callus was defined and then compared with the pulp and peel of fruits. The obtained results by combining MS and NMR data show that the callus is rich in ursane and oleane triterpenoids, unlike pulp and peel. Furthermore, flavonoids, amino acids, sugars, and phenolic acids were also identified. The conspicuous presence of specialized metabolites, especially pentacyclic triterpenes, suggests the potential bioactivity of callus, thus the evaluation of antimicrobial activity on different Gram positive and negative bacterial strains is in progress.

Keywords: *Mespilus germanica,* callus biomass, LC-HRMS/MS, NMR, metabolomics

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S2.P199 The phytochemical diversity of wild hops from northern France for potential brewing application

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Hops, *Humulus lupulus* L., is one of the ingredients used in brewing, particularly for its antiseptic, bittering and aromatic properties. Breeding programs are looking for new varieties with original aromatic properties adapted to their terroir in a context of climate change. In our local scale, a study has highlighted the genetic and chemical diversity of wild hops harvested in northern France, suggesting an interest for brewing purposes (Paguet et al. 2024). The present study investigated the chemical diversity of 29 wild accessions collected in northern France and replanted *ex-situ* in an experimental hop field, in comparison with 19 commercial varieties as well as two heirloom varieties. Hop phytochemical analysis included quantification of major prenylated phenolic compounds by UHPLC-MS, untargeted metabolomic and molecular networking studies by UHPLC-HRMS/MS, and analysis of volatile compounds by HS-SPME GC-MS. In addition, experimental beers were brewed and hopped with 13 wild hops from our collection, 14 commercial varieties and 2 heirloom varieties. In order to evaluate the brewing potential of wild hops, experimental beers were analyzed by sensory characterization, performed by a trained panel of 19 people, associated to physico-chemical characterization including analysis of volatile compounds by SBSE GC-MS and bitterness quantification by UV spectroscopy. Wild hops from northern France presented lower cohumulone contents within total alpha acids and a greater quantity and diversity of non-oxygenated sesquiterpenes. The brewing potential of wild hops has been confirmed by sensory analysis and several accessions were comparable to commercial hops on specific descriptors such as yellow fruits or floral.

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Keywords: Hops, untargeted metabolomics, volatile compounds, sensory analysis, brewing

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S2.P200 Effect of the aquaponic system on productivity and quality of *Acmella oleracea* R.K. Jansen

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Acmella oleracea R.K. Jansen (Asteraceae) has recently aroused great interest in several fields (Rondanelli et al., 2020; Uthpala et al., 2021). Thus, great quantities of biomass are required. The *A. oleracea* biological properties are ascribed to *N*-alkylamides, being spilanthol the main representative (Spinozzi et al., 2022). To date, *A. oleracea* production mainly involves in field and hydroponic cultivations, which require large amounts of water, fertilizers, and pesticides. Aquaponics, characterized by a symbiotic ecosystem in which fish, plants and nitrifying bacteria take part, represents an environmentally friendly technique to produce crops. This system potentially overcome environmental issues minimizing nutrient and fertilizer requirements, and water wastage (Yep and Zheng, 2019). On this basis, the quality of two cultivars of *A. oleracea*, i.e., cv. yellow and cv. purple, grown in aquaponic systems was evaluated every two weeks, from 4th July to 29th September 2023. Specifically, capitula production, chlorophyll content by spectrophotometric assay and *N*-alkylamides content by HPLC-DAD-MS analyses were assessed. Monitoring of the main water nutrients through ICP-MS analyses was also carried out. The obtained data were compared to those obtained for plants grown in hydroponics. Statistical analysis revealed that cv purple exhibited notably higher levels of spilanthol (14.85-48.0 mg/g of dried flowers) than cv yellow (10.93-40.77 mg/g). Moreover, a significant increase in flowerhead numbers and *N*-alkylamides concentration was observed during full flowering period (18th August -15th September 2023). Overall, the results suggested that aquaponic systems offer similar efficiency to traditional hydroponic methods, making them a viable alternative for large-scale *A. oleracea* production.

Keywords: *Acmella oleracea*, aquaponics, cultivar, eco-friendliness, *N*-alkylamides

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S2.P201 Comparison of free volatile compounds of wild and cultivated species *Veronica catenata* Pannell

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Plants of the genus *Veronica* and their extracts are used worldwide in traditional medicine and nutrition (Salehi et al., 2019; Xue et al., 2019). Extracts from the edible species *Veronica catenata* Pennell (Plantaginaceae), the pink water speedwell, are the subject of the present study. The compositions of the extracts comprising the free volatile compounds are compared between plants collected in their natural habitat and those that have been cultivated. The cultivated speedwell was grown from seeds collected the previous year. The isolation of free volatile compounds was performed by microwave-assisted extraction, and each extract obtained consists of two phases: a lipophilic (essential oil) and an aqueous phase (hydrosol). The chemical compositions of the isolates were determined by gas chromatography-mass spectrometry. The essential oil of *V. catenata* collected in the natural habitat was dominated by phytol 42.26% and hexahydrofarnesyl acetone 17.22% (Dunkić et al., 2021). Hexahydrofarnesyl acetone is present in a similar percentage (17.86%) in the essential oil obtained from cultivated plants. With content of 6.47%, phytol is significantly less present in cultivated plants. In the hydrosol phase of *V. catenata* from wild species, the dominant compounds are α-muurolol (35.12%) and hexadecanoic acid (12.85%) (Nazlić et al., 2022). These two compounds also dominated the composition of hydrosol in the cultivated plant with a similar percentage of identification. Water speedwell is an edible species and because of this it is desirable for cultivation. Volatile compounds are a part of specialized metabolites and also contribute to the plant overall biological activity. This study showed that, regardless of whether it is a wild-collected or a cultivated *V. catenata* species, the same basic volatile compounds have been identified, making the cultivated species also suitable for further study.

Keywords: *Veronica catenata,* Plantaginaceae, essential oil, hydrosol

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S2.P202 Urban parks biowaste as a sustainable source of new antidiabetics

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Biowaste produced in urban parks is composed of large masses of organic matter that is only occasionally used economically. In this work, we aimed to investigate if urban parks biowaste contains phenolic compounds that can be used for production of high-value antidiabetic products. Seven plants, widely distributed in urban parks in Central Europe (*Achillea millefolium, Cichorium intybus, Centaurea jacea, Malva sylvestris, Medicago sativa, Plantago lanceolata, and Trifolium pratense*) were extracted using 10% and 50% ethanol. Chemical composition of the prepared extracts was determined using HPLC and UV-VIS methods, while their antioxidant, anti-inflammatory and antidiabetic activity were determined using chemical and *in vitro* enzymatic methods. The extracts were rich in total phenols, flavonoids and phenolic acids, including caffeic acid, quercetin, kaempferol, luteolin and apigenin derivatives. They demonstrated moderate antioxidant activity as evidenced by their oxygen radical absorbance capacity (ORAC), reducing power and DPPH antiradical activity. The extracts were able to inhibit the activity of lipoxygenase, as well as heat- induced ovalbumin coagulation, albeit weaker than the positive controls nordihydroguaiaretic acid and diclofenac. On the other hand, several extracts demonstrated excellent antidiabetic activity, assessed through inhibition α-glucosidase and amylase. For example, anti- α-glucosidase activity of 10% and 50% ethanol extract of *M. sativa* extracts (9.35 ± 0.07 µg/mL and 3.44 ± 0.05 µg/mL, respectively) was higher than the activity of the positive control, acarbose (156.59 ± 0.80 µg/mL). The results indicate that the biowaste obtained from urban parks represents an ecologically acceptable alternative to conventional cultivation for the preparation of ecologically acceptable, high-value antidiabetic products.

Keywords: α-glucosidase, amylase, antidiabetic, phenolics, urban parks

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S2.P203 Unravelling the secrets of fungicides through actinobacteria-phytopathogen interactions

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Phytopathogenic fungi and fungi-like oomycetes contribute to substantial global yield losses in agricultural production annually, with several fungi known to produce toxins that have severe impacts on animal and human health (Ristaino *et al.*, 2021). While synthetic pesticides have been widely used to chemically control microbial infestation in agricultural fields, their extensive use has raised serious concerns such as residual effects and toxicity to human health, biodiversity decline in agricultural areas, and environmental pollution (Kim *et al.*, 2017). Therefore, phasing out synthetic pesticides with proper substitutes is essential to create more resilient and sustainable food systems to feed a growing world population. Biological controls, involving the introduction of naturally occurring agents such as beneficial microorganisms or crude extracts from natural products directly into a natural ecosystem, have emerged as one of the most promising approaches to mitigate crop diseases. As *actinobacteria* are the most important antibiotic producer and co-exist with fungi in nature, we aim to investigate the biotechnological potential of actinobacteria and their secondary metabolites for use in the biocontrol of phytopathogens through advanced chemical ecology and natural product chemistry. This presentation will cover the isolation of the actinobacteria from Danish soil and the antifungal evaluations of the isolated strains against *Alternaria solani* and *Phytophthora infestans*, the causative agents of early blight and late blight diseases, respectively. Additionally, LCMS-based metabolomics analysis of the prioritized strains will be described, and the on-going workflow involves large-scale fermentation and bioassay-guided isolation for the identification of antifungal compounds.

Keywords: actinobacteria, antifungal, *Alternaria solani*, *Phytophthora infestans*, metabolomics

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S2.P204 The involvement of cyclotides in plant response to heavy metals in *Viola* spp.

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Heavy metals are a significant pollutant resulting from the extraction and combustion of fossil fuels (Munawer, 2018). The genus *Viola* is rich in metallophyte species, that can be used in phytoremediation of heavy metals from polluted soils (Sychta et al., 2020). Cyclotides are plant cyclic cysteine knot peptides involved in the host plant's response to various types of stress. Some studies have shown that some cyclotides may have the ability to bind metals (Skjeldal et al., 2002). All the *Viola* species screened for cyclotides showed the production of a wide variety of these peptides (Burman et al., 2015). The present study reviews the results of several studies we have carried out in recent years to test the hypothesis that cyclotides may be involved in mechanisms of tolerance to heavy metals in *Viola* spp. The studies involved several species and populations of violets, both metallophytes and non-metallophytes: *V. uliginosa, V. westwalika, V. tricolor*, and *V. arvensis*. The effects of heavy metals on cyclotide production were tested *in vitro* and *in vivo*. Quantitative and qualitative analysis of cyclotides was based on mass spectrometry (LC-MS and MALDI-MS). All the studies clearly showed that heavy metal treatment prompts plant response in terms of the change in the production of cyclotides. This indicates that cyclotides play a role in the mechanisms of tolerance. However, the complexity of cyclotide involvement in the tolerance mechanisms requires mechanistically focused studies on the interaction of these peptides with heavy metal ions. Funding: NCN Opus 2021/41/B/NZ8/01737

Keywords: plant peptides, tolerance to heavy metals, abiotic stress

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S2.P205 Applying the global nature products social molecular networking (GNPS) platform to identify natural products involved in tolerance to heavy metals in *Viola* spp.

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The extraction and combustion of fossil fuels are one of major sources of heavy metal pollution. Various potential strategies for coping with heavy metal pollution involve the usage of plants. One of the methods – phytoremediation – involves the use of heavy metal-resistant plants to extract the pollutants from the soil. The genus *Viola*, Violaceae is rich in metallophyte species such as *V. tricolor* L. displaying high resistance to heavy metals, that live and thrive on polluted soils. Multiple classes of different molecules are involved in the mechanisms of tolerance to heavy metals in plants, e.g. phytochelatins or flavonoids. GNPS is a platform that identifies molecules or their classes based on mass spectrometry – LC-MS and LC-MSMS. Our recent study on the metabolomic basis of heavy metal tolerance in *V. tricolor* yielded hundreds of LC-MS peaks corresponding to unknown compounds potentially involved in the response. The current study aimed to identify these molecules using GNPS. Samples (roots and leaves) from two populations (metallicolous, nonmetallicolous) were extracted through maceration in methanol and analyzed using data-dependent analysis (DDA) LC-HR-MSMS. The collected data was subjected to GNPS workflow. The constructed networks allowed for the theoretical identification of several compound classes in the dataset: e.g. flavonoids, lipids, and sterols.

Keywords: tolerance to heavy metals, abiotic stress, metabolomics, bioinformatics

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S2.P206 Screening plant extract for biological activity. A work spanning 60° Latitude South

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This presentation will cover the achievements of accessing the potential of Antarctic mosses' biological activities. The development of the work was based on the methodological algorithm developed during our 30-year experience in prospecting plants in the Amazon rain forest made under rigid protocols involving extract preparation by maceration, disk-diffusion and microdilution-broth assays, thin-layer chromatography, bioautography tests, antioxidant assays, A*rtemia salina* toxicity assay, statistical analyses (Suffredini *et al.*, 2023), and phenolic compound quantification by the Folin-Ciocalteu method. So, 24 extracts obtained from the Antarctic moss *Sanionia uncinata* were evaluated for their biological-active potential and their chemical profiles, obtained by gas- chromatography coupled to mass spectrometry (GC-MS). Only two out of 24 extracts showed antibacterial activity against *Staphylococcus aureus*. The extracts showed strong antioxidant activity and high levels of phenolic compounds. Diterpenes, triterpenes, steroids, and fatty acid metabolites have contributed to the biological/chemical achievements. The experience previously acquired with the Brazilian plant extracts was primordial to studying the Antarctic plants due to the small amount of biomass that was possible to collect during fieldwork, and one species was used as a model to be adapted to the Antarctic plants. This species showed antibacterial and antioxidant activities. Carrying out prospecting projects is based on persistence, systematization, and resilience as a way of understanding the biodiversity potential of tropical forests and more distant places in the era of climate change.

Acknowledgments: CNPq, CAPES, FAPESP.

Keywords: Antarctic, Brazilian biodiversity, *in vitro* assays, large-scale screening, statistical analyses

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S2.P207 Polyphenol and anthocyanin content in early producing maqui hybrids (*Aristotelia chilensis*)

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Maqui berries are known for their high content of polyphenols and anthocyanins (Schreckinger et al., 2010). *Aristotelia chilensis* (Mol.) Stuntz, Elaeocarpaceae, is a bush or tree native to Chile and the western Argentine Patagonia. Its outstanding antioxidant properties (Araya et al., 2006) triggered the overexploitation of natural populations through wild collection to satisfy the growing demand for different maqui berry-based products. To ensure sustainable production, commercial varieties have been developed (Vogel et al., 2016). 69 hybrids have been selected from individuals obtained by crossing plants with outstanding fruit yield and quality characteristics. These genotypes have been evaluated for the proportion of pulp in fruits, and their concentration of polyphenols and anthocyanins. At the same time, the ripening period of each hybrid was recorded to detect early producing genotypes for agricultural production, especially in regions with less water availability during the summer months. The hybrids showed a range of polyphenol concentrations from 0.4 to 6.0 EAG/100 g dry matter (DM), and anthocyanin concentrations from 0.7 to 2.2 g cy-3-glu/100 g DM. The proportion of pulp in the berries ranged from 14 to 82% with an average of 51%. For future agricultural production in regions with water scarcity, early producing genotypes were identified, with a short ripening period, high proportions of pulp, and high concentrations of polyphenols and anthocyanins.

Keywords: maqui berry, proportion of pulp, ripening period, adaptation to water scarcity

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S2.P208 Effect of different ethylene levels on other phytohormones and *Arabidopsis* resilience

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Ethylene, a distinctive gaseous phytohormone, plays a pivotal role in various developmental processes and frequently interacts with other phytohormones. It governs essential plant processes, including fruit ripening and senescence, highlighting its significance in plant physiology. Understanding the nuanced effects of ethylene is crucial for optimizing agricultural practices and effective crop management (Lin et al., 2009). Plants, being immobile, encounter challenges posed by abiotic factors such as drought, salinity, and extreme temperatures. These challenges significantly limit their adaptability, alter growth and development, and consequently reduce crop yield (Zhang et al., 2022). We aim to explore the impact of reduced or elevated ethylene production in *Arabidopsis* mutants on the content of other phytohormones and also possibly explore their relationship under stress conditions. This research introduces a sensitive methodology for assessing levels of ethylene or its direct precursor 1-aminocyclopropane-1-carboxylic acid (ACC) by means of a new liquid chromatography-tandem mass spectrometry method, employing a fully validated method with a specific derivatization technique for accurate ACC and phytohormone profiling. Our results show a clear influence between elevated ethylene and ACC levels and other phytohormones and their selected metabolites content.

Keywords: *Arabidopsis thaliana,* ethylene, phytohormones, abiotic stress

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S2.P209 Copper-induced siderophore production and copper complex formation in vitamin-producing bacteria at a Vermont copper mine

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Ely Copper Mine in Vermont, USA, a metal-polluted Superfund site, has been accumulating acidic water (pH > 2) or acid rock drainage (ARD) for over 50 years, contributing to contamination of nearby water bodies (Fig. 1). Such contamination is exacerbated by rising flooding events associated with climate change. We previously characterized its Proteobacteria-dominated ARD microbiome and isolated amoebae and associated bacteria (*Dyella*) from this copper-contaminated (2 mg/L water; 2 mg/kg sediment) site (Giddings, 2020; Giddings 2022).

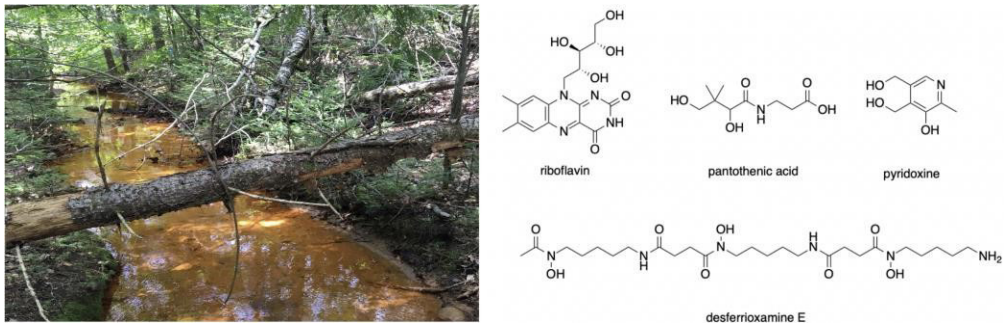

Fig. 1. ARD at Ely Copper Mine in Vershire, Vermont and bacterial metabolites detected.

To develop site-specific strategies for remediation, we explored the ARD microbial community for producers of metal-chelators or siderophores. Siderophore-producing *Dyella* were identified using a colorimetric chrome azurol S plate assay and isolates were grown in complex media with increasing copper concentrations (0–100 μM). Metabolomics analyses of organic extracts of *Dyella* showed an abundance of pantothenic acid (vitamin B5), riboflavin (vitamin B2), and pyridoxine (vitamin B6), which are likely why these bacteria associate with amoebae within this extreme environment (Fig. 1). Notably, siderophores, such as desferrioxamine E, were detected and observed to increase with increasing copper concentrations. Copper also formed stable complexes with several flavins. Our data provide insight into the symbioses and adaptations amoebae employ to survive in nutrient-deficient, metal-polluted environments and offer the potential to find new bioremediation methods.

Keywords: metal pollution, siderophores, *Dyella,* copper-induced metabolites

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S2.P210 Disentangling metabolite dynamics in extreme environments: life detection strategies for extraterrestrial worlds

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Microbes from terrestrial extreme environments offer an opportunity to test biosignature production in conditions relevant to astrobiological targets. In Antarctica, iron-rich brine (~3X seawater salinity) leaks through a glacier forming a bright red 'waterfall' known as Blood Falls (Fig. 1). This feature provides a portal into ecosystems hidden beneath the Antarctic ice sheet, one of the least explored biomes on Earth. This site is also analog for ecosystems beyond Earth such as on Enceladus, a moon of Saturn, that harbors a liquid ocean beneath its ice shell, or possible Martian groundwater systems (Sklute et al. 2022).

Figure 1. Blood Falls, McMurdo Dry Valleys, Antarctica (credit: P. Rejeck).

To identify biosignatures produced during microbial iron transformations, we grew *Shewanella* sp. (strain BF02_schw), originally isolated from Blood Falls (Boles et al. 2021), in minimal marine media with amorphous iron hydroxides (100 mM) and analysed the volatilome, metabolome, transcriptome, and mineralogy for indicators of microbe-mineral interactions. Significant tryptophan degradation was observed in iron treatments, suggesting potential Fenton reaction mediated nutrient loss, however this loss may be moderated as genes regulating tryptophan biosynthesis were concomitantly overexpressed. Altering membrane fluidity is a known strategy for growth at low temperatures in psychrotolerant *Shewanella* species (Bowman et al. 1997). In iron treatments, we observed overexpression of eicosapentaenoic acid (EPA) biosynthesis genes, suggesting EPA may also help manage iron toxicity. Collectively our results show the poly- extremophile strain BF02_schw produces diverse molecular biosignatures indicative of growth in a metal-rich environment. Metabolites produced during growth in astrobiological analogs, such as those identified here, may provide potential agnostic biomarkers for life detection.

Keywords: Antarctica, *Shewanella*, iron-mediated metabolism, subglacial environments

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produce eicosapentaenoic acid (20: 5ω3) and grow anaerobically by dissimilatory Fe (III) reduction. *International Journal of Systematic and Evolutionary Microbiology*, *47*(4), pp.1040-1047.

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S2.P211 Effect of green-based extraction approaches on antioxidant activity and phenolic compounds of Oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart)

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Greek Oregano (*Origanum vulgare ssp. hirtum (Link) Ietswaart*) is a Mediterranean culinary herb, traditionally used in folk medicine, as far as in food, animal feed and in pharmaceutical industry. The species contain high quality essential oil and is rich source of phenolic compounds, known for their bioactivity, attributed to health-promoting properties; antioxidant, antibacterial, antifungal, etc. Nowadays, there is a growing interest for natural antioxidants that could be used as alternative to the synthetic ones, using at the same time green-based extraction approaches. The impact of microwave- assisted extraction (MAE), ultrasound-assisted extraction (UAE), and accelerated solvent extraction (ASE) on the total phenolics, total flavonoids and total antioxidant activity of oregano extracts was investigated. Dried and ground oregano extracts were subjected to extraction with 60% aqueous methanol. The antioxidant activity of oregano extracts was evaluated using ABTS, DPPH and FRAP assays. MAE proved the most effective extraction technique in exploring the bioactive compounds from oregano. Specifically, MAE extracts showed more total phenolics, total flavonoids and antioxidant activity on ABTS, DPPH and FRAP assays in comparison to ASE and UAE extracts. The HPLC/PDA/MS analyses revealed that rosmarinic acid, salvianolic acid B and carvacrol were the major phenolic compounds in all the obtained extracts, followed by flavonoid glycosides, with their content being highest in MAE extracts, obtained at 70°C, over 10 min. However, higher carvacrol content was favored by UAE and less by ASE. Summarizing, MAE could be employed as green and efficient method for producing oregano extracts rich in bioactive compounds, exhibiting strong antioxidant properties for developing nutraceuticals and pharmaceuticals.

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Keywords: *Oregano*, green extraction, microwave, phenolics, LC-MS analysis

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S2.P212 Enhancing polyphenol production in *Cistus albidus* L. through strategic mycorrhization and irrigation management: An off-season approach

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Polyphenols, valuable for their health benefits, face challenges in synthetic production, including low yields and toxicity issues (Ofosu et al., 2020). Mediterranean semideciduous shrubs, adapted to abiotic stress, are significant sources of these metabolites. *Cistus albidus* L., a species renowned for its medicinal attributes, thrives under the harsh conditions of the Mediterranean climate, producing a wide array of natural metabolites with minimal resource inputs (Raus de Baviera et al., 2023). Our study focused on enhancing *C. albidus's* polyphenol production through *Glomus* sp. inoculation and controlled irrigation. We discovered that maintaining water availability at field capacity modulates the plant-mycorrhiza symbiosis so that *Glomus* sp. behaves different as expected, significantly increasing polyphenol and antioxidant levels compared to not inoculated plants. Especially during autumn, season with limited polyphenol production in *C. albidus*, we observed values over four times higher than the control, indicating that strategic water management and *Glomus* sp. inoculation can substantially boost polyphenol and antioxidant content in *C. albidus*. After the plants were exposed to drought stress, typical symptoms of mycorrhizal symbiosis returned. As observed by (Ortuño et al., 2018) mycorrhiza may improve the water status of *C. albidus*, allowing to devote more energy to secondary metabolism and the synthesis of phenolic compounds under well-watered conditions, rather than just focusing on survival. This approach, particularly effective during seasons with limited polyphenol production, highlights the potential of combining mycorrhization with irrigation strategies to enhance the productivity, presenting a viable method for optimizing the production of pharmacological compounds.

Keywords: polyphenols, *Cistus albidus* L., *Glomus* sp. inoculation, water availability

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S2.P213 The potential of chitosan from *Hermetia illucens* as a sustainable biopesticide

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The long-standing use of chemical pesticides by farmers has enhanced pest resistance while continuing to pose serious environmental risks. As a result, there has been an upsurge in research and development of biopesticide formulations. Farmers are searching for safer, more efficient ways to maintain their harvests while preserving the environment. The use of frass from the *Hermetia illucens* (Black soldier fly) as a potential biofertilizer and biopesticide for crops has previously been crudely reported. Frass is a waste product from the Black soldier fly farm and it is made up of various organic wastes including molten skins and pupal shells of the Black soldier flies from which chitin can be extracted. Chitosan, which is deacetylated chitin is an important commercial molecule with desirable properties and holds immense promise as a sustainable biopesticide. This study aimed at exploring the extraction and application of chitosan in pest management, addressing environmental concerns associated with conventional pesticides. The chitosan extraction process involved pretreatment of the skins and shells from black soldier flies, demineralization, deproteinization followed by the deacetylation of chitin to obtain chitosan while following rigorous quality control parameters, ensuring optimal yield and purity. The resulting chitosan exhibited notable antifungal, antibacterial and antipesticidal properties specifically noted for *Colletotrichum sublineolum* (which causes anthracnose in sorghum), *Escherichia coli* (a common pathogenic bacteria and contaminant) and *Aleurodicus dispersus* (whiteflies that transmits begomo viruses which cause Tomato yellow leaf curl) respectively establishing its potential as a multifunctional biopesticide. Our investigations reveal the efficacy of chitosan against a range of agricultural pests. Furthermore, the use of *Hermetia illucens* frass for chitosan production aligns with sustainable practices. The larvae of *Hermetia illucens* thrive on organic waste, providing a dual benefit of waste management and biopesticide production. In addition, chitosan is as well a biodegradable molecule. This integrated approach contributes to a circular economy and reduces the environmental impact associated with conventional pesticide production making it a compelling choice for sustainable agriculture.

Keywords: *Hermetia illucens,* chitosan, biopesticide, sustainable

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S2.P214 Screening of Nettle Clones with the Ph Eur HPTLC method for identification testing

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The study compares the health-related compounds in 20 clones of stinging nettle leaves (*Urtica dioica* L.) at different harvest times. The cultivars were dedicated for textile fibre production, the leaves are assessed for valorization. High performance thin layer chromatography (HPTLC) fingerprint comparison was used and commercial nettle leaves from a local pharmacy and collected in the wild from the Swiss mountains included. During the growth phase, the morphology of the nettles changed in size as well as in the colour of the leaves and stems. After drying at 40 °C, the nettle leaves were extracted with 70 % methanol (70:30 V/V) in an ultrasonic bath for 15 min. The HPTLC plates derivatized with anisaldehyde sulfuric acid (AS) and ferric chloride (FC) were compared to the PhEur Identity testing prescribed Natural product (NP/PEG) reagent derivatization in order to assess different natural product classes. The ubiquitious reference compounds rutin and chlorogenic acid were identified in every nettle examined, while hyperoside, quercetin, and the Ph Eur relevant scopoletin were not or only faintly detected in any nettle of the summer harvest. Assessment for Scopoletin, as a identity marker content was performed using LC-MS analysis. Complementary to the metabolic comparison, the HPTLC-DPPH radical scavenging assay showed that activity was higher with increasing growth of the plants. Overall a late harvest of the leaves is recommended to achieve the necessary secondary metabolite content for health related benefits.

Keywords: nettle leaves, fibre side stream valorization, HPTLC, DPPH, scopoletin

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S2.P215 Creation of a medicinal plants collection and research consortium to catalyze drug discovery in San Diego and beyond: An update from 2022-2024

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As many as 40% of all drugs found in modern pharmacies today are directly or indirectly derived from plants (USDA, 2024). Yet, the rate of plant-based drug discovery has declined in recent decades, in part due to lack of readily available medicinal plant collection resources. At the same time, the threat and reality of biodiversity loss has been overwhelmingly increasing. It is now nearly ten years since the Nagoya Protocol went into effect. Many drug discovery programs have yet to effectively implement the aims and scope of the Nagoya Protocol for enhancing the conservation and sustainable use of biodiversity or to recognize the value of traditional ecological knowledge of plants and their uses. The Department of Science and Conservation at San Diego Botanic Garden has undertaken a project initiative "Creation of a National Medicinal Plants Collection and Research Consortium to Catalyze Drug Discovery in San Diego and Beyond" that aims to demonstrate a new way forward for plant natural product drug discovery. As we have embarked on a journey to build a medicinal plants collection in the USA for the purposes of both plant conservation and scientific research, we attempted to proactively create equitable partnerships with meaningful societal contributions including plant conservation efforts, ecosystem restoration projects, and joint development of phytochemical knowledge that aims to bridge traditional and modern medical practices. This project accordingly includes partnerships with members or governments of indigenous communities (i.e. Native American Indians), which brings great opportunities along with operational challenges, and a paramount ethical responsibility. As a non-profit organization, we have created a consortium of research partners that includes other non-profits, academia, industry, and tribal governments to find ways to work together to improve outcomes and remove barriers from more traditional past research programs. We have developed early educational programming to engage community members from diverse backgrounds both in-person and online. Finally, we have undertaken multi-institutional natural product drug discovery and development projects studying two native medicinal plants, California yerba santa (*Eriodictyon californicum*) and California sagebrush (*Artemisia californica*). These plants were grown under varied sets of controlled conditions in our medicinal plant greenhouse and nursery areas to then measure and evaluate the modulation of genomic, transcriptomic, and metabolomic expression. In vitro pharmacological testing has been conducted by partner institutions. Examples from all of these activities will be presented in the conference.

Keywords: Native American ethnobotany, consortium, phytochemistry, *Eriodictyon*, *Artemisia*

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S2.P216 The cannabis/marijuana (*Cannabis sativa* L.) landscape in Africa: an overview of its cultivation and legal aspects

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Cannabis is the most widely used illicit substance globally. However, it remains an important crop in Africa and was originally cultivated by poor farmers who could not benefit from legal agriculture (Merz, 2018). Currently, African farmers produce enough cannabis to meet continental demand while exporting small quantities. We reviewed the literature on cannabis cultivation, legalization, farming, export, production, history, trade, medical and recreational effects of cannabis in Africa between 1975 to 2021. We searched databases such as Web of Science, Google Scholar, PubMed, Scopus for research papers, reports, and books. Also, we searched local newspapers and court rulings for relevant cannabis content. Although many countries in Europe and the Americas have liberalized controls of different aspects along the cannabis value chain. However, most African nations upheld pre- independence laws against cannabis in compliance with the 1925 International Opium Convention, although the legal terrain has and continues to evolve (Carrier and Klantschnig, 2018). Consequently, some African governments, including South Africa, the Democratic Republic of Congo (DRC), Uganda, Lesotho, Malawi, eSwatini, Zimbabwe, Rwanda, Kenya, and Egypt, have authorized limited cannabis farming. We recommend a more inclusive and regulated cannabis farming system so that local farmers benefit from its export while governments from a broader tax base. African governments need to invest more in cannabis research and development. This will pave the way for their citizens to benefit from the therapeutic effects of medical cannabis and value addition which can fetch higher returns than the raw cannabis materials.

Keywords: medicinal cannabis, marijuana, Africa, legalization, trade, farming

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S2.P217 A comparative toxicity study depending on type of clinical use of the roots of *Asarum heterotropoides* var. *mandshuricum* (Maxim.) Kitag

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Natural products are used for various purposes such as foods, health supplements and medicines. In the case of the safety assessment of natural products, it is determined based on known toxic substances, but may change depending on the manufacturing method, so there is a limit to simply evaluating them based on toxicity results of the natural product raw materials themselves. The root of *Asarum heterotropoides* var *mandshuricum* (Maxim.) Kitag (AR) is widely used as a traditional herbal medicine mainly in East Asia. In this study, we presented toxicity comparison results for the main forms of use of AR root, powder and water extraction. Genotoxicity, single- and repeated-dose toxicity studies were conducted in accordance with the OECD testing guidelines. As a result of the study, toxicity changes including positive results in genotoxicity and adenoma in the liver were observed in the powder form of AR. However, no specific toxicity changes were observed in water extraction form of AR. As a result of HPLC analysis for the main components, methyl eugenol and safrole, known as toxic substances, were detected only in powder form. This means that the corresponding component is removed during the water extraction process of AR, and it was considered to be decreased a toxicity effect.

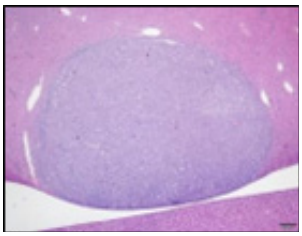

Fig. 1. Hepatocyte adenoma in the liver after administering 2000 mg/kg/day AR powder

Keywords: *Asarum heterotropoides var. mandshuricum,* acute toxicity, subchronic toxicity, genotoxicity, toxic components

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S2.P218 Determination of triterpenoids of the native *Centella asiatica* L. from the Jeju Island, South Korea

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Centella asiatica L. (CA) is a (sub)tropical herbal medicine belonging to the Apiaceae (Antoine N Nicolas et al., 2009). It is widely distributed throughout tropical and subtropical regions of Africa, Asia, Australia, and islands in the western Pacific Ocean (Nora E Gray et al., 2018). However, it has been found to have different total triterpenoids depending on the growing ecological environment (Boju Sun et al., 2020). In recent years, its extracts and bioactive components have been reported to have anti-inflammatory, anti-tumor, anti-oxidant, wound healing, cardioprotective and improving- memory effect (Changhe Wang et al. 2022).

In the present study, the aerial part of CA was collected native to Jeju Island, Republic of Korea with Voucher No. MFDS-C-8705, and its dried extract was quantified by high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) focusing on four triterpenoids, asiaticoside, madecassoside, asiatic acid, and madecassic acid.

Analysis by HPTLC of the samples was performed on HPTLC aluminum precoated plate (60 F254) by using mobile phase dichloromethane, methanol, water (14:6:1 v/v/v) with 10% sulfuric acid. and we developed a HPLC simultaneous analysis of four triterpenoids in *C. asiatica*.

All the results obtained from in this study help to determine the characteristics and analysis of standardization of CA native to Jeju Island.

Keywords: *Centella asiatica* L., triterpenoids, HPTLC, HPLC, herbal medicine

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S2.P219 Streoselective inhibitory effects of ginsenosides on six uridine 5'-diphosphoglucuronosyl transferases in human liver microsomes

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This study investigated the inhibitory potential of both protopanaxadiol (PPD)- and protopanaxatriol (PPT)-type ginsenosides (Fig. 1.) on the activities of six uridine 5'-diphospho- glucuronosyltransferase (UGT) isoforms (1A3, 1A4, 1A4, 1A6, 1A9, and 2B7) in pooled human liver microsomes (HLMs). Among the various active ingredients of ginseng, ginsenosides are the main chemicals responsible for the pharmacological effect of ginseng such as anticancer, antioxidant, and cholesterol-lowering activity. Ginsenosides inhibited the UGT isoform activities in a structure- dependent manner. For example, PPT-type ginsenosides showed minimal inhibition against the activity of the 6 UGT isoforms evaluated (IC_{50} > 37.1 μM). The activities of UGT2B7 and UGT1A9 were inhibited by the PPD-type ginsenoside having two sugar moieties at the carbon-3 position of the ginsenoside structure (e.g., ginsenoside Rg3) and PPD-type ginsenosides having two sugar moieties at the carbon-3 position of ginsenoside regardless of the presence or absence of the sugar group at carbon-20 position of ginsenosides (e.g., ginsenoside Rb1, Rb2, Rd, and Rg3), respectively. These results suggest that ginsenosides have a structure-specific inhibitory effect on UGTs in HLMs.

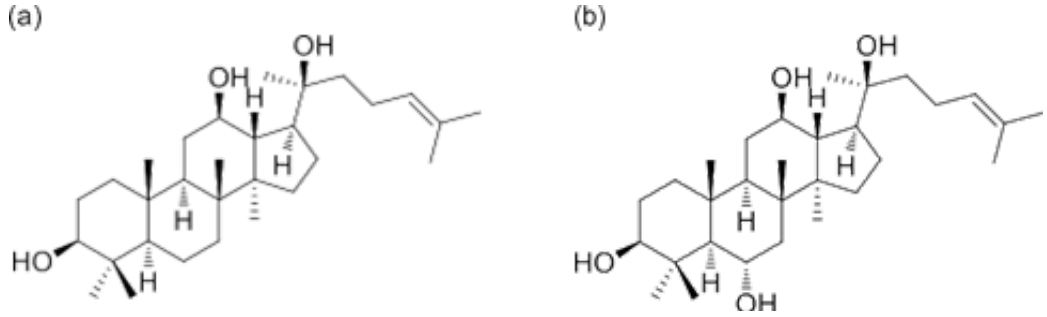

Fig. 1. Structure of (a) protopanaxadiol and (b) protopanaxatriol

Keywords: ginsenosides, inhibition, microsomes, uridine 5'-diphospho glucuronosyltransferase

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S2.P220 Discrimination of herbal medicines originated from soy beans (*Glycine max* Merrill.) using high-performance thin-layer chromatography

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Glycine Semen Nigra (GN), Glycine Semen Preparata (GP) and Glycine Semen Germinatum (GG) are herbal medicines derived from *Glycine max* Merrill, exhibiting distinct clinical properties due to varied processing methods. GN is recognized for its diuretic and detoxifying effects, while GP alleviates symptoms such as headaches, chest tightness, and insomnia resulting from colds. GG is employed for summer colds, addressing abdominal pain and dysuria by reducing fever and inducing diuresis. This study focuses on the development of a High-Performance Thin-Layer Chromatography (HPTLC) method for the discrimination of GN, GP, and GG. The optimal mobile phase comprised a solvent mixture of dichloromethane, methanol, water, and glacial acetic acid in a ratio of 10:4:1:0.5 (v/v/v/v). HPTLC results revealed a consistent blue spot at an Rf value of 0.6, identified as genistin. (Kim, J. A. et al. 2007). Additionally, spots at Rf values 0.35, 0.55, and 0.65 distinguished the three groups. The identification of compounds within these spots, through TLC-Mass Spectrometry- interface, aimed to correlate them with the specific activities of each herbal medicine. In conclusion, our developed HPTLC method facilitates the simultaneous differentiation of three herbal medicines from the same species, *Glycine Max* Merrill. This approach is expected to contribute to the standardization of herbal medicines through a rapid and cost-effective method.

Keywords: high-performance thin-layer chromatography (HPTLC), *Glycine max* L., glycine semen nigra, glycine semen preparata, glycine semen germinatum

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S2.P221 Analytical Quality by Desgin(AQbD) methodology apporach for simultaneous quantification of Hesperidin and Magnolol in Daekumeum-ja using HPLC-PDA

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Daekumeum-ja (DKJ) is known for its efficacy in preventing alcohol-induced inflammatory mucosal damage and cognitive impairments (J.J.Kim et al., 2010). To standardize the quality of DKJ, an HPLC-PDA analysis method was developed for the simultaneous quantification of two major components, hesperidin and magnolol. The robustness of this method was improved with the Analytical-Quality-by-Design strategy (AQbD), enhancing efficiency in both time and cost. Initially, a risk assessment was conducted to identify the Critical-Analytical-Procedure-Parameters (CAPPs), such as the ratio of the organic mobile phase and the column oven temperature. To evaluate the impact of CAPPs on Critical-Analytical-Procedure-Attributes (CAPA), peak tailing and resolution, a Design- of-Experiments (DOE) approach using Central-Composite-Design (CCD) was implemented. The optimal chromatographic analysis conditions were established for optimal column oven temperature, detection wavelength of 290 nm, flow rate, and mobile phase composition and gradient condition. In alignment with the International Conference on Harmonization (ICH) guideline, the developed method underwent validation. This study conclusively affirmed the capability for robust and reliable simultaneous quantitative method of DKJ components, thereby proving the successful development of the method.

Keywords: daekumeum-ja, HPLC-PDA, simultaneous quantification, analytical quality by design (AQbD), central composite design (CCD)

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S2.P222 Discrimination between similar herbal medicines of Campanulaceae by high-performance thin layer chromatography (HPLTC)

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Adenophorae Radix (AR), Adenophorae Remotiflori Radix (ARR), and *Codonopsis lanceolata* root (CL), members of the Campanulaceae family, face misidentification due to their morphological similarities, leading to confusion regarding their appropriate utilization in the herbal medicine markets in Korea (Park, E. S., et al., 2017). In response, this study aimed to establish a rapid discrimination method utilizing High-Performance Thin Layer Chromatography (HPTLC). 16 as AR, 7 as ARR, 10 as CL, and 7 samples distributed under unclear names were purchased from the herbal markets. Analysis of components identified in previous research and samples was conducted under the same HPTLC conditions. Results revealed two key components for classification: β-sitosterol with a retardation factor (Rf) value of 0.41 and α-spinasterol with an Rf value of 0.37. Distinct tendencies were confirmed, such as the exclusive presence of the β-sitosterol band in AR, exclusive α-spinasterol in CL, and both components in ARR. Furthermore, AR was discerned by the absence of a blue band with an Rf value of 0.61. Based on this classification, the samples were categorized into three groups: 17 as AR, 5 as ARR, and 18 as CL, leading to the correction of the labeling errors in the origin of 40 samples. Consequently, this study has successfully devised a straightforward and efficient analytical method to differentiate between AR, ARR, and CL, addressing the prevalent issue of misidentification. The implementation of this method is anticipated to play a pivotal role in enhancing the standardization of herbal medicine quality.

Keywords: *Adenophorae Radix, Adenophorae Remotiflori Radix, Codonposis lanceolata* root, Campanulaceae, HPTLC

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Park, E. S., Lee, S. R., Joeng, J. M., Song, M. G., Yoon, J. H., & Ju, Y. S., 2017. Discriminative criteria of Adenophorae Radix, Codonopsis Lanceolatae Radix and Adenophorae Remotiflori Radix. The Korea Journal of Herbology, 32(2), 33- 40.

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S2.P223 Quantification of saponin-rich plants mixture in complete feed with UHPLC-MS/MS using a "one-point" calibration

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Quantification of additives in complete feeds is necessary to estimate the rate of incorporation and homogeneity of the feed, which guarantees the effectiveness of the additive. However, for botanical compounds this remains a challenge due to the complexity of the matrix and the level of incorporation, requiring sensitive and specific methods.

A specific and sensitive method for the quantification of saponin-rich mixtures in complete feed, based on UHPLC-MS/MS, was developed. The additive studied was a natural commercial feed additive (Norponin® XO2-300 Nor-Feed, France) used in animal nutrition for the management of coccidiosis. A mixture consisting of a standardized *Yucca schidigera* and *Trigonella foenum- graecum*, in which protodioscin has been identified and used as a phytomarker for the quantification of the additive at a level of 300 ppm in complete feeds.

Quantification of the sample is carried out with an optimized sample preparation, an internal standard and with a "one-point" calibration. Using the pure additive for calibration permits the direct determination of the additive content in the sample by integrating the area of the phytomarker. The use of an internal standard with a structure close to that of the phylomarker allowed us to overcome the problems of recovery and matrix effects encountered in sample preparation and UHPLC-MS/MS. The analysis method, which is rapid and suitable for routine use, has been validated in-house (Thomspon et al., 2002). This method meets all the prerequisites for use by European authorities as part of the registration of a food additive (FEEDAP, 2012).

Keywords: saponins, additive, complete feed, coccidiosis, UHPLC-MS/MS

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S2.P224 Interactions of ivy (*Hedera helix*) with other herbs or medicines: a systematic review

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Cough is a predominant medical condition and general practitioners are regularly consulted by patients to treat it. Ivy (*Hedera helix* L., Araliaceae) is a commonly used for respiratory disorders. It includes saponins which exhibit bronchodilator, mucolytic, spasmolytic, and antibacterial properties. Although it is widely used, the potential interactions with other herbs or medicines are not fully investigated. Through a systematic review of the literature, this study examines the interactions of ivy with other herbs and medicines.

This review utilized multiple databases. Initially, a systematic literature search was performed in PubMed, Embase, and Cochrane Library. Another search was in the databases of several regulatory agencies with excellent records regarding herbal products interactions. These agencies are mainly the European Medicines Agency (EMA) and Health Canada. The search terms used were "ivy", "*Hedera helix*", "drug interactions", "herb-drug interactions", and "phytochemical interactions". The search covers the period between January 1990 and December 2021.

In this review, 23 studies were included. These studies (randomized controlled trials, observational studies, case reports and *in vivo* and *in vitro* studies) investigated the interactions of ivy with various herbs and medicines, including anticoagulants, bronchodilators, and corticosteroids. The majority reported no significant interactions between ivy and the other herbs or medicines studied. However, some studies reported potential interactions, such as increased bleeding risk when ivy was used with anticoagulants or antiplatelet agents. Overall, the available evidence suggests that ivy can be used safely with other herbs or medicines, although caution should be used when using ivy with anticoagulants or antiplatelets.

Keywords: ivy, *Hedera helix*, interactions

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S2.P225 Brazilian regulation for food supplements containing bioactive substances from plant extracts

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The interest in food supplements is increasing in Brazil. Although the regulation for food supplements has been updated by Brazilian Health Regulatory Agency (ANVISA) in recent years, the regulated sector suffers from challenging requirements for providing compliant products.

Below, we present the latest regulatory changes related to the use of bioactive plant extracts in food supplements and highlight the challenges in approving new ingredients in Brazil.

The Regulation for approving novel food ingredients has existed since 1999 (RES16/1999), however, the food supplement category was not created before 2018 (RDC243/2018). It provided a clear application procedure for such ingredients. Moreover, RDC839/2023 will replace RES16/1999 from March 2024 on, while other older regulations will remain valid, which results in a complex scenario. RDC839/2023 determines the safety requirements for e.g. bioavailability and toxicokinetic in food ingredients as well as in their products of degradation. The chemical characterisation should be as complete as possible. Moreover, it is not clear whether the use of extracts from some species in food supplements will be accepted if the same species have also been used in herbal medicinal products. Insufficient or missing information must be justified, and may or may not be accepted by ANVISA. Lastly, only plant extracts that have been approved by ANVISA can be used in supplements. The approved ingredients are listed in IN28/2018 and its updates. ANVISA's decision normally takes around 2 years after application. Publishing the summarised manufacturing process and specifications will be a disadvantage for own developed ingredients.

Keywords: Brazilian regulation, food supplement, plant extract, bioactive substances

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RDC 16/1999 – Regulamento referente a procedimentos para registro de alimentos e ou novos ingredientes. Brazil, 30 April 1999.

RDC 243/2018 – Dispõe sobre os requisitos sanitários dos suplementos alimentares. Brazil, 26 July 2018.

RDC 839/2023 – Dispõe sobre a comprovação de segurança e a autorização de uso de novos alimentos e novos ingredientes. Brazil 14 December 2023.

IN 28/2018 – Estabelece as listas de constituintes, de limites de uso, de alegações e de rotulagem complementar dos suplementos alimentares. Brazil, 26 July 2018.

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S2.P226 Chemical profile of natural musk (*Moschus moschiferus*) by GC-MS/MS

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Since 'natural musk' is one of the most expensive herbal medicine, it's essential to develop an adequate quality control method in order to prevent forgery and maintain its quality in the view of regulatory authorities. Analytical methods using indicator compounds have been reported, but with limitations that make it difficult to reflect the overall chemical characteristics of musk. In this study, we presented a standardized chemical profile, which is expected to be utilized as a basis for the development of quality evaluation methods based on chemical similarity. Musk samples were collected without removing the abdominal connective tissue from *Moschus moschiferus* of different areas and ages. Twelve standard samples were obtained by confirming that no traces of artificial manipulation were found through genetic analysis and organoleptic tests. Samples were analyzed using GC-MS/MS (scan mode), which can efficiently identify the constituents of musk. Seven peaks that were detected at 1% area of the total peak area or more, were selected as the standard chemical profile. The sum of the areas of the seven peaks was more than 75% of the total peak area and the relative standard deviation (% RSD) of the relative retention time [reference: IS (methandienone)] of each peak was consistent at less than 5%. Three peaks were identified as etiocholanolone, dihydroandrosterone, and epicholestanol by comparing retension times and mass spectra with commercial standards. Among the seven peaks in the standardized chemical profile, four peaks were not indivisually identified, but they are commonly detected peaks that accounts for 45% of the total area and are judged to be very important in reflecting the overall chemical properties of musk. Also, the relative standard deviation (% RSD) of the relative peak area of each peak [reference: IS (methandienone)] was 25% or less, indicating that there is a difference in the composition of each individual musk.

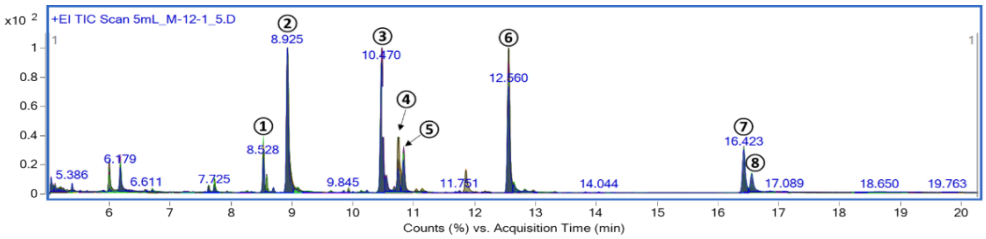

Fig. 1. Chromatogram of a strandardized chemical profile for 'natural musk'. **(1)** unknown 1, **(2)** unknown 2, **(3)** etiocholanol one, **(4)** unknown 3, **(5)** dihydroandrosterone, **(6, I.S.)** methandienone, **(7)** unknown 4, **(8)** epicholestanol,

Keywords: Natural musk*(Moschus moschiferus)*, Chemical fingerprint analysis, Herbal medicine

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S2.P227 Advances in the Brazilian Regulation for the use of plant extracts in medicines

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The use of herbal medicines dates back to ancient times and is now increasing globally. Street markets in Brazil offer plants for any treatment, but the regulated sector must meet requirements to register herbal medicines.

We aim to present the regulatory advances related to herbal medicines, compare them with European Regulations, and highlight the main difficulties in approving new products in Brazil.

Herbal medicinal products are separately regulated and different from other medicinal products. As in Europe, they are distinct categories related to traditional use or scientific evidence. ANVISA (Brazilian Health Regulatory Agency) is updating the legislation regarding herbal medicines to harmonise with European concepts (DOU95/2021; Port1.409/2023).

The current RDC26/2014 and IN02/2014 focus on the chemical markers and do not take into account the classification into standardised, quantified and other extracts. On the other hand, HMPC- monographs are accepted for a simplified process, and many registered herbal active ingredients come from the European tradition. The harmonisation of concepts will make many aspects of quality and pharmacological interpretations very similar to those in Europe.

However, there are still challenges in terms of distinguishing the concepts and characteristics that differentiate herbal medicines from those that are synthetic. This has led to a discussion that has now lasted for more than 6 years. This period of uncertainty, coupled with the lengthy registration process, discourages the development of new herbal medicines and slows down the market potential.

Regulatory harmonisation bodes well for a promising development of herbal medicines in Brazil in the near future.

Keywords: herbal medicines, plant extracts, ANVISA

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S2.P228 Comparing the volatilome of Spanish-style and natural cv. Chalkidiki green table olives at industrial scale

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Table olives are one of the most popular fermented foods, with great nutritional and sensory characteristics. For the preparation of cv. Chalkidiki green table olives**,** Spanish-style processing is almost exclusively used despite diminishing the content in health-promoting compounds and increasing the content in salt of the edible part (Mastralexi et al., 2019). On the contrary, the natural processing of olives involves minimal chemical input and retains better the nutritional value of olive drupes (Conte et al., 2020). These different practices also shape the fermenting microbial communities that are implemented in the quality attributes of the products (Ruiz-Barba et al., 2023). Volatilomics approach has been used to identify specific molecules, originating from the microbial metabolism taking place on table olive fermentation and affecting table olives' aroma and flavor (Alvanoudi et al., 2023).

The present study aims to shed light on the compositional characteristics of volatile organic compounds (VOCs) of Spanish-style and natural cv. Chalkidiki green table olives at industrial scale. VOCs were determined using headspace SPME-GC-MS. Microbiological analyses and sensory evaluation of the final products were also conducted via appropriate methods. More than 50 VOCs were detected (acids, alcohols, aldehydes, esters, phenols, etc.) and the final products were differentiated depending on the process applied. Furthermore, results from the microbiological and sensory quality assessment of the products showed that all of them were safe and organoleptically acceptable. Consumers' preference for the odor attribute found to be significantly different between the samples. The Greek table olive industry may benefit from the current findings.

Keywords: Spanish-style green olives, natural olives, volatile organic compounds, headspace SPME- GC-MS, Chalkidiki cultivar

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S2.P229 Addressing regulatory challenges and ensuring safety in the e-commerce of herbal remedies in Mexico

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In Mexico, plant-based products are classified as herbal medicines, herbal remedies (HR), and food supplements. Regulatory scrutiny focuses solely on herbal medicines, complicating oversight of remedies (Rojas et al., 2022). Ambiguous standards contribute to quality issues, such as mislabeling and adulteration, posing risks to safety and public health (Biesterbos et al., 2019). Online purchases through e-commerce platforms worsen the situation by lacking essential information, such as plant identity and origin (Alwhaibi et al., 2021). Producers frequently exploit legal loopholes to prioritize economic gains over rigorous studies.

This study aimed to evaluate HR obtained from physical health food stores and online platforms, adhering to Mexican regulations. Three HR were purchased nationwide: dry plants (MV-A, MV-B, MV-S), and infusion drops (ID-A, ID-B, ID-S) online. Compliance with NOM-072-SSA1-2012 for labeling and General Methods of Analysis for foreign matter, botanical identification, and thin-layer chromatography (TLC) following the guidelines of the Mexican Herbal Pharmacopoeia (FHEUM, for its acronym in Spanish).

Results revealed labeling discrepancies, with MVs lacking labels and IDs having deficient ones. MV- S samples exceeded the 2 % foreign matter limit, while the other samples complied. Only two MV- B samples did not match the specified species, affecting their macroscopic characters and chromatographic profiles. TLC analysis of IDs showed lower concentrations, indicating dilution but consistent species identification.

In conclusion, the analyzed samples do not fully adhere to national regulations, posing a significant issue as they are distributed nationwide in both physical and online stores.

Keywords: e-commerce, general methods of analysis, Herbal Mexican Pharmacopeia, herbal remedies, safety

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S2.P230 Questions for consumer reporting on risks and benefits from herbal medicine usage: A preliminary international Delphi study

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Information about rational uses of allopathic drugs exists in abundance, yet global primary healthcare continues to make use of herbal medicines (HMs) for which data are sparse. We began to address this issue by validating a questionnaire on consumer experiences.

Thirty-nine questions were constructed from existing pharmacovigilance (PV) reporting forms that could ask HM consumers about 5-domains: reporter information, consumer's health status, the HMs being consumed, perceived HM benefits, and adverse experiences. Experts in PV and HMs identified from the International Society of Pharmacovigilance (ISoP) and online resources were recruited into a 2-round Delphi study to score importance of questions on herbal medicine usage.

The 16 experts were recruited with ~20 yr experience in PV or HMs. Both rounds had 100% response rates. Twenty-one consensus questions from 39 questions were highly important. Most other questions were scored as moderately important. Experts made more than 400 comments about reasons for scores, use of the reported data, and recommendations for effectively reporting.

In conclusion, the international Delphi indicated important questions for consumers to report HM experiences. These needed formatting to help reporters to easily provide structured and reliable data and could be implemented in countries lacking HM reporting. The study demonstrated the value of small cohorts of experienced panelists who freely comment.

Keywords: herbal medicines, consumers, spontaneous reporting system, pharmacovigilance, Delphi technique

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S2.P231 Analysis of the chromatographic profile and *in vitro* antiurolytic activity of species of the genus *Phyllanthus* (Phyllanthaceae), endemic and non-endemic of the flora of Santa Catarina

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Phyllanthus spp. popularly known as "stone breaker," is used in folk medicine for kidney stones. However, the interchangeability between different species of the genus is described in reports of popular use, also stimulated by the difficulty of botanical differentiation, especially by laypeople. Therefore, the objective of this work aims to evaluate the chemical profile of *Phyllanthus* species and the antiurolytic activity in an in vitro model. For this, the aerial parts of *Phyllanthus* species were studied, including *P. niruri, P. tenellus, P. urinaria*, *P. hyssopifolioides*, and two recently described species endemic to the State: *P. eremitus,* and *P. timboensis*. The 70% ethanolic extracts were obtained by maceration from dried plant material. For subsequent analyses, the extracts were freeze- dried. The chromatographic profiles were obtained by High Performance Liquid Chromatography coupled to Diode Array (HPLC-DAD). The antiurolytic activity was carried out using the model described by Zanovello et al., 2021. The evaluation of the chromatographic profiles obtained showed different chemical compositions between the species. By comparing the profiles with standards (gallic acid, corilagin, luteolin and quercetin), it was possible to identify quercetin and luteolin in *P. tenellus*. Gallic acid was found in *P. niruri* and *P. tenellus*, corroborating with Brazilian Pharmacopeia (BF) Regarding the prediction of antiurolytic activity measured *in vitro*, the species *P. niruri, P. tenellus, P urinaria, P. hyssopifolioides* significantly decreased ($p<0.001$) the number of calcium oxalate crystals at concentrations of 0.1 and 1.0 mg/mL, respectively, relative to the negative control.

Keywords: *Stonebreaker,* antiurolytic activity, chromatographic profile

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S2.P232 Hot chips - is it a real ´challenge´?

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Capsaicinoids are biologically active secondary metabolites found in the placental tissue of chili peppers. These fruits are utilized globally in cuisine to impart a range of flavors and levels of "heat," with the character and intensity varying across different varieties. The most common capsaicinoids include capsaicin, dihydrocapsaicin and nordihydrocapsaicin. The Scoville Heat Scale (SHU) serves as a conventional method to evaluate the pungency of chili-pepper-based products, spanning from 0 to 16,000,000 SHU for pure capsaicin (Angelov T., et al.,2023) The increasing interest in spicy foods among the youth has motivated producers to incorporate highly pungent chili powders into their products. An example of this trend is the "Hot Chips Challenge", which led to severe health issues, including gastrointestinal pain and discomfort, after consuming just one chip. This incident led to alerts through the RASFF and the subsequent removal of this product from the EU market.

Employing a validated U-HPLC-HRMS/MS method, we analyzed the capsaicinoid profile in a selection of individual chips. The total capsaicinoid content was found to exceed 2000 mg/kg. In the absence of regulatory caps for capsaicinoid content in chili-based products, guidance documents from the BfR and EFSA are available for reference. Given the BfR's proposed NOAEL (No Observed Adverse Effect Level) for capsaicin at 0.083 mg/kg and LOAEL (Lowest Observed Adverse Effect Level) at 0.25 mg/kg of body weight, our findings indicate that the NOEL was surpassed by 20% for adults and 100% for young people.

Keywords: chilli peppers, capsaicinoids, health risk

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S2.P233 Botanical adventure in pharmacy education: implementing an escape game for quality control training

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Pharmacy education continuously seeks inventive methods to engage and educate students in response to the dynamic pharmaceutical landscape. Active and experiential learning approaches have emerged as effective strategies in this pursuit. This study introduces a novel application of escape game principles in pharmacy education, tailored specifically for third-year bachelor's students.

Our escape game intricately simulates quality control procedures in phytomedicines, with a focus on analyzing mint leaves and essential oils in accordance with European Pharmacopoeia guidelines. Quality control plays a pivotal role in pharmaceuticals, particularly those derived from botanical sources, necessitating a thorough understanding from students. Traditional teaching methods often lack immersive experiences in this specialized field.

Comparative analysis utilizing Gaussian curves demonstrates discernible differences in student performance between the years 2022 and 2023. The introduction of the escape game resulted in improved grades and reduced variability, indicating its positive impact on student learning outcomes. Importantly, consistent outcomes for a standardized task across both years, including unchanged practical assignments, underscore the game's influence on grades, rather than solely students' inherent skill enhancement. Our study underscores the potential of innovative pedagogical approaches in pharmacy education, suggesting further investigation into their long-term efficacy across diverse educational settings.

Keywords: escape game, pharmacognosy education, *Mentha piperita*

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S2.P234 Call for a contextual reform of the regulations on traditional herbal medicine in Burkina Faso

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Medicinal plants and traditional phytotherapy were legally recognised in Burkina Faso in 1994, under the Public Health Code, as being able to contribute to access to healthcare. Subsequently, a regulatory framework on the conditions for practising traditional medicine, traditional plant-based medicines and traditional health establishments was adopted with a view to promoting their integration into the health system. However, adherence to these regulations by traditional medicine practitioners has not been documented. The aim of this study was to assess the regulatory compliance of the practices of these traditional practitioners in relation to traditional herbal medicine and to formulate prospects for improvement. A mixed cross-sectional study using individual semi-directive interviews was conducted with traditional practitioners in four health regions of the country.

Sixty-seven (67) traditional healers, with an average age of 56 years, the majority of whom were men (71.6%), took part in the study. Herbalists (43.3%) and naturopaths (37.3%) were the main professional categories. The majority (82%) did not have legal authorisation to practise traditional medicine, and most herbalist establishments (92%) did not have a licence to open. The main reason for these regulatory non-compliances was that the regulation of traditional herbal medicine is very bureaucratic and orthodox, and is not adequate to the socio-cultural realities of traditional health practitioners. The results of this study therefore call for national reform of the regulations on traditional herbal medicine in Burkina Faso, with a view to facilitating its integration into the conventional health system.

Keywords: Traditional herbal medicine, Access to healthcare, Regulation, Burkina Faso

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S2.P235 The in vitro modulatory effects of *Artemisia sieberi* on phase-I cytochrome P-450 isoenzyme gene expression using human hepatoma cells (HepG2)

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The modulation of cytochrome P-450 (CYP) gene expression by natural products can lead to an unwanted clinical situation and drug adverse effect(s). We investigated if the consumption of *Artemisia sieberi* (*A. sieberi*) could modulate five CYP enzymes using Hep G2 cells. The plant was extracted twice as boiled water and water by sonication to mimic the traditional consumption methods. The endotoxin levels were determined in the aqueous extracts using the LAL assay. The modulation of CYP gene expression was determined using the RT q-PCR assay. The aqueous extracts were screened for Pregnane X Receptor (PXR) ligands using the competitive binding assay. The endotoxin levels in the plant extracts were determined to be below the maximum allowable endotoxin concentration and deemed suitable for Hep G2 cell culture. The IC_{50} values for the boiled and sonicated extracts of *A. sieberi* extracts were 110.6 and 191.9 µg/mL, respectively. Thus, an extract concentration of 16.5 µg/mL, and lower, were chosen for the subsequent gene expression experiments. The aqueous extracts were found to modulate the gene expression of two CYP isoenzymes significantly. The aqueous extract of boiled *A. sieberi* were able to induce the gene expression of CYP 1A2 and 3A4 for more than two-fold in comparison to untreated control. The receptor binding assay show that constituent(s) of *A. sieberi* boiled extract bind strongly to PXR. The study warns consumers from the concomitant consumption of boiled *A. sieberi* aqueous extract and narrow-therapeutic indexed pharmaceutical drugs that are mainly metabolized by CYP 1A2 and 3A4 enzymes.

Keywords: *Artemisia sieberi*, cytochrome P-450 enzyme induction, PXR, herb-drug Interaction

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S2.P236 Comparison of qNMR and HPLC-UV techniques for quantitation of secoiridoid phenolics in olive oil

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Extra virgin olive oil (EVOO), a principal component of the Mediterranean diet, is distinguished by its rich concentration in secoiridoid phenolic compounds with antioxidant properties. Despite its recognized association with cardiovascular disease prevention (Health claim EU 432/2012), the lack of a standardized analytical method in EU legislation for the measurement of the secoiridoid phenolics, creates a notable gap. The International Olive Council requires the application of an HPLC-UV method for phenolic compounds quantitation in *Olea europaea* L.

The aim of this study was the comparison of HPLC-UV (1) and qNMR (2) techniques for the quantitation of secoiridoid phenolics in EVOO. qNMR directly measures signals proportional to the number of atoms and molecules, while HPLC-UV indirectly correlates signals with the number of molecules using tyrosol equivalents or other response factors (1). Notably, discrepancies in quantitation results between the two techniques were observed. For instance, equimolar quantities of certain phenolic compounds, verified by NMR, yielded significantly different peak areas when measured by HPLC-UV due to different light absorption capacities for each compound (Fig. 1). For the first time we have clarified the quantitative correlation between NMR and HPLC signals for the EVOO phenolics related with the EU health claim regulation. Additionally, some EVOO secoiridoid phenolic compounds may undergo transformation or isomerization reactions during chromatography which may result in inaccurate assessment of their true content in olive oil when measured by HPLC.

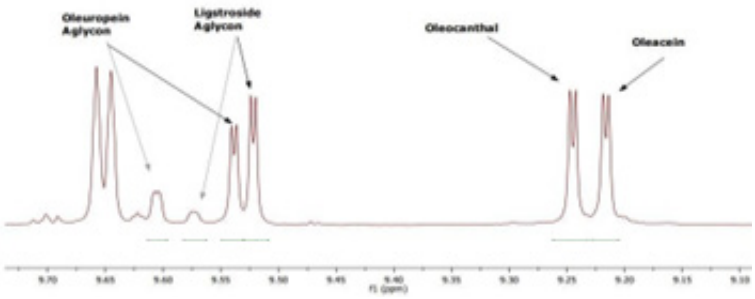

Fig. 1. Comparison of NMR and HPLC-UV techniques for quantifying secoiridoids in equimolar quantities in olive oil.

Keywords: secoiridoids, olive oil, liquid chromatography, nuclear magnetic resonance, UV-Vis spectrometry

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S2.P237 Anticancer efficacy of Korean medicine and sorafenib (concomitant drug) for hepatic tumors

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This study evaluated the therapeutic effects of a Dioscoreae rhizome and Polygalae radix mixture in combination with sorafenib (MIX) on a transplantation tumor model induced using hepatocellular carcinoma HepG2 cells.

The HepG2 cells transplantation tumor model has been established through HepG2 subcutaneously injection in Balb/c nude mice and the effect of MIX on the growth of the transplantation tumor was observed. Natural killer (NK) cell activity was measured using Granzyme B. Telomerase reverse transcriptase (TERT) and alpha-fetoprotein (AFP), known as prognostic markers of hepatocellular carcinoma, were analyzed by real-time PCR. Also, apoptosis and inflammation-related protein expressions were assayed by Western blotting.

Among the MIX groups, MIX25 (sorafenib 10 mg/kg + Dioscoreae rhizome and Polygalae radix mixture 25 mg/kg) showed surprisingly the best effect. MIX25 inhibited the tumor volume and weight. MIX25 enhanced apoptosis-promoting proteins such as Bax, cytochrome c, and caspase-3 and decreased apoptosis-inhibiting proteins such as survivin. In addition, MIX25 inhibited MAPK inflammatory response and inflammatory markers including TNF-α and COX-2. NK cell activity in the MIX25 group showed a significant difference compared with other drug-treated groups. Moreover, cancer marker genes including AFP and TERT in the MIX25 groups showed a better suppressive effect compared with MIX-treated groups.

MIX25 inhibited HepG2 cell transplantation tumor proliferation in Balb/c nude mice and could be suggested as a potential candidate for the treatment of patients with hepatocellular carcinoma.

Keywords: *Dioscoreae rhizome, Polygalae radix*, sorafenib, HepG2 cell, anticancer

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S2.P238 *Erythrina* spp. as natural anti-inflammatory ingredients for cosmeceuticals: An *in-silico* study

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Many inflammatory skin conditions depend upon activation of the nuclear factor NF-κB pathway which triggers the transcription of genes coding for pro-inflammatory mediators (Bell *et al.*, 2003). Considering the rising demand for natural cosmeceuticals, developing products containing anti- inflammatory botanical ingredients capable of modulating the NF-κB pathway is worth investigating. *Erythrina* spp. are used traditionally to treat skin ailments and alleviate inflammation. Their extracts and phytochemicals have demonstrated anti-inflammatory activity (Jiménez-Cabrera *et al.*, 2020; Khumalo *et al.*, 2021; Susilawti *et al.*, 2023). These anti-inflammatory phytochemicals include flavonoids, a class of compounds already known to target the NF-κB pathway (Kadioglu *et al.*, 2015). In this study, an *in-silico* molecular docking approach using AutoDock Vina was employed to predict the binding affinities and ligand efficiencies of 19 flavonoid derivatives, from *Erythrina* spp. with anti-inflammatory activity, against three key proteins of the NF-κB pathway. These included the NF- κB p50/p65 heterodimer, the IκB kinase subunit b (IKKb) and the NEMO/IKK association domain protein.

The isoflavonoids alpinumisoflavone (**1**), alpumisoflavone (**2**) and wighteone (**3**) showed the best ligand efficiencies towards the NF-κB p50/p65 complex, while the pterocarpans erybraedin A (**4**) and phaseollidin (**5**) showed the strongest ligand efficiencies towards IKKβ and NEMO/IKK, respectively. To the best of our knowledge, this is the first report on the interactions of *Erythrina* flavonoids with proteins of the NF-κB pathway.

Further *in-vitro* studies are warranted to validate the effects of these compounds on the NF-κB pathway and confirm the potential of *Erythrina* spp. as natural anti-inflammatory ingredients for cosmeceuticals.

Keywords: *Erythrina*, anti-inflammatory, NF-κB, *in-silico*, molecular docking

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Medicinal Plants and Natural Products in Animal Healthcare and Veterinary Medicine

S2.P239 Chemical composition of resin extract from *Corymbia spp.* an indigenous Australian native plant

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Corymbia spp. an indigenous Australian plant found across regions from northern Queensland to New South Wales, has attracted considerable attention due to its medicinal properties, particularly its essential oils (Schuster et al., 2018). However, there remains a dearth of research concerning the compounds derived from its resin. To bridge this knowledge gap, this study employed various techniques such as column chromatography, size exclusion, and multiple rounds of preparative high-performance liquid chromatography (HPLC) to isolate compounds from the resin. The isolated compounds were determined using mass spectroscopy and Nuclear Magnetic Resonance (Bruker 600 MHz AVANCE III NMR spectrometer). 1D NMR (1H and 13C NMR) and 2D NMR (COSY, HMBC, HSQC, NOESY) experiments were used in this identification (Ritmejerytė et al., 2022). The analysis revealed the presence of (E)-3-(4-hydroxyphenyl) prop-2-enoic acid **(C1)**, 3,5-dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-chroman-4-one **(C2)**, 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-chroman-4-one **(C3)**, 3,5-dihydroxy-2-(4-hydroxyphenyl)-6-[1-(4-hydroxyphenyl)ethyl]-7-methoxy-chroman-4-one **(C4)**. The physiochemical properties of these compounds agreed with Lipinski rule of 5 for small molecule therapeutics, making them promising candidates for the development of new drugs (Daina et al., 2017).

Keywords: *Corymbia spp.,* HPLC, NMR, physicochemical properties

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S2.P240 *Melissa officinalis* (Lemon balm) extract to promote calming effect before stressful events

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Behavioral disorders, due to stress, are common during veterinary consultation for dogs. Even if plant-based solutions are commonly used to calm dogs, data supporting their efficacy are scarce. Melissa officinalis is not an exception. This study aims to highlight its calming effect on dogs.

Fifteen dogs with behavioral disorders linked to stress were divided into 3 groups:

- Control group (CTL) fed with standard diet and supplemented with placebo (dextrose, 5 mg/kg/day)
- Lemon Balm (LB) group fed with standard diet and supplemented with *Melissa officinalis* extract (5 mg/kg/day)
- Positive control group (PC) fed with standard diet and treated with α cazosepin (15 mg/kg/day).

In collaboration with a local veterinary clinic, 15 dogs were recruited and randomly allocated to the 3 treatment groups. The treatment duration was 3 days. Physiological parameters (heart rate, respiratory rate, and body temperature) and behavioral parameters (body signs and special behavior) were monitored before and after the treatment.

After 3 days of treatment, LB dogs significantly reduced the intensity of the expression of their behavioral signs of stress between the two consultations compared to CTL dogs. The observed effect was at least similar to PC dogs. However, there is no significant differences between the 3 groups in terms of physiological parameters.

This study demonstrates the calming effect of *Melissa officinalis* before stressful events. However, the sample size was quite small. It may be worthwhile to extend this study by replicating it on a larger number of dogs to confirm the initial results obtained.

Keywords: *Melissa officinalis*, stress, behavior, pet

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S2.P241 Equine Stromal Cells: effects of non-ribosomal peptides on proliferation and viability

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Equine stem cells (ESC) are used extensively in treatment of osteoarthritis and tendon and ligament injuries in horses. Two of the most common cell types used in treatments are bone marrow derived mesenchymal cells (BMSC) and articular chondroprogenitor cells (ACP) (McCarthy et al., 2012). Non-ribosomal peptides (NRP) have also been used throughout the medical field in drug development and antibiotic use (Felnagle et al., 2008). Virginiamycin is a pure NRP that was specifically fed to cattle to increase growth and milk production (McEwan and Fedorka-Cray 2002). The objective for this project is to compare doses of both non-pure and pure NRP's on the proliferation and viability of ESC's when growing in culture. The hypothesis is that proliferation and viability of ESC's will be increased with the addition of NRP's.

A non-pure crude NRP and Virginiamycin were added to the ESC's at doses 5ug, 10ug, 20ug, and 40ug for either 3 or 6 days. ESC's were then harvested and counted using a hemocytometer. A live/dead stain measured by flow cytometry was performed for viability. The flow cytometry revealed all cells maintained good viability. Proliferation results showed significant increases for doses of 10ug and 20ug Virginiamycin on day 3 ACP's. In the BMSC's, all dose amounts of Virginiamycin increased proliferation at day 3 but only the 5ug dose continued to increase proliferation by day 6. Based on these results, Virginiamycin can be added to culture to increase proliferation of ACP's and BMSC's. This can be beneficial when high cell counts are needed.

Keywords: Non-ribosomal peptides, stem cells, equine, *Virginiamycin*

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S2.P242 Extracts from the roots of *Scutellaria baicalensis* reduce honeybee death caused by nosemosis

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Honeybees are very important pollinators and they role in the ecosystems can not be overestimated. *Apis mellifera* L. workers pollinate approximately 80% of flowering plants and only in the United States they effort is estimated up to $15 billion worth of crops annually. Moreover, they produce honey, wax, pollen, royal jelly, and propolis, products eagerly used by many people. Unfortunately, honeybees suffer from pesticides, immunodeficiencies, beekeeping practice in which antibiotics are used, malnutrition, and disease. These factors can drastically reduce honeybee lifespan (Ptaszyńska and Załuski, 2020).

The aim of the study was to check if *Scutellariae baicalensis radix* extract can prolong honeybee lifespan especially after infected with pathogenic fungi (*Nosema* spp.). The 75% ethanolic extracts were prepared from the freshly collected roots after their lyophilization using classical maceration (CM) and ultrasound-assisted extraction (UAE).

After 20 days of administration of extracts (0,01; 0,1; 1,0; 10%) to non-infected honeybees, no toxic changes were observed, it did not shorten the honeybee lifespan.

After treatment of *Nosema*-infected honeybees with the extracts, the number of *Nosema* spores decreased to 2,05 x 10^6 and 4,20 x 10^6 for CM and UAE comparing to a control group 15,20 x 10^6. The 10% extracts appeared to reduce spores the strongest.

To sum up, the extracts decreased the level of honeybee nosemosis in infected insects, as well as when used as prophylaxis. All these state-of-the-art research findings, from *in vitro* to nature, should be taken into account when drafting protocols for prospective honeybee medicament tests.

Keywords: *Scutellaria baicalensis,* honeybees, *Nosema*

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S2.P243 Larch (*Larix decidua* L.) fibre waste: characterization of a functional feed ingredient

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In the larch woodworking industry, wood shavings or sawdust is a fibre waste product. In previous studies, larch fibre waste (*Larix decidua* L.) was evaluated for health-beneficial compounds, flavonols (taxifolin, TXF; dihydrokaempferol, DHK), and terpenoides (larixol, LX; larixyl acetate, LXA) and the antioxidant and anti-inflammatory potential effects (Pferschy-Wenzig et al., 2008; Stockhammer et al., 2009) also enhancing its use in animal nutrition (Tedesco et al., 2015; Tzika et al., 2017).

This study aimed to evaluate and compare the bioactive compounds and antioxidant potential of larch fiber waste (native to the mountains of central Europe) obtained from five woodworking companies, to verify possible differences in their composition. The hexane, methanol and water extracts were fractionated by UPLC-PDA instrument, to quantify the presence of TXF, DHK, LX, and LXA. ABTS assay was used to determine the antioxidant activity (EC_{50} mg/mL).

In the hexane extract, LX and LXA were quantified in the range 0.80-15.7 % w/w, and 8.70-14.50 % w/w respectively, while in the methanol extract, TXF and DHK were present in the range 16.20-23.20 % w/w and 6.40-24.00 % w/w respectively. The mean antioxidant activity of the methanol extracts was EC_{50} = 0.002 mg/mL, and EC_{50} = 0.012 mg/mL of the water extract.

Although there were differences in bioactive compound content, it can be concluded that larch fibre waste from woodworking companies requires further attention as a functional feed ingredient in supporting metabolism and animal health.

Keywords: wood waste, biomass valorization, sustainable feed

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S2.P244 Identification of novel caffeoyl- and feruloyl-derivatives in *Solanum glaucophyllum*

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Solanum glaucophyllum Desf. (SG) is one of the few plants that produce the source of the bioactive form of vitamin D as $1,25(OH)_2D_3$. However, plants are always a multi-component system with synergistic effects, and it is difficult to correlate efficacy data with a unique component. The aim of this study was to expand the knowledge of phenolic compounds in SG, which have mainly been reported with a focus on quercetin derivatives (Rappaportt et al., 1977). For the first time, several chlorogenic acids were detected in leaf extracts of SG. Specifically, 3-*O*-caffeoylquinic acid (chlorogenic acid) and 5-*O*-caffeoylquinic acid (neochlorogenic acid) were unequivocally verified via reference material, collision-induced dissociation (CID) experiments and high-resolution mass spectrometry (HR-MS).

The workup included extraction with acetone-water, size exclusion and reversed phased chromatography leading to further caffeoyl- and feruloyl-derivatives. LC-MS/MS experiments indicated conjugations to glucose and glucaric acid. Interestingly, fragmentation of the molecular ions led to the very same patterns at different retention times suggesting several isomers, e.g. those known for tomatoes (Larbat et al., 2014). Independent organic syntheses performed for various isomers of caffeoyl glucoside, feruloyl glucoside, caffeoyl glucaric acid and feruloyl glucaric acid esters allowed verification of these substances in SG for the first time. All substances were fully verified by NMR, CID and HR-MS experiments. Compared to current literature the phenolic spectrum of SG was significantly increased by the work reported here.

Keywords: *Solanum glaucophyllum*, polyphenols, caffeoyl derivatives, feruloyl derivatives

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S2.P245 Phytochemical screening and study of the hypotensive effect of the aqueous extract of *Rubus ulmifolius* Schott leaves

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Hypertension (hypertension) is a cardiovascular pathology characterized by systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg (Yousefi et al., 2021). Several antihypertensive agents have many side effects, hence herbal medicine as an alternative (Landazuri et al., 2017). *Rubus ulmifolius* Schott (*Rosaceae*) is a thorny shrub, used in traditional medicine for the treatment of several pathologies such as hypertension (Ali et al., 2017; Fakchich and Elachouri, 2021).

The objective of this study is to investigate its hypotensive effect and analyze its chemical composition. The study of the hypotensive effect of the aqueous extract of *R. ulmifolius* leaves (AERu), administered intravenously, was conducted on anesthetized Wistar rats maintained at a temperature of 37°C. Compound separation from AERu was conducted using ultra-high performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). The results showed that the intravenous administration of the aqueous extract in anesthetized normotensive rats induced a dose-dependent hypotensive effect, with a reduction in systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) by 47.77%, 51.98%, and 48.73%, respectively, at a dose of 50 mg/kg of body weight. Furthermore, the extract showed no effect on heart rate. Phytochemical analysis revealed the presence of chlorogenic acid, quercetin-3-O-β-D-glucuronoside, and kaempferol-3-O-β-D-glucuronoside.

In conclusion, our results demonstrated that the aqueous extract of *R. ulmifolius* leaves has a significant hypotensive effect. The richness of this plant in bioactive phytochemical compounds may be responsible for this effect.

Keywords: *Rubus ulmifolius*, hypertension, hypotensive, polyphenols, tannins

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S2.P246 Evaluating the antibacterial properties of macroalgae against swine pathogens

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The EU swine industry is the second largest in the world, while also being the largest exporter of pork and pork products (€13.8bn in 2022). However, the recent banning of zinc oxide as an antibacterial agent in pig production may have a knock-on effect in the sustainment and future growth of this industry. Although the supplementation of animal feed with seaweed is common for increasing key performance indicators (i.e. Feed intake, weight gain and prebiotics), less is known about their efficacy as antibacterial agents. In this study, the antibacterial properties of extracts, derived from thirteen macroalgae species, were investigated against five priority swine pathogenic bacteria, using a broth microdilution assay. Of the 65 extracts tested, 38 were shown to have widespread activity at minimum inhibitory concentrations (MICs) ≤ 1 mg/ml. Overall, extracts from Rhodophyta and Phaeophyceae genera macroalgae demonstrated greater activity (1000 – 31.25 µg/mL) compared to the Chlorophyta genus macroalgae, respectively. In particular, non-polar extracts of *Alaria esculenta* (Phaeophyceae), *Asparagopsis armata* & *Asparagopsis taxiformis* (Rhodophyta) displayed the strongest antibacterial activity against the Gram-positive *S. aureus* (62.5 – 31.25 µg/mL). While the dichloromethane (DCM) extracts of *A. esculenta* & *A. taxiformis* displayed the strongest antibacterial activity against the Gram-negative *S. enterica* subsp & *E. coli* (31.25 µg/mL). The results of this study highlight the antibacterial potential of macroalgae, which may further support its future application as an antibacterial agent in supplemented animal feed.

Keywords: Macroalgae, swine, antibacterial, supplemented animal feed

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S2.P247 A survey-based study on knowledge, attitude, and perception of pet owners towards the use of plant extracts from bio-refinery in pet food formulations

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Plant biomass extracts contain bioactive compounds that can be used in pet food, and therefore promote production cycle sustainability of biorefineries. Therefore, the use of plant extracts in pet food formulation can improve pet's gut-health while contributing to environmental sustainability (Buff et al., 2014). Understanding pet owners' thoughts on pet food quality is critical to knowing how much interest they demonstrate towards more sustainable solutions (Goswami et al., 2022). This study used a web based cross-sectional survey to evaluate Italian pet owners' knowledge, attitudes, and perceptions of innovative pet food containing plant based functional feed ingredients. Preliminary findings based on 169 responses revealed a predominantly female respondent base (63.3%), aged–51-64 years (38%), mainly from the northwest region of Italy (80%), and primarily employed (47%). Most respondents owned one pet (48.2%), predominantly dogs (50.6%), and gastrointestinal issues were the most reported (39.2%) health disorders. The majority sought advice from veterinarians (80.1%) and purchased pet food from specialized shops (65.1%). Notably, a significant proportion expressed unfamiliarity with natural extracts in pet food (61.4%) but showed interest in extracts, particularly from pomegranate (33.7%) and olive leaves (22.3%), to enhance gastrointestinal health (72.9%). A noteworthy percentage (42.2%) expressed willingness to pay a premium of 5-10% for pet food containing plant extracts.

This study highlights the growing interest among Italian pet owners in the addition of natural plant extracts to pet foods. Despite limited knowledge amongst consumers, there is a positive trend toward using plant extracts, particularly pomegranate and olive leaves, to improve gut-health.

Keywords: natural by-products, pet owners, questionnaire, animal-health

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S2.P248 Anti-inflammatory effects of Cinnamon essential oil assessed on diverse cell models

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The One Health approach advocates cutting antibiotic use in livestock feed to boost human and animal health. Phytochemicals, with anti-inflammatory properties, offer valuable health benefits as natural alternatives. Cinnamaldehyde is a compound naturally found in Cinnamon (*Cinnamomum cassia*) with various therapeutic properties (Da Silveira et al., 2014). In the context of designing functional feed in antibiotic-free diets for animals, it is mandatory to evaluate its effects on livestock models, thereby reducing *in vivo* studies.This study aimed to assess the efficacy of a Cinnamon essential oil (Cinna; 5 to 100 µg/mL) in modulating inflammation *in vitro* across three cell models. Chicken macrophage-like HD11 and porcine enterocyte IPEC-J2 cells were used to study cytokine gene expression in livestock cell models. Mechanisms underlying such effects in enterocytes were further investigated with a human reference cell line (Caco-2), by cell imagery. Cinna alleviated inflammation by significantly reducing IL-1β and IL-8 gene expressions in HD11 cells (50 and 100 µg/mL, $p<0.01$), and IL-6, IL-8, TNF-α, CCL20 gene expressions in IPEC-J2 cells (-2 to -30-fold depending on cytokines and doses; $p<0.05$), compared to pro-inflammatory group (Fig. 1). In the Caco-2 cells, Cinna (50 and 100 µg/mL) alleviated inflammation by significantly reducing the % of NF-κB positive nuclei (-70%; $p<0.01$).Using cellular models, these results demonstrate Cinna's anti-inflammatory effects by regulating pro-inflammatory cytokines in livestock models. This regulation involves the NF-κB pathway, as confirmed in Caco-2 cells. Cinna shows promise as a candidate for developing functional feed for livestock. The authors declare no conflicts of interest.

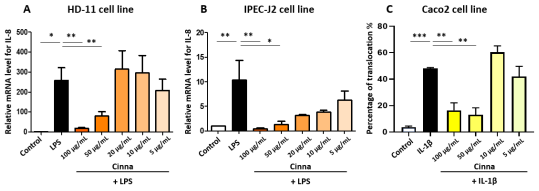

Fig. 1. Effect of Cinnamon essential oil on induced inflammation in 3 different cell models. HD-11 cells (A) or IPEC-J2 cells (B) were pre-treated with 5 to 100 µg/mL of Cinnamon essential oil (Cinna) for 2h (HD11) or 1h (IPEC-J2) and then co-incubated with LPS (10 ng/mL) for 2 or 1h respectively (n=3). IL-8 gene expression (A and B) was measured by real-time qPCR. Caco2 cells (C) were pre-treated with 5 to 100 µg/mL of Cinna for 1h and then co-incubated with IL-1β for 1h. Inflammation was assessed using fluorescent NF-κB immunocytochemical labeling (C).

Keywords: Cinnamon essential oil, HD11, IPEC-J2, cytokines, NF-κB

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S2.P249 Induction of apoptosis in pancreatic cancer cells by *Albizia julibrissin* extract through inhibition of an oncogenic signaling pathway

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Pancreatic cancer is one of the most fatal solid tumors due to poor prognosis. *Albizia julibrissin* exhibits anti-inflammatory and antioxidant against a variety of diseases. However, little is known about its anti-cancer effects in human pancreatic cancer cells. In the present study, we aimed to investigate the anti-cancer potential of *Albizia julibrissin* extract against human pancreatic cancer cells and define its molecular mechanism of action. Anti-cancer effects of *Albizia julibrissin* extract were determined by WST-1 assay, colony formation assay, western blot analysis, Annexin V/PI staining, and animal experiments using xenograft mouse model. *Albizia julibrissin* extract significantly suppressed the growth and colony formation of pancreatic cancer cells. *Albizia julibrissin* extract also induced apoptosis in pancreatic cancer cells by enhancing the expression levels of cleaved PARP and cleaved caspase-3 as well as inhibiting oncogenic signaling such as MAPK and PI3K/AKT pathway. In addition, *Albizia julibrissin* extract suppressed tumor growth and induced apoptosis in xenograft mouse model used human pancreatic cancer cells. Our findings provide crucial evidence that *Albizia julibrissin* extract may have potential as a therapeutic drug for pancreatic cancer.

Keywords: natural product, *Albizia julibrissin*, npoptosis, pancreatic cancer

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S2.P250 Antibacterial, antibiofilm and quorum sensing inhibitory activities of fractions of the ethanol extract of *Searsia lancea* against pathogens causing bovine mastitis

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Bovine mastitis is a significant problem in the dairy industry exacerbated by antibiotic-resistant pathogens able to form protective biofilms via quorum sensing. This study aimed to determine antibacterial, antibiofilm and anti-quorum sensing activities, and *in vitro* safety, of fractions of the ethanol extract of *Searsia lancea* against isolates of major multidrug resistant bacteria of mastitis origin. Antibacterial, antibiofilm and anti-quorum sensing activities of the fractions were determined against pathogenic bacteria isolated from clinical cases of mastitis. Cytotoxicity was determined against bovine dermis cells. A Waters UPLC-QTOF-MS (XEVO-G2) instrument was used to tentatively identify compounds in the most active fractions.

DCM and ethyl acetate fractions had MIC values of 0.001 and 0.01 mg/mL respectively, better than that of the antibiotic ciprofloxacin. All fractions were relatively non-cytotoxic. The DCM fraction had excellent selectivity index values up to 90, but the ethyl acetate fraction was least toxic. The ethyl acetate fraction had the best antibiofilm and preformed biofilm disrupting activities. Notably, the DCM and water fractions demonstrated the lowest minimum quorum sensing inhibitory concentration (MQSIC), with DCM $MQSIC_{50}$ = 0.01 mg/mL, suggesting that fractions have potential to modulate virulence factors. Compounds including myricetin 3-O-glucoside were identified in active fractions.

These findings underscore the potential of fractions of *S. lancea* extract as valuable resources in combating antimicrobial resistant mastitis pathogens. Further investigation of their mechanisms, identification of active compounds, and thorough *in vivo* assessments are essential steps toward their development as effective preventative and treatment options for microbial infections such as bovine mastitis.

Keywords: bovine mastitis, antibiotic resistance, quorum sensing, antibiofilm, *Searsia lancea*

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S2.P251 Potential use of *Paulownia* leaves as feed additive to enhance animal health condition

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Paulownia is used as a medicinal plant in traditional Chinese medicine against numerous diseases (Dzugan et al. 2021). Previous studies have reported that the use of Paulownia leaves (PL) is also suitable for feeding domestic animals and has similar nutritional values to alfalfa (Al-Sagheer et al 2019). PL are reported to be slightly bitter but palatable to sheep and cattle, can be used in feed for pigs without adverse effects, and are processed into pellets for fish and chicken feed containing up to 20% PL (Al-Sagheer et al 2019) The use of up to 15% PL meal in the diet of rabbits had no negative effects on performance, nutrient digestibility, and blood components, in addition, a remarkable reduction in pathogenic bacteria was observed in both the caecum and the feed itself (Al-Sagheer et al 2019). In feeding trial in sheep, the number of leukocytes and erythrocytes was significantly reduced and the blood glucose level decreased significantly (Vaslyakov et al. 2013). Studies with broilers showed that Paulownia polysaccharides can improve the immune system by enhancing cellular and humoral immunity (Wang et al. 2019). Enriched broiler feeds with 0.5 g PL/kg diet is the most acceptable dosage for supplementation in broilers to optimize performance, improve oxidative remarks activity, and enhance immunity without compromising productivity (Sakr, et al. 2022). However, further studies are needed to provide insights into the role of PL in alleviating oxidative stress, modulating intestinal microbial biodiversity, and mediating metabolic activities associated with gut function activity in animals.

Keywords: *Paulownia* leaves, animal feeding, health condition

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S2.P252 Exploring ethnoveterinary medicine in dogs in Ireland, Norway and Poland

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The study aimed to investigate the use of ethnoveterinary medicine by dogs' owners. It has been studied in various regions worldwide (Mayer et al., 2017), but ethnoveterinary medicine's application in Northern and Central-Eastern Europe remains relatively understudied (Disler et al., 2014). A survey was conducted in in late 2023 and early 2024, in three countries: Ireland, Poland and Norway. A questionnaire about the use of medicinal plants (MP) and their preparations in pets, was distributed to dog owners who visited veterinary clinics. The results were not surprising; out of 102 responders less than 30% used medicinal plants as therapy in their dogs but in those that had used these therapies, there was a report of more than 85% in effectiveness. According to the survey, the owners learn about the use of medicinal plants mostly from internet and in case of Poland also from the veterinarians who take care of their pets. In total the owners indicated the use of 17, 5 and 8 different herbs in Poland, Ireland and Norway, respectively. Linseed, valerian and chamomile were the most frequently mentioned plants, whereas digestive issues, skin problems and anxiety were the most common circumstances of MP application. In general, the owners indicated interest but lack of knowledge about how to use herbal medicines. The survey indicates high need of promotion of veterinary herbal medicine among animal owners and urgent need of education of present and future veterinarians in phytotherapy.

Keywords: ethnoveterinary medicine, dogs, medicinal plants

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S2.P253 Polish perspective on phytotherapeutic approaches to pet anxiety: an analysis of popular preparations

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Herbal medicines and supplements are gaining growing popularity among veterinarians and animal owners in Poland. They are thought to be effective, safe and environmentally friendly, with causes their high social acceptance. The common belief, especially among animal owners, is that remedies of natural origin have mild calming effect without causing serious sedation. This leads to many cases of self-introduced therapy of pets, as herbal supplements are also easily and widely accessible. There are several dozen preparations of this type available on the market in Poland.

This work aims to compare popular calming preparations for dogs and cats available on the market in Poland and their composition in terms of the substances of plant origin used and its concentration (as stated by the producer). They are available in different formulations: oral preparations, diffusers, sprays, calming collars, or rectal suppositories. We decided to narrow our list only to various oral preparations: tablets, twist off capsules, oils and suspensions. We analyzed 50 products, some of which were medicinal products, others complementary food or dietary supplements. All of them are available in pet stores and online without a prescription.

The compared products differed considerably in terms of composition and available information on the content of individual additives/substances. Most of them were multi-component. The most commonly used extracts were: *Valeriana officinalis*, *Melissa officinalis*, *Passiflora incarnata*, *Camellia sinensis* and *Chamomilla recutita*. The popularity of CBD oils is also noteworthy: among the 50 products analyzed, there were as many as 16 oils from various manufacturers.

Keywords: calming preparations, pet anxiety, herbal extracts, CBD oil

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S2.P254 Anti-tumor effects of *Auricularia polytricha* extracts on human breast cancer cell lines

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Auricularia polytricha (AP), a cloud ear mushroom, has been reported for several medicinal properties such as anti-inflammation (Xiang et al., 2021), anti-oxidation (Chiu et al., 2014), and immunomodulation (Yu et al., 2009) as well as anti-human lung cancer activity (Yu et al., 2014). In this study, anti-tumor activity of AP was demonstrated *in vitro* against human breast cancer cell lines. APH, APE and APW were extracted by sequential maceration with hexane, ethanol, and water, respectively. All extracts were investigated for their toxicity against human breast cancer cells (MDA-MB-231 and MCF-7). The cytotoxicity of APH on MDA-MB-231 and MCF-7 after 24 h of treatment showed IC_{50} values at 24.31 ± 0.80 and 13.13 ± 1.60 mg/mL, respectively. Moreover, the biological results showed that the APH inhibited migration, invasion, and colony formation and induced apoptosis. In addition, the pro-apoptotic genes (*CASP3*, *CASP8* and *CASP9*) were upregulated by APH treatment on breast cancer cells. In conclusions, APH displayed the anti-tumor effect on breast cancer cells by apoptosis induction. These results will lead to the future development of drug discovery of AP.

Keywords: *Auricularia polytricha*, anti-tumor activity, apoptosis, colony formation

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S2.P255 Analysis of veterinary historical German-language textbooks regarding medicinal plants used for the treatment of gynecological diseases and fertility disorders

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Diseases of the female genital tract cause antibiotic and hormonal treatments as well as high culling rates in cattle. We analyzed five books published in Germany and Switzerland between 1878 and 1921, and handwritten notes of the veterinarian Ammann-Honegger (1879–1960) (Steiner et al., 2022). We systematically examined the sources regarding use reports (UR) based on medicinal plants to treat gynecological diseases. Herbal ingredients, target animal species, type of administration and indication were detailed documented for each UR. The sources contained 103 UR (79 administered orally (o), 13 locally (l), and 11 both o and l). Fifty-five different plant species were recorded. The most frequently mentioned medicinal plants were *Juniperus communis* L. (19 UR, l and o), *Linum usitatissimum* L. (18 UR, o), *Juniperus sabina* L. (13 UR, l and o), *Matricaria chamomilla* L. (13 UR, l and o) and *Gentiana lutea* L. (12 UR, o). *Retentio secundinarum* was most frequently mentioned as indication (44 UR), followed by parturition preparation (17 UR) and endometritis (15 UR). Based on their ingredients, the recorded plant species could be divided into (a) high-quality fodder, (b) appetizer and digestives, (c) specific gynecologicals and (d), predominantly toxic plants. Active defense against bacterial infections as well as a stable barrier of the endometrium contribute both to uterine health (Sheldon et al., 2019). The plants listed under a)-c) could at least indirectly support these functions.

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S2.P256 Phyto-synergy: a holistic approach to tackle *paratuberculosis*

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The presented work will provide a comprehensive overview of our research methodology, key findings associated with the development of a potential herbal therapy against *Mycobacterium avium* subspecies *paratuberculosis* (MAP). MAP is considered to be the causative agent of Johne's disease, an intestinal chronic infection in ruminants (Feller et al., 2007). These bacilli upon transmission through milk and milk products lead to development of Crohn's disease like autoimmune disorders in human beings (Naser et al., 2004). Till date, no reliable treatments are available for this pathogenic infection.

The conducted research focuses on the development of a novel herb-based synergistic combination as a potential anti-MAP therapy. Our study involved screening a diverse range of medicinal herbs for their anti-MAP potential using *in vitro* Resazurin microtitre assay (REMA). The obtained best active extracts (BAEs) i.e. hydroalcoholic extracts of *O. sanctum* and *S. xanthocarpum* were further assessed for synergy using fractional inhibitory concentration index (FICI). Subsequently, a synergistic combination was formulated to enhance the overall efficacy through the potentiation of individual herb properties. The results demonstrate significant antimycobacterial activity of the herb-based synergistic combination against MAP, with no significant cytotoxic effects on host cells. Moreover, the combination exhibited promising immunomodulatory properties, suggesting a dual mechanism of action for enhanced efficacy.

Keywords: *Mycobacterium avium* subspecies *paratuberculosis*, Johne's disease, Crohn's disease, REMA assay

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S2.P257 Preliminary study of the neuroprotective capacity of *Corema album* (L.) D.Don ex Steud (Ericaceae) juice and its butanolic fraction

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Corema album (L.) D.Don ex Steud (Ericaceae) berries are an edible fruit which have traditionally been consumed in those geographical areas where the plant is endemic (Iberian Peninsula). In the present study, the ability of this fruits juice and its butanolic extract to decrease ROS levels in SHSY-5Y cell line culture was analysed. Both its direct effect on this cell line, and its preventive effect against H_2O_2-induced oxidative stress were assessed (León-González *et al.*, 2012). On the other hand, SH-SY5Y cells were differentiated into dopaminergic (Retinoic acid) and cholinergic (BDNF), to replicate an *in vitro* model of both Parkinson's and Alzheimer's disease respectively (de Medeiros *et al.*, 2019). Dopaminergic differentiated SHSY-5Y were stressed with 6-hydroxydopamine which induce a massive oxidative stress in this cell line (Lopes *et al.*, 2017) in the presence or absence of *C. album*. On the other hand, for mimicking tau and amyloid-β pathology associated with Alzheimer´s disease, BDNF-differentiated cells were treated with okadaic acid, with or without the juice and the extract of the fruits. The plant capacity to increase cell viability under these toxic conditions was studied. Finally, the inhibitory effect of juice and butanolic juice extract on different enzymes related to neuroprotection was also investigated. The results show an interesting neuroprotective capacity of *C. album* by being able to reduce induced oxidative stress, to inhibit MAO-B *in vitro*, and to increase the survival of differentiated cholinergic or dopaminergic cells under toxicity conditions. UHPLC–MS/MS analysis of the juice and the extract was performed.

Keywords: *Corema album,* SHSY-5Y, neuroprotection, MAO, antioxidant

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S2.P258 Anti-inflammatory and antioxidant ability of *Vitis vinifera* L. leaves

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Vitis vinifera L. is a species belonging to the Vitaceae family, which fruit has been extensively studied due to its use since ancient times. Grapes and their seeds are considered source of pharmacological and cosmetic products. However, other parts of the plant such as the leaves, have been less studied, despite having been used for different therapeutic purposes (Mansour *et a*l. 2013). Currently, the European Medicines Agency accepts the well-established use of a dried aqueous grapevine leaf extract for the treatment of chronic venous insufficiencies symptoms. It is interesting to deepen the study of this part of the plant to take advantage of the large quantities of them that are generated as agricultural waste (Maia *et al.*, 2018). In the present study, the anti-inflammatory ability of a methanolic extract of the *V. vinifera* leaves has been analysed. Radical scavenging ability *in vitro* and in cell-based assays was determined. The direct effect and the protective effect of the extract after induced oxidative stress were assessed in HepG2 cell line. On the other hand, its anti-inflammatory potential was evaluated through the impact upon NO levels produced by RAW 264.7 stimulated with *E. coli* lipopolysaccharides. In addition, different concentrations of the extract were approached on its effect upon lipoxygenase activity *in vitro*. The phytochemical profile of the extract was analyzed using UHLC-QTOF-MS. Results showed an interesting antioxidant and anti-inflammatory ability of the leaves, pointing out this part of the grapevine plant as a valuable resource for phytochemical metabolites of pharmacological and nutritional value.

Keywords: Vitis vinifera, antioxidant capacity, anti-inflammatory, phenolic content

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S2.P259 Pre- and post- treatment effect of water-soluble phenolics from date palm (*Phoenix dactylifera* L.) fruits in cisplatin-induced hepatotoxicity in rats

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This presentation will cover the pre- and post-treatment for the effects of water-soluble phenolics from date (*Phoenix dactylifera L)* fruits (DFP) on cisplatin-induced hepatotoxicity in rats. Natural products have been proposed to be better agents in the suppression of chemotherapy-induced toxicities due to their inherent bioactive values and lack of toxicity. (Asma et al., 2022; Dziadek et al., 2019; Zhang et al., 2018).

As part of continuing search for novel natural products with potent bioactive principles capable of suppressing toxicities associated with cancer therapy, we employed the pre- and post-treatment strategies with DFP on cisplatin-induced rats. Treatment of rats with DFP before or after exposure to a single therapeutic dose of cisplatin (5mg/kg) led to enhanced antioxidant status, in addition to significant ($p < 0.05$) suppression of lipid peroxidation in cisplatin-induced rats. Furthermore, a concomitant reduction in activities and levels of liver function markers was observed after treatment with DFP. Photomicrographs from hematoxylin/eosin stained liver tissues revealed the reversal of cisplatin-induced pathology at both time points. Chromatographic characterization of DFP revealed peaks of phenolics that have been widely reported for medicinal values (Fig. 1). DFP at both time points suppressed cisplatin-induced hepatotoxicity in this study, though, pre-treatment strategy showed greater significance than post-treatment strategy. Hence, pre-treatment with DFP may provide protection from the hepatic damage induced as a result of cisplatin-based chemotherapy.

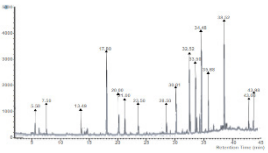

Fig. 1: GC-MS chromatogram of Date Fruit phenolics (DFP)

Keywords: hepatotoxicity, cisplatin, DFP, liver markers, antioxidant status

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S2.P260 Determination of anticancer properties of *Moringa oleifera* seed bioactive fraction and the interaction effect of its combination with the classical cytotoxic drugs

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This presentation will cover the determination of anticancer properties of *Moringa oleifera* seed bioactive fraction (MSBF) and the interaction effect of its combination with 5-Fluoro-uracil, Cisplatin or CX-4945 (CK2-inhibitor) in breast and colon cancer cell lines. Moringa oleifera is known for its diverse range of biological activities including anticancer. Recently, drug combination strategy has been recommended to defeat the associated challenges with chemotherapy and combination of natural products with conventional anticancer drugs has been supported by several studies (Naeem et al., 2022; Gilad et al., 2021; Talib et al., 2021)

As part of continuing search for novel natural products as drug combination candidate in cancer therapy, we investigated the interaction effects of MSBF with 5-fluorouracil, Cisplatin or CX-4945 (a CK2-inhibitor) in breast cancer (MDA-MB-231 (triple-negative breast cancer (TNBC)) and MCF-7 (estrogen receptor-positive (ER+)) and colon cancer (HCT-116 and HT-29) cell lines. MSBF was obtained through defatting, depigmentation and ethanol precipitation. The results reveal the synergistic interaction between MSBF with 5-FU, or Cisplatin, while antagonism was observed between MSBF with CX-4945 in both cancer cells lines. However, treatment with MSBF/5-FU combination potentiated the highest significant ($P < 0.05$) DRI (103.7 ± 5.63) for 5-FU at Fa of 0.96 with CI, 0.72±0.01 in MDA-MB-231 (Fig.1). Assessment of the effect of MSBF /5-FU treatment on pro-apoptotic properties and cell cycle arrest on MDA-MB-231 cell lines is ongoing to understand the mechanism behind this interaction. Hopefully, treatment with MSBF/5-FU combination may cause dose reduction and consequently, reduction in toxicity of 5-fluorouracil, thereby, improving chemotherapy.

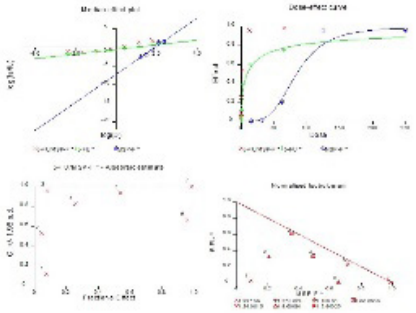

Fig 1. Synergistic interaction between MSBF and (5-FU) in MDA-MB-231 cell lines

Keywords: Chemotherapy, *Moringa oleifera* seed, 5-fluorouracil, combination index, synergism

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S2.P261 Fertility parameters of dairy cows after intrauterine treatment of clinical endometritis with an antibiotic or an herbal veterinary medicinal product

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Reproductive disorders represent challenging problems for dairy cattle. We compared fertility parameters of cows after the intrauterine treatment of clinical endometritis (CE) with cephapirin (Metricure®; cefapirin benzathin 500 mg per dosis; CEPH) or an herbal product (25 ml of EucaComp®PlantaVet containing alcoholic extracts of *Calendula officinalis* L., *Mellissa officinalis* L., *Origanum majorana* L. and the essential oil of *Eucalyptus globulus* Labill. (EUC)).

Totally, 169 cows between 21 and 35 days after calving were included and randomly assigned to one of the both treatment groups (Menoud et al., 2024). For final analysis, the reproductive performance of 140 cows (73 CEPH and 67 EUC) from 27 farms was monitored from 24 days after the first treatment until the next calving or culling date. No significant differences between treatment groups were found regarding first service conception rate, calving to conception interval for cows pregnant at 120 days p.p., pregnancy rate at 120 days p.p., number of inseminations per calving, intercalving period, calving rate, culling reasons and number of gynaecological treatments later than 24 days after the first treatment of the clinical endometritis. There was a trend (p=0.06) for a slightly longer first service period in the treatment group for CEPH (80 days postpartum (p.p.)) compared to EUC (74 days p.p.). In conclusion, EUC is with a high probability not inferior to CEPH. This finding could help to reduce the use of antimicrobials in the daily routine veterinary treatment of CE.

Keywords: Veterinary phytotherapy, on farm study, clinical endometritis treatment

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S2.P262 Medicinal plants for animal health care: translating tradition into modern veterinary medicine - the COST Action MedPlants4Vet

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The demand for natural products supporting animal health and welfare is increasing in the post-antibiotic era. This is in line with the goals of national and international action plans on antimicrobial resistance, the One Health paradigm and the European green deal. It addresses not only farm animals, but also companion animals living in proximity with their owners. Herbal veterinary medicinal products (HVMP) are instrumental in achieving these goals. In the current EU legislation, HVMP are only mentioned to the extent that there is insufficient information for a simplified authorization (Regulation (Eu) 2019/6).

Therefore, the interdisciplinary and international network MedPlants4Vet was founded to identify and close significant gaps in knowledge about the use of traditional HVMP. The network consists of over 250 people from science, industry, practice and regulatory authorities from around 39 countries. MedPlants4Vet aims to significantly promote interdisciplinary cooperation as well as professional training and further education. The creation of a comprehensive decision tree that could serve as a guide for future regulations and authorization procedures is a key focus. In line with current EU legislation, the creation of a basis for a simplified registration of traditional HVMP at EU level with a corresponding legal framework is a matter of priority. In human medicine, a corresponding legal framework was already established in 2004 (Directive 2004/24/Eg).

To achieve these goals, MedPlants4Vet is organized in six working groups (WG; Fig. 1).

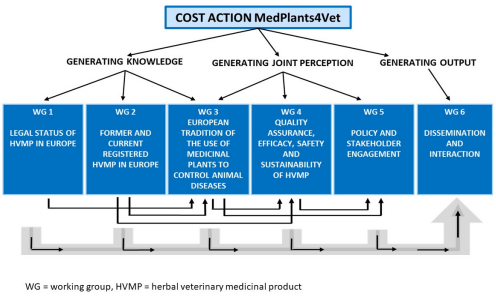

Fig. 1. MedPlants4Vet is organized in six working groups.

Further information is available on the homepages www.medplants4vet.eu and www.cost.eu/actions/CA22109/ . MedPlants4Vet (CA22109) is supported by COST (European Cooperation in Science and Technology).

Keywords: herbal veterinary medicinal products, legal framework, ethnoveterinary research - veterinary phytotherapy, phytochemistry

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December 2018: https://eur-lex.europa.eu/eli/reg/2019/6/oj

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S2.P263 Effectiveness of replacing antibiotics with botanicals in the treatment of bovine mastitis

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Due to the growing resistance to antibiotics in the human population, it is necessary to use antibiotics prudently in controlling diseases in humans and animals. Bovine mastitis is considered the most common disease leading to economic loss in dairy industries. The goal of this research was to check whether antibiotic therapy can be successfully replaced with botanicals that inhibit biofilm formation (Singh et al., 2017), to reduce or disrupt quorum sensing (Boyen et al., 2009) in the mammary gland.

Cows with clinical signs of mastitis in one or more quarters were divided into two groups. The first was treated intramammary beta-lactam antibacterial Ampicillin sodium+Cloxacillin sodium (A), second with AHV Extra bolus (AHV International) for per os one-time (B). Some cows from the first treatment got combined therapy intramammary with Amoxicillin 150 mg/ml parenterally, (A+A). On the first day after the appearance of clinical signs of the disease and day 5~~th~~ following parameters in milk were determined: somatic cell count (SSC), haptoglobin, lactoferrin, total protein, albumin, and aspartate aminotransferase (AST) activity.

Lower ($P<0.05$) SCC and haptoglobin concentration after 5 days of treatment were found in cows from the B and A+A groups. Considering that in the A+A group, the therapy was for 4 days, and in the B group only once, we conclude that botanicals had a prominent effect in treating mastitis following standard parameters like SCC and haptoglobin in milk. The use of antibiotics did not appear to be a factor of choice. The potential of supportive therapy with botanicals should be explored.

Keywords: bovine mastitis, botanicals, antimicrobials

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S2.P264 Pumpkin seed cake waste as a sustainable functional feed ingredient

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The valorization of biomass residuals as feed ingredients is a key aspect of the circular economy. This study aimed to evaluate the characteristics of pumpkin (*Cucurbita pepo* L.) seed waste, commonly called pumpkin seed cake in the feed industry, obtained after cold-press oil extraction.

Using green chemistry methodologies, the biomass was characterized in its apolar and polar fractions by supercritical CO_2 and water extraction, respectively. The qualitative and quantitative characterization was conducted using GC-MS and UPLC-PDA. Klason lignin (KL), and acid-soluble lignin (ASL) were determined following the ISO standards (ISO, 2020). ABTS assay was used for the antioxidant capacity evaluation.

Among apolar components, fatty acids composition indicates high relative percentage (81%) of unsaturated fatty acids, with a higher percentage of oleic (36%) and linoleic acid (45%). The unsaponifiable fraction (4.4% of the apolar extract), was represented by 97-98% of sterols (spinasterol, Δ7,22,25-stigmastatrienol, Δ7,25-stigmastadienol, β-sitosterol), and squalene traces (3%). The characterization of the polar fraction detected the presence of cucurbitin at 1% of the extract, 54.51 $mg/g_{biomass}$ of total lignin, 1.98 $mg/g_{biomass}$ of KL, and 52.52 $mg/g_{biomass}$ of ASL. The antioxidant assay showed a higher antioxidant capacity of the polar fraction (EC_{50} = 0.075 mg/mL), compared to the apolar fraction (EC_{50} = 0.24 mg/mL), confirming that the presence of added-value compounds could be beneficial in the development of sustainable feed ingredients. *In vivo* studies are needed to expand and confirm the effectiveness of pumpkin seed cake waste in the perspective of the use of this waste as a functional feed ingredient.

Acknowledgements

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Keywords: *Cucurbita pepo* L., biomass valorization, sustainable feed

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S2.P265 Herbal bioenhancers: an example of inhibition of ABCB1 using *Brugmansia* tincture

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The therapeutic efficacy of numerous pharmacological agents is often compromised by the active efflux of drugs across cellular membranes, mediated by the ATP-binding cassette (ABCB1) transporters (Yurdakok-Dikmen et al., 2018). This study explores the potential of Brugmansia (angel's trumpet) tincture, derived from *Brugmansia suaveolens* (Willd.) Sweet known for its tropane alkaloids majorly scopolamine (De Feo, 2004; Oğraş et al., 2022), as a bioenhancer through the inhibition of ABCB1 expression *in vitro*. *Brugmansia suaveolens*, belonging to the Solanaceae family, is widespread throughout the world, mainly in areas with tropical, subtropical, and temperate climates, both as a spontaneous species and as an ornamental (Petricevich et al., 2020). Using the L929 mouse fibroblast cell line as a model system, we investigated the effects of the tincture on the mRNA expression levels of ABCB1 using quantitative PCR (qPCR). We applied ethanolic tincture at 9.68-0.005 mg/ml concentrations and determined percent cytotoxicity regression curve by MTT. At tested concentrations (Inhibitory Concentration15-25) a dose dependent decrease by 68.57-76.27% of mRNA expression at 302.42- 604.85 ug/ml concentrations were observed; which is also confirmed at protein level by western blot assays. Our preliminary results indicate a dose-dependent decrease in ABCB1 expression upon treatment with Brugmansia tincture, suggesting its potential role as a bioenhancer by facilitating greater intracellular drug accumulation. These findings provide a promising foundation for further research into the application of natural compounds in overcoming drug resistance mechanisms, potentially leading to more effective therapeutic strategies.

Keywords: Brugmansia tincture, L929 cell line, ABCB1 expression, *Brugmansia suaveolens*

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S2.P266 Antibacterial activity of essential oils against streptococci and staphylococci causing bovine mastitis

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Bovine mastitis is a major concern for the dairy cattle community worldwide, leading to high economic loss (Gomes et al., 2016). Mastitis, subclinical or clinical, can be caused by contagious or environmental pathogens. Although the use of antibiotics is still the primary approach for treatment, its efficacy is limited. Moreover, the development of antimicrobial resistance (AMR) leads to the need for alternatives to antibiotic therapy (Yang et al., 2019). Due to their antibacterial effects, essential oils (EOs) have gained much attention in this field as candidates to fight against bacterial infections and control further development of AMR (Arbab et al., 2022).

This research aimed at testing the antibacterial activity of eleven EOs and two EO blends against *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, by evaluating the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), using microbroth dilution assay. The MIC values of the EOs tested ranged between <0.001% and >3.125% v/v. The effect was higher against *Streptococcus spp* than *Staphylococcus spp* bacteria in general. The EOs of *Origanum vulgare*, *Cinnamomum zeylanicum*, *Thymus vulgaris*, and Blend BR were the most effective against *S. agalactiae*, *S. dysgalactiae*, and *S. uberis* showing MIC and MBC values between <0.001% and 0.390% v/v, and <0.001% and 0.780% v/v, respectively. Against *S. aureus* and *S. epidermidis*, the lowest MICs occurred with *Cinnamomum zeylanicum*: 0.098% and 0.195% v/v, respectively.

This study highlights the importance of evaluating EOs as effective antibiotic alternatives and complementary resources for treatment of bacterial infections.

Keywords: Essential oils, bovine mastitis, antimicrobial resistance, streptococci, staphylococci

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S2.P267 Absorption, distribution, accumulation and excretion of thymol after its sustained administration in rabbits

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The metabolic pathway of thymol, a major constituent of *Thymus vulgaris* L., was studied for the first time in the rabbit organism after its sustained oral administration.

Forty-eight rabbits were allocated to control and experimental groups with thymol addition (250 mg/kg feed) for 21 days and withdrawal for 7 days. Significant correlation of the levels of thymol determined by GC/MS after solid phase microextraction ($r_s = -1.000$, $p < 0.01$) between intestinal wall (IW) and plasma points to the intensive absorption of thymol from the intestine. The significant correlation between plasma and liver ($r_s = 0.786$, $p < 0.05$) indicates intensive biotransformation and excretion processes in liver, and between liver and kidney ($r_s = 0.738$, $p < 0.05$) the intensive metabolism of thymol in the kidney. Even though thymol was determined only in trace amounts during the period without thymol addition, its amount in IW was significantly higher than in plasma. Thymol as lipophilic substance was found only in trace amounts in fat and muscle. In faeces it was detected in both periods above trace amounts showing significantly higher levels than in colon as a consequence of its conversion into hydrophilic metabolites and efficient elimination from organism. Our results showed some metabolic processes also after thymol withdrawal as consequence of caecotrophy, a characteristic feature of rabbit digestion processes responsible for better utilization of nutrients from feed.

Insufficient information about bioactivity of plant compounds in the animal organism represents a challenge for scientists to understand their metabolic processes at molecular level.

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Keywords: thymol, rabbit, bioavailability

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S2.P268 *In-vitro* investigation of the gastroprotective effects of a natural extract blend: a promising avenue for canine gastritis prevention

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Gastric hyperacidity is a common ailment in dogs that requires gastroprotection. Natural compounds, such as (psyllium, *aloe vera*) have anti-inflammatory properties (Szweda et al., 2014). The purpose of this study was to evaluate the gastroprotective efficacy of a natural extract (NE) blend containing inactivated yeast, psyllium seed cuticle (*Plantago ovata* L.), lupin protein flour, fenugreek, licorice, and *Aloe vera* (L.) Burm.f. extracts, in gastric adenocarcinoma (AGS) cells under hyperacidic conditions. Three NE concentrations were investigated based on a medium-sized dog's (15-20kg) stomach volume to deliver a maximum 2 g dose (0.3/0.15/0.075 mg/ml). AGS cells (10^4 cells/well) were treated with NE in 96 well plates and examined with the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) using a Tecan M NANO+ plate reader (Ghazanfari et al., 2013). Cell viability was quantified against a positive control (sodium dodecyl sulfate,1 mg/ml), followed by an acid pH gastroprotection test to determine NE's effectiveness under established positive control, hyperacidic conditions (pH=2). One-way ANOVA with Dunnett's post-hoc test was used for statistical analysis. NE showed 100% cell viability (0.554 ± 0.009 mg/ml, $p<0.05$), while the positive control had 40% viability (0.213 ± 0.003 mg/ml) compared to the negative control (0.534 ± 0.013). NE showed considerable gastroprotection (0.837 ± 0.009, $p<0.05$) at 0.3 mg/ml compared to negative control (0.852 ± 0.057) and positive control (0.686 ± 0.027). In conclusion, NE showed promising gastroprotective properties in AGS cells, although in vivo experiments are required to confirm NE's efficacy for clinical use in the prevention of gastritis in dogs.

Keywords: bioactive-compunds, dogs, gastroprotection

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S2.P269 Acaricidal activity of essential oil of *Satureja montana* L. against *Dermanyssus gallinae in vitro* conditions

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Dermanissus gallinae commonly known as the poultry red mite causes great damage in the poultry industry. Plant-derived acaricides offer a strong alternative to chemical acaricides in the control of ectoparasites such as *D. gallinae* (Radsetoulalova et al., 2020; Ratajac et al. 2024). The aim of this study was to investigate the acaricidal activity of essential oil (EO) *Satureja montana* L. from the Balkan Peninsula and to evaluate their potential and limitations to be included in formulations for rational control of *D. gallinae* in the poultry industry. The chemical profile of EO was analyzed by gas chromatography coupled with mass spectrometry. The acaricidal efficacy of EO (0.1; 0.3; 0.5; 1; 3; 5 and 6% concentration) was tested on adult mites using the Petri-dish method, through direct exposure for 1 minute (contact toxicity) and subsequent exposure for 1 hour (residual toxicity). The EO efficiency ranged, in direct exposure-contact, after 48 hours of observation, from 3 to 100% toxicity, depending on the tested concentration, while the residual effect ware negligible. George et al. (2010) also demonstrated efficacy after 24h exposure as 77.80% for *S. montana* L. Thus, the results of the present and other studies suggest a strong effect of EO against *D. gallinae* through direct contact and absence of prolonged effect. Plant based formulations are most likely less susceptible to resistance development and more acceptable in terms of the residues in animal products and the environment in comparison with chemicals (Abbas et al., 2018).

Keywords: Acaricides, *Dermanyssus gallinae, Satureja montana* L., Contact effect, Residual effect

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S2.P270 A novel phytogenic supplement that prevents coccidiosis in chickens

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Coccidiosis, caused by a protozoan parasite of the genus *Eimeria*, is one of the most severe contagious parasite diseases affecting the poultry industry worldwide. Using phytogenics to prevent chicken coccidiosis is a novel strategy aimed at combating the increasing issue of drug-resistant strains of *Eimeria* spp. This study demonstrated the anticoccidial activities of a *Leguminosae* medicinal herb TP showing significant activity against *Eimeria* spp. The chemically defined TP extract exhibited significant suppressive activity against *E. maxima* oocyst sporulation and *E. tenella* sporozoite invasion and reproduction in Madin-Darby bovine kidney (MDBK) cells. Furthermore, administration of basal chicken diets containing TP to *Eimeria*-infected chickens significantly reduced the output of oocysts and severity of intestinal lesions. Dietary supplementation with TP significantly improved relative weight gain in *E. tenella*- and *E. acervulina*-infected chickens. The anticoccidial activities of TP on *E. acervulina*, *E. tenella* and *E. maxima* were further supported by anticoccidial index scores, which showed greater efficacy than those of amprolium, a commercial coccidiostat used in poultry. Furthermore, TP supplementation positively impacted the primary metabolism of chickens challenged with *E. tenella* or *E. acervulina*. In conclusion, the TP-derived chemical ingredients are demonstrated to be a novel phytogenic supplement that can be used to control *Eimeria*-induced coccidiosis in chickens.

Keywords: *Eimeria* spp., Coccidiosis, Anticoccidial index, Phytogenic supplement, Primary metabolism

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Cutting edge technologies in natural product drug discovery, formulation and development

S3.P1 *In silico* investigation of *Pterocarpus marsupium* against type-2 diabetes mellitus through network pharmacology approach

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Pterocarpus marsupium heartwood (PMH) is a widely used plant in the management of diabetes in India. This presentation will highlight the potential of PMH-derived phytochemicals to control T2DM through network pharmacology approach. In this study, the bioactive compounds were retrieved from authentic scientific domains related to heartwood. Thereafter, the scientific tools were used to identify phytochemicals that meet Lipinski's Rule of Five parameters and oral absorption standards. Target identification for these phytochemicals was conducted using databases such as Binding DB, SwissTargetPrediction, STITCH, and TargetNet. Targets associated with T2DM were obtained from databases such as GeneCard, TherapeuticTargetDisease, NCBI Gene, and DrugBank. The STRING database was used for the PPI study. KEGG and GO enrichment studies were performed using the DAVID database to identify the pathways, biological pathways, cellular components, and molecular functions. The common targets between the PMH-phytochemicals and T2DM were used for network formation, including phytochemical targets, protein-protein interactions, and target-pathway networks. Finally, molecular docking between anti-T2DM targets and PMH phytochemicals was performed. The results of this study revealed that PMH may regulate blood glucose levels by directly interacting with target proteins such as SLC5A2, DPP4, DRD2, PPARG, and AKR1B1, which was verified using the molecular docking studies. Moreover, several networks like PT, TT, and TP were constructed and introduced modifications to each network. Subsequently, these networks were integrated to form a phytochemical-target-pathway network, which we hypothesize to offer enhanced precision in information retrieval. Several PMH targets showed protein-protein interactions which are involved in the signaling pathways such as insulin resistance and the T2DM signaling pathway. These findings generate further interest in the drug development process against T2DM.

Keywords: Network pharmacology, *Pterocarpus marsupium*, T2DM, Insulin resistance, molecular docking, biotargets, phytochemicals

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S3.P2 Integration of High-Throughput Screening, AI-ML Strategies, and ECD/DP4+ for the Discovery and Elucidation of Novel Natural Products

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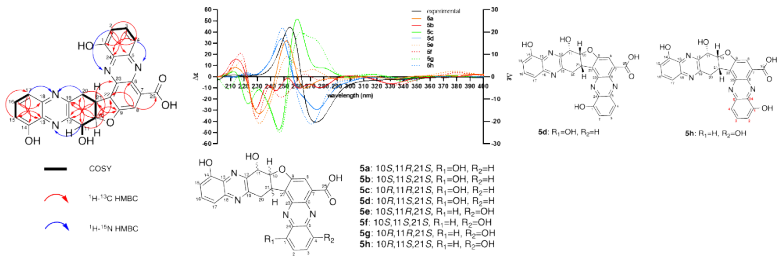
Identifying and characterizing novel natural products holds immense potential for discovering bioactive compounds with diverse therapeutic applications. This presentation focuses on the integration of high-throughput screening (HTS), artificial intelligence-machine learning (AI-ML), and electronic circular dichroism (ECD)/DP4+ for the efficient discovery and structural elucidation of novel natural products. HTS enables the rapid evaluation of sizeable natural product libraries against biological targets, facilitating the identification of potentially bioactive compounds. Furthermore, combining HTS with advanced separation techniques allows for the isolation and purification of active compounds.

AI-ML strategies enhance the discovery process by predicting de novo structures and bioactivities and prioritizing compounds for further investigation. These approaches aid in exploring chemical space and identifying novel structural motifs. AI-ML algorithms trained on large datasets of known natural product structures and activities enable efficient compound screening.

Once potential hits are identified, ECD coupled with DP4+ becomes crucial for structural elucidation. ECD spectroscopy provides information on absolute configuration, aiding in stereochemistry determination. DP4+ is a probabilistic method for relative configuration determination, enabling accurate structural elucidation, even in complex mixtures.

In this pipeline, we incorporated ECD and GIAO NMR calculations coupled with a DP4+ probability measure, enabling the structure revision of phenazinolin D (4), izumiphenazine A (5), and baraphenazine G (7) and the structure characterization of two new diphenazines, baraphenazine H (3) and izumiphenazine E (6). The advancements discussed have the potential to revolutionize the field, facilitating the identification of compounds with significant therapeutic potential.



Keywords: *ECD, NMR/DP4+*, cytotoxicity, eIF4e, high-throughput screening, natural product

References

An ECD and NMR/DP4+ Computational Pipeline for Structure Revision and Elucidation of Diphenazine-Based Natural Products. Yihao Zhuang, Fei Yang, Arya Menon, James M. Song, Rosa V. Espinoza, Pamela J. Schultz, Amanda L. Garner, and Ashootosh Tripathi. J. Nat. Prod. 2023, 86, 7, 1801– 1814.

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S3.P3 On-tissue derivatization and MALDI–MS Imaging reveal hemlock alkaloid distribution in *Conium maculatum*

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The plant *Conium maculatum* is well-known for containing piperidine alkaloids, mainly coniine (**1**) and γ-coniceine (**2**). Its ingestion has strong effects on the central nervous system of mammals, causing ataxia and convulsions. (López et al., 1999; Talaty et al., 2005). However, the distribution of these alkaloids in plant tissues has not been studied in detail, yet. To close this knowledge gap, we analyzed the distribution of the hemlock alkaloids using MALDI mass spectrometry imaging (MSI), a technique that allows the determination of the localization of specific compounds in samples. However, it has limited applications for low molecular weight compounds, as the matrices can have background interference as well as overlapping signals with the analytes (Chen et al., 2012). Also, the hemlock alkaloids are rather volatile, which makes them difficult to analyze using MALDI-MSI. To overcome these limitations by modifing the physicochemical characteristics of these compounds, three derivatizing agents were studied. Derivatization with coniferyl aldehyde (CA), showed the best results, and the alkaloids were successfully visualized in the plant tissues, detecting **1** mainly in the midrib and margin of the leaf and in the endocarp of the fruit, while **2** was mainly detected in the dorsal ribs of the fruit (Fig. 1).

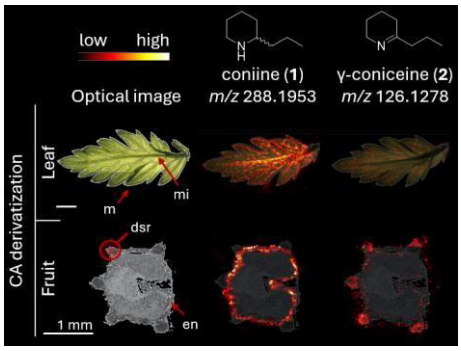

Fig. 1. MS Images of hemlock alkaloids in leaf and fruit tissues of *C. maculatum*. Midrib (mi), margin (m), dorsal secondary rib (dsr) and endocarp (en)

Keywords: *Conium maculatum*, spatial distribution, piperidine alkaloids, derivatization

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S3.P4 New world and old world *Salvia* species (sages): searching of diterpenoids with cytotoxic activity

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This presentation will cover the isolation, structure elucidation and cytotoxic activity of diterpenoids of three *Salvia* species, two from the New World (NW) *S. involucrata* (*SI*) and *S. fulgens* (*SF*) (subgenus *Calosphace*), and one from the Old World (OW) *S. rosmarinus* (*SR*, subgenus *Rosmarinus*). *Salvia* is the most abundant genus of the Lamiaceae family, and it is divided into 11 subgenera (Kriebel et al., 2019). The main secondary metabolites isolated from these plants are diterpenoids, mainly *neo*-clerodanes from the NW species, and abietanes from the OW species (Wu et al., 2012).

We made collections of the three species, *SI* collected at Xilitla, San Luis Potosí (May 2017), *SF* at Los Azufres, Michoacán (November 2018), and *SR* at Milpa Alta, Mexico City (August 2022). The dried and powdered leaves of the collected species were extracted by percolation with CH_2Cl_2. The isolation was carried out by column chromatography, preparative TLC and semipreparative HPLC to produce eight diterpenoids (**1**-**8**, Fig. 1). The chemical structures were elucidated by extensive analysis of NMR spectroscopy and HR-DART-MS. The absolute configuration was established by experimental and theoretical ECD data or single-crystal X-ray diffraction.

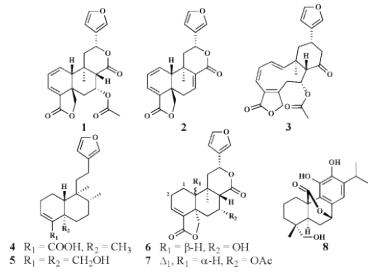

Fig. 1. Isolated compounds from *SI* (**1**-**6**), *SF* (**7**) and *SR* (**8**).

The isolated diterpenoids were screened *in vitro* against six human cancer cell lines: MCF-7, K562, U251, SKLU-1, HCT-15 and PC-3. The highest growth inhibition was observed against K562, U251 and SKLU-1 (with values of 33.0-81.4 %), and the most active diterpenoid was **8** with 81.4 % against K562 (IC_{50} = 22.04±1.0 μM).

Keywords: *Salvia*, diterpenoids, abietane, *neo*-clerodane, cytotoxicity

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S3.P5 Use of *Galleria* moth larvae model to assess the pathogenesis of AIEC and the therapeutic potential of Geraniol as an antibacterial agent

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Crohn's disease (CD) is an inflammatory bowel disease (IBD) caused by abnormal immune responses to microbial infections in genetically susceptible individuals. Antibiotic-resistant adherent-invasive *Escherichia coli* is related to Crohn's disease (AIEC). Antibiotic resistance is one of the most important problems in modern healthcare, especially when it comes to illnesses of the gastrointestinal tract. There is an urgent need for new medications because the effects of these bacteria cannot be treated with current antibiotics. To address the issues of bacterial resistance and the decline of beneficial gut bacteria, the study concentrated on substances known as terpenoids (geraniol), which can reduce the action of bacteria rather than exterminate them. Gene deletions were put together utilizing the phage red gamma recombination technique in the adherent invasive *E. coli* background (Datsenko & Wanner 2000).

The moth larvae were bought from Live Food UK. Each larva weighed somewhere between 250 and 300 mg. To reach the doses of 10^5, 10^6, and 10^7 CFU/larvae, bacteria obtained from the mid-log phase were diluted with 1xPBS to 10^5, 10^6, 10^7, and 10^8 CFU/ml, which is nearly equivalent to OD= inapplicable, 0.01, 0.02, and 0.13 respectively based on the free online Lab tools (https://www.labtools.us/bacterial-cell-number-od600/). Following injection, the larvae were kept at 37°C and kept in the dark. Over the course of five days, the coloration and mortality of the larvae were monitored twice daily. If the larvae did not react to touch, it was considered that the larvae had died. A minimum of three different iterations of each experiment were conducted. The Kaplan-Meier estimator was used to examine survival data.

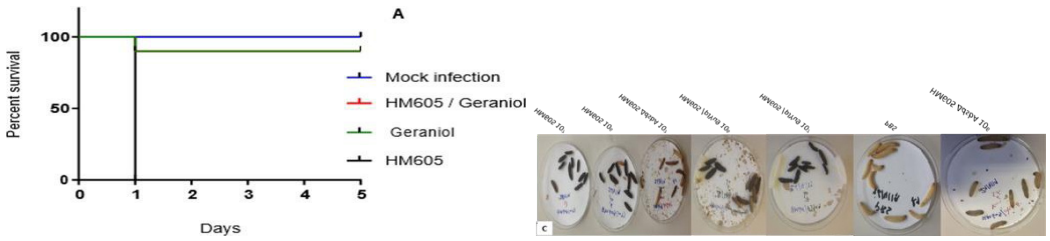

Fig. 1 AB. (A) In the larvae geraniol safety test, 10 µL of the mixture (HM605-107 + geraniol) was injected at the prescribed doses to the right of each larva's final proleg. Each larva has been observed for five days. Death of larvae is predicted by melanisation and loss of mobility. There were three replications of each experiment (n=3), and the graphs were constructed using pooled data. Using a significance level of P 0.0001, the Gehan-Breslow-Wilcoxon test was used to compare untreated and treated groups infected with AIEC (HM605).

(B) Image of *Galleria mellonella* larvae. *Galleria mellonella* larvae were injected with AIEC 10^7CFU — HM605(WT) and indicative doses of geraniol or geraniol alone in the last proleg.

As a result, it was shown that the wild type of AIEC was more effective at killing larvae at the recommended dose than the *dsbA*-mutated strains. DsbA is required for the HtrA/DegP protein's protease activity, which is essential for AIEC survival in phagosomes. Geraniol can target the *dsbA* gene and prevent bacterial growth by competitive inhibition. This implies that the substance geraniol was discovered to possess antibacterial activity against the strains employed in this experiment.

Keywords: Adherent invasive *E. coli* (AIEC), Crohn's disease, geraniol, antibiotic resistance, Larvae, *Galleria mellonella*

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S3.P6 Inhibition of transforming growth factor β signaling by prodigiosin through disruption of receptor recycling and subcellular translocation in epithelial cells

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Prodigiosin (PG), a naturally occurring polypyrrole red pigment produced by various microorganisms, including certain *Serratia* and *Streptomyces* strains, has shown promising anticancer activity (Perez-Tomas et al., 2003). However, the molecular mechanisms underlying its action on malignant cells remain unclear. Transforming growth factor-β (TGF-β) is a multifunctional cytokine that governs various cellular processes in development and tissue homeostasis, with dysregulation of TGF-β signaling associated with numerous human cancers. Recent evidence highlights the importance of internalized TGF-β receptors and their intracellular trafficking in initiating signaling cascades (Heldin and Moustakas, 2016).

In this study, we identified PG as a potent inhibitor of the TGF-β pathway. PG hinders TGF-β signaling by targeting multiple sites in this pathway. It facilitates the sequestration of TGF-β receptors in the cytoplasm by impeding the recycling of type II TGF-β receptors to the cell surface. Furthermore, PG induces a reduction in receptor abundance on the cell surface by disrupting receptor glycosylation. In lung cancer A549 and HepG2 cells, nanomolar concentrations of PG significantly reduce TGF-β-triggered phosphorylation of Smad2 protein. This reduction is also reflected in the suppression of downstream target gene expression, including genes encoding fibronectin, plasminogen activator inhibitor-1 (PAI-1), and N-cadherin.

Keywords: Prodigiosin, cancer, TGF-β, intracellular trafficking

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S3.P7 Assessing intra- and inter-variability of Macedonian wild-types and commercial *Cannabis* strains using short tandem repeat markers

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Developing consistent Cannabis varieties capable of reliably producing a wide range of potentially beneficial cannabinoids is crucial for the research and medical communities (Nutt et al., 2013). Macedonian wild-growing cannabis possess cannabidiol potential and can be prospectively registered as a variety, but extensive UPOV (International Union for the Protection of New Varieties of Plants) descriptor-based testing is required. Genetic individuality and stability can be assessed utilizing STR (short tandem repeat) marker analysis as an alternative method. The aim of this study was to assess intra and inter-variability of outdoor cultivated Macedonian wild-types and commercial Cannabis strains. Automatic DNA extraction using MagCore kit was performed on 52 cannabis samples (flower/leaf tissue material) from five groups of wild-type plants and four groups of commercial strains that were outdoor cultivated from seeds. The wild-type seeds were collected from areas in Eastern Macedonia. Polymerase chain reaction and fragment analysis conditions were according to Houston et al. (2017) using 12 STR markers (ANUCS501, 9269, 4910, 5159, ANUCS305, B05- CANN1, 1528, 3735, 9043, D02-CANN, C11-CANN1, H06-CANN2). All of the samples were same homozygotes for marker 9269. STR markers ANUCS501, 4910, ANUCS305, 1528, 3735 and C11-CANN1 exhibited high inter- and intra- group variability, whereas only wild plants from Dolni and Gorni Podlog, were distinctive from all other groups with different alleles of ANUCS501, 1528 and D02-CANN. The STR analysis of cultivated seed plant material reveals significant variability within groups, underscoring the necessity for thorough genetic analysis correlated with phenotypic and phytochemical traits to identify the optimal genotype for registering wild plant groups as varieties in the European Union database.

Keywords: *Cannabis sativa*, hemp, DNA, molecular markers, genetic variability

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S3.P8 Preclinical development of natural products into agents for pediatric brain cancers

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Pediatric CNS tumors are the most common malignancy in childhood and adolescence, and occur with highest incidence in infants and children 5 years of age or younger. Therefore, anticancer therapeutics for the treatment of pediatric brain tumors are urgently needed. Selective human constitutive proteasome inhibitors have shown early stage promise for treating CNS tumors. We present here a project that was initiated with cytotoxic natural products, such as the carmaphycins and salinosporamide, as leads for the synthetic medicinal chemistry development of novel brain blood barrier penetrant proteasome inhibitors. These synthetic small molecules possess improved profiles of selectivity and metabolic stability in several different cell lines and microsome experiments. Extensive SAR studies, including incorporation of fluorinated amino acid residues, have achieved the production of highly potent small molecules at 5-6 μM fully inhibiting constitutive proteasome at 0.5-1 nM range with favorable pharmaceutical properties for the development of a CNS pharmaceutical agent.

Keywords: proteasome inhibitors, cytotoxicity, cell lines

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* Presenting author. E-mail address: eglukhov@ucsd.edu (Evgenia Glukhov)

S3.P9 Exploring the anti-mycobacterial potential of French Guiana's flora through a metabolomic study

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Among the unique flora of the Amazonian Forest, numerous species have captured researcher's attention for their therapeutic potential, demonstrating promising activities against infectious diseases. Notably, mycobacterial infections such as tuberculosis stand as a significant health concern in French Guiana. With a high incidence rate and the emergence of drug-resistant strains, there is an urgent need to explore therapeutic alternatives (Chaptal et al., 2022; Succo, 2020).

This study delves into French Guiana's biodiversity, to explore the chemical composition of 11 plant species, and uncover novel metabolites showcasing anti-mycobacterial properties. Drawing from the literature, preliminary results indicate interesting activities in the selected plant species (Pavan et al., 2009).

The use of a methodology combining recent computational metabolomics tools and a MS-based Molecular Network approach, facilitates structural dereplication and allows to work on large datasets, ensuring rapid and accurate characterization of metabolites of therapeutic interest. Using multiple analytical techniques, such as UHPLC-HRMS/MS and NMR, we conducted a comprehensive metabolomic analysis to profile the chemical composition of the plant extracts. Simultaneously, bioassays against multiple strains of mycobacteria were performed. The integration of bioassays results on a Molecular Network facilitated the identification of bioactive molecules, which were prioritized for further investigation and bioassay-guided purification.

This multidisciplinary approach provides insights into the diverse chemical landscape of the studied plant extracts, and proposes a systematic approach to uncover novel anti-bacterial agents. This presentation will highlight the methodologies employed, initial findings, and the potential contribution of these plants in discovering therapeutic alternatives against mycobacterial infections.

Keywords: French Guiana, medicinal plants, mycobacteria, metabolomics, molecular network

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S3.P10 Arnica of Provence: an alternative to *Arnica montana* L.? Investigations about polyphenolic composition and antioxidant potential

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In the Provence region (France), an Asteraceae species named *Pentanema montanum*m (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort. *(*syn. *Inula montana* L.*)* is traditionally used with the same indications as *Arnica montana* L., against bruises, wounds, rheumatisms and muscular pain, and is even locally called "Arnica de Provence" (Garayev et al., 2018; Gyawali et al., 2022). Both species contain mainly sesquiterpene lactones and phenolic compounds, which are usually implicated in the defence against oxidative stress, involved in acute or chronic diseases (Vuolo et al., 2019).

Hydro-ethanolic extracts of these two species underwent evaluation of antioxidant potential and phytochemical studies. The radical scavenging activity was assessed using both 96 well-plate DPPH and DPPH-On-Line-HPLC assays. Well-plate DPPH assay showed higher activity for *P. montanum* extracts, with an IC_{50} twice lower than *A. montana* extracts, respectively 23.66 ± 0.75 µg/mL and 45.71 ± 3.05 µg/mL. DPPH-On-Line-HPLC assay allowed to identify 1,5-dicaffeoylquinic acid as the main compound responsible for the anti-oxidant activity in both extracts. This compound is more abundant in *P. montanum* extracts, supporting the well-plate DPPH assay results.

Metabolomic data were obtained through UHPLC-HRMS/MS analysis and processed using computational metabolomics and MS-based Molecular Networking. The two species only share 16% of their chemical composition, most metabolites being species-specific.

This presentation will emphasize the comparative analysis by molecular networking of *A. montana* and *P. montanum* chemical profiles, with a focus on exclusive metabolites of each species and those contributing to radical scavenging activity.

Keywords: metabolomics, molecular networking, antioxidant, DPPH, traditional medicine

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S3.P11 Biosynthesis of anti-inflammatory compounds from *Calendula officinalis*

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Plants have been used in traditional medicine for thousands of years. In some cases, therapeutic properties have been attributed to specific metabolites, enabling their use as drugs in modern medicine. However, the exact molecule(s) responsible for the bioactivity of many plant extracts remains unknown. Further, access to natural products is often complicated by low abundance, occurrence in complex mixtures, or by structural complexity, which can complicate chemical synthesis. In this project, we are investigating the previously reported anti-inflammatory bioactivity of *Calendula officinalis* (pot marigold) floral extracts. Studies have suggested bioactivity may be associated with the presence of a class of molecules known as triterpene fatty acid esters (TFAEs) (Colombo et al., 2015; Nicolaus et al., 2017). To investigate this, and to identify specific molecules that contribute to bioactivity, we combined comparative metabolic profiling of Asteraceae species with bioactivity assays in model human cell lines. Our data suggests that TFAEs with C:16 hydroxylated scaffolds may exert an anti-inflammatory effect via tumour necrosis factor (TNF) and the interleukin 6 (IL-6) pathways. Additionally, we have combined metabolomics, genomics, and transcriptomics with rapid, transient expression in *Nicotiana benthamiana* to identify and characterize the biosynthetic pathways of *C. officinalis* TFAEs (Fig. 1). Our work provides a route to the identification and production of novel plant bioactives.

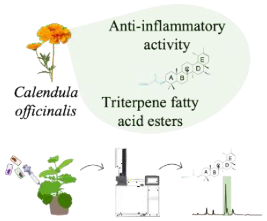

Fig. 1. *Calendula officinalis* flowers are rich in triterpene fatty acid esters (TFAEs) which have been proposed to confer anti-inflammatory bioactivity. Heterologous expression of candidate genes in *Nicotiana benthamiana* enabled us to elucidate the biosynthetic pathways of these molecules and investigate their bioactivity.

Keywords: *Calendula officinalis*, anti-inflammatory bioactivity, bioassays, triterpenes, *Nicotiana benthamiana*

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S3.P12 Development and validation of an analytical method for the analysis of standardized red propolis extract and its chemopreventive effect against colon carcinogenesis

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Nowadays, Brazilian red propolis (BRP) has gained prominence in the scientific literature due to its several pharmacological applications. Despite this fact, some toxicological effects have been observed from BRP, in which is credited to the presence of polyprenylated benzophenones, such as gutiferone E, xanthochimol and oblongifolin B (Ccana-Ccapatinta et al. 2020).

In view of this context, our research group has obtained a standardized red propolis extract without the presence of polyprenylated benzophenones using a sequence of *n*-hexanes and then methanol (SRPE). In addition, an analytical method using RP-HPLC-PDA was developed and validated according to the guidelines proposed by Brazilian National Health Surveillance Agency (ANVISA, 2017). For this purpose, the main compounds from SRPE were isolated, identified, and considered as chemical markers (Fig. 1). The developed method showed to be precise, sensitive, and reliable for analyzing SRPE.

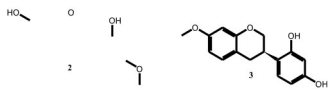

Fig.1. Compounds used as chemical markers in the development and validation of the analytical method: **1**: isoliquiritigenin; **2**: vestitol; **3**: neovestitol; **4**: medicarpin; **5**: 7-O-methylvestitol.

Considering the biological properties of SRPE, different doses of this extract (3, 6, and 12 mg/kg b.w.) were administered in combination with the carcinogen 1,2-dimethylhydrazine (DMH, 40 mg/kg b.w.) using an *in vivo* colon carcinogenesis model (da Silva et al., 2022). The animals treated with 6 mg/kg b.w. showed a significant reduction (41.57%) in pre-neoplastic lesions induced by DMH, thus revealing a preventive effect against colon carcinogenesis of SRPE.

Keywords: Brazilian red propolis, chemopreventive effect, colon carcinogenesis, analytical method

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S3.P13 Extract rich in polyprenylated benzophenones from Brazilian red propolis: obtaining, analytical characterization and *in vitro* evaluation against breast cancer lines

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The present work describes the obtaining of an extract rich in polyprenylated benzophenones from Brazilian red propolis (ERPB), the development and validation of an HPLC-UV method to characterize it as well as its evaluation against breast cancer cell lines MCF-7 and MDA-MB-231 and a normal counterpart MCF-10A. A mixture of gutifferone E + xanthochymol **(1+2)**, and isolated oblongifolin B **(3)** (Fig. 1) were used as chemical markers of ERPB.

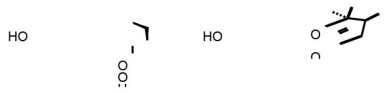

Fig. 1. Structure of the polyprenylated benzophenones gutifferone E **1**, xanthochymol **2**, and oblongifolin B **3**

ERPB was obtained by maceration of Brazilian red propolis using *n*-hexanes followed by concentration and suspension in cold methanol to remove waxy material. The RP-HPLC-DAD analytical method was established based on initial experiments using gradient scouting run evaluation (Snyder and Dolan, 1996) and validated according to the guidelines of the Brazilian National Health Surveillance Agency (Santos et al., 2021). The concentrations of **1+2** and **3** were 101,822 µg/mL and 257,918 µg/mL, respectively, corresponding to 16.68% and 42.25% of the total content of the extract and the validation parameters evaluated were satisfactorily met. The cytotoxic effects of ERPB were assessed through XTT assay (Gonçalves et al., 2021) and the obtained IC_{50} were 19.58 µg/mL (MCF- 10A), 11.56 µg/mL (MCF-7) and 5.22 µg/mL (MDA-MB-231) thus resulting in selectivity index values of 3.75 (MCF-10A/MDA-MB-231) and 1.69 (MCF-10A/ MCF-7). In conclusion, ERPB exhibit promising cytotoxic effects on the tested breast cell lines. However, further investigation to elucidate its potential therapeutic applications and safety profiles should be conducted.

Keywords: Brazilian red propolis, polyprenylated benzophenones, analytical method, breast cancer cell lines

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S3.P14 Computational NMR enhances metabolomic analysis and sustainability

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In the absence of crystals for diffraction measurements, rigorous stereospecific structural analysis of natural products (NPs) is the domain of NMR and chiroptical spectroscopies. The stereochemical significance and near ubiquitous occurrence of hydrogen atoms make ^{1}H NMR a powerful analytical tool, but the wealth of information comes at the price of complexity - even in seemingly simple small molecules.

The solution to this conundrum is applying the NMR theory. Its foundation was laid in the 1920s and proven experimentally in the 1950s. Since then, the lack of computational resources has been the main, if not only, reason why applying NMR theory to spectral analysis has been unfeasible. This has now changed dramatically: adequate computational power and software tools are available that allow automated computational spectral analysis - even of highly complex ^{1}H NMR spectra - in seconds to minutes. While 2D NMR is invaluable for spectral analysis, much of the valuable coupling information in NPs remains ignored in current practice, as is evident from the abundant use of "multiplet" annotations. However, the ability to extract the entire ^{1}H NMR information can transform both qualitative and quantitative analysis of NPs for several reasons:

1. Couplings provide essential stereochemical information and allow independent verification of chemical (shift) assignments from 2D correlation experiments.
2. As sets of chemical shifts and couplings must be self-consistent and coherent with the observed spectra, confidence in the correctness of the results increases dramatically.
3. Even overly complex spectral patterns and peak overlap can be fully explained using the additional constraints provided by the NMR theory.
4. Chemical shifts and especially couplings are instrument independent and represent highly reproducible physical constants under well-defined experimental conditions.
5. Collectively, this allows automated and highly specific dereplication of known NPs based on shared raw 1D ^{1}H spectra - regardless of NMR instrumentation.

Results from computational analyses of newly discovered and challenging NPs with available raw NMR data will be shared to explain the methodology and its capabilities. We will exemplify the growing experimental evidence for capability of the fastest and most affordable NMR measurement to extract new structural and quantitative insights and potentially transform metabolomic and biomedical NP analysis. Applying computational analysis particularly to 1D ^{1}H NMR spectra serves scientific sustainability for several reasons: (A) Exploitation of the full NMR information content enhances the re-use of essential prior knowledge. (B) Increased throughput and reduced per-sample costs of today's capable NMR instruments. (C) Widened capability of NMR as a non-destructive analytical technique with reduced solvent footprint, especially on benchtop instruments without deuterated solvents.

Keywords: Structural analysis, quantitative analysis, dereplication, NMR

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S3.P15 Exploring on the networking of chemical compositions and their cytotoxicity toward KBCC *Nepenthes* plants

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In collaboration with the Dr. Cecilia Koo Botanic Conservation Centre, we collected 60 *Nepenthes* species for further investigation. Preliminary activity screening showed that the methanol extract of *Nepenthes mirabilis* suppressed elastase release, indicating anti-inflammatory activity.

In addition, activity-guided isolation, molecular networking and mass spectrometry were used to isolate, purify and identify potentially active natural products. Various analytical experiments were performed using instruments such as HPLC-PDA, spectrophotometer, and LCMS/MS. The study used activity-guided isolation, chemical analysis, and supplemented by computer simulation of the Global Natural Product Molecular Network (GNPS) for ancillary analysis.

This study found that the naphthoquinone component, plumbagin, in *Nepenthes* was effective in inhibiting the growth of human liver cancer cells (HepG2), human triple negative breast cancer cells (MDA-MB-231) and human non-small cell lung cancer cells (A549) (Wójciak et al., 2023). The compound also exhibited anti-inflammatory (human neutrophil) properties. The dried methanol and *n*-hexane partition layers of 60 *Nepenthes* species were screened for cytotoxicity and anti- inflammatory activity, and the chemical composition and cytotoxic network were explored with the indicator compound, plumbagin. Besides, we carried out the carbon dots synthesis of plumbagin and found that it became a nano-probe with wide spectral performance.

Keywords: *Nepenthes mirabilis, Nepenthes, carbon dots,* cytotoxicity, anti-inflammatory, molecular networking

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S3.P16 Chemical composition and bioactivity of *Uvaria micrantha* from Vietnam

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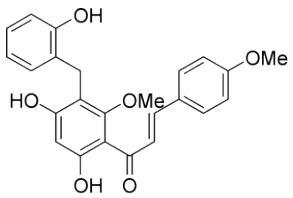
Uvaria (Family Annonaceae) was reported to have anti-inflammatory, anti-cancer, and anti-malaria activities. Among them, *Uvaria micrantha* is distributed in Southeast Asia and Australia. However, only one paper revealed the isolated compounds from the stems of *Uvaria micrantha* and their potential effects on anti-leukemia and cervical cancers (Boonsombat et al., 2021). Traditionally, this plant can be used to treat back pain and digestive problems. In this study, 18 folk Vietnamese herbal plants were collected from Ho Chi Minh City, Vietnam. After extraction and partition, the *n*-hexane, *n*-butanol, water, and methanol extracts of these plants were screened for their anti-Alzheimer's disease, anti-osteoporosis, anti-stroke, and anti-inflammatory activities, in collaboration with National Research Institute of Chinese Medicine, Taiwan. This preliminary screening found that VN018 showed good activities in the multiple assays, especially its methanol layer. After gene sequencing and plant specimen identification, VN018 was identified as *U. micrantha*. Hence, the methanol extract of branches and leaves from *U. micrantha* was chosen for further separation, purification, and exploring their potential bioactivities. In the current study, one new and twelve known compounds were isolated (Fig. 1). The activities of the extracts and pure compounds for anti- stroke activity will be shown.



1

Fig. 1. Structure of compound **1**

Keywords: *Uvaria, Uvaria micrantha,* uvarisinyicone, Alzheimer's disease, osteoporosis, stroke, anti-inflammatory

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S3.P17 Ontology based ethnobotany data to support natural product-based drug discovery – growth of data, large-scale insights and case studies

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For millennia and across cultures plants have been used to treat human diseases. Despite efforts to organize this knowledge, attempts have so far been either limited to specific types of traditional medicines or unable to practically support drug discovery tasks. In this work we describe our work of creating ontologies and controlled vocabularies with the aim to achieve interoperability of ethnobotany data with chemical, biological, and medical datasets. This dataset comprises >500m data points, >2m species-disease links, >500,000 natural products, and >34m target-disease links, covering areas from botany, historical use, plant metabolites, bioactivity, to target-disease links. We performed analyses on the data to understand and quantify chemotaxonomy principles as well as to develop real-world use cases of relevance for drug discovery. We investigated to what extent plants within the same genus (or family) contain the same metabolite, as well as to estimate the novelty of the chemistry that can be discovered in each plant (both in terms of absolute novelty, and novelty in a given organism). Furthermore, we established a real-world use case for the pain disease area, and quantified in which way we can re-identify chemistry associated with *in vivo* activity from data across different mode-of-action classes. Overall, compiling ethnobotany information on a large scale allowed us to quantify relationships between historical use, plants, chemistry, compounds, and bioactivity, and to use such data in real-world drug discovery projects. Conflict of interest statement: The authors are employees of Pangea Botanica GmbH, Pangea Botanica Ltd and Wilde Ventures GmbH.

Keywords: ethnobotany, chemotaxonomy, drug discovery, artificial intelligence, machine learning

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S3.P18 Optimization formulation of *Rhodiola rosea* L. nanoemulsion containing natural oil using response surface method (RSM): preparation, characterization, stability test and antioxidant activity

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The oil phase plays a vital role in nanoemulsion formulation. *Rhodiola rosea* L. root extract offers a wide range of pharmacological advantages, such as antioxidative, anti-colllagenase, anti-aging, neuroprotective, anti-inflammatory, cardioprotective, and anti-cancer activities. Sunflower oil, chosen as the natural oil phase, offers various advantages such as its antioxidant properties and its safety for skin application. This study involved preparing 13 variations of *Rhodiola rosea* L. nanoemulsions with sunflower oil and a combination of surfactant and co-surfactant using the spontaneous emulsification method. The nanoemulsion was analyzed for several parameters, such as Particle Size, Polydispersity Index (PDI), and Zeta Potential. The analytical parameters for the nanoemulsion of *Rhodiola rosea* L. were calculated using Design Expert software. The nanoemulsion was optimized using the response surface method (RSM) to determine the optimal amounts of surfactant and cosurfactant. The optimized formulation consists of 21.5% transcutol, 30% labrasol, and 3% sunflower oil. These proportions yield optimal response values of 177.63 nm for particle size,

0.25 for PDI, and -47.69 for zeta potential. The optimized *Rhodiola rosea* L. nanoemulsion exhibits the characteristics of a nanoemulsion, viscosity, stability studies and demonstrates antioxidant activity. Therefore, this study provided the guidelines for a method and combination of formulation stable nanoemulsions for cosmetic and pharmaceutical fields in the future by a combination of surfactant-cosurfactant.

Keywords: nanoemulsion, *Rhodiola rosea* L., response surface method, natural oil

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S3.P19 Oxidoreductase FSP1-mediated redox-regenerating natural product E18C6 potently suppresses ferroptotic cell death

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Ferroptosis, a form of regulated cell death dependent on iron and driven by lipid peroxidation, contributes to degenerative diseases and organ injury (Stockwell, 2022; Angeli et al., 2017; Koeberle et al., 2023). Agents with anti-ferroptotic properties hold great promise in preventing excessive cell death in these pathologies. Bioactivity-guided isolation from 19 traditional Vietnamese medicinal plant extracts identified the natural product E18C6 as a potent anti-ferroptotic compound (EC_{50} = 10 nM) standing out among the most efficient ferroptosis inhibitors described to date. Subsequent screening of 59 structural analogues from our in-house library identified E18C6 as the most potent derivative in this structural compound class. Mechanistically, the oxidoreductase FSP1 converts E18C6 to its reduced form, a potent radical scavenger that efficiently prevents lipid peroxidation and ferroptosis. The FSP1-dependent inhibition of lipid peroxidation was confirmed in the FENIX assay (Shah et al., 2019), which assesses radical scavenging efficiency within phospholipid bilayers *in vitro*. These results strongly suggest that E18C6 acts as a robust inhibitor of ferroptosis through FSP1- dependent reduction to its active radical-scavenging form, with continuous efficient regeneration by FSP1. *In vivo* efficacy of E18C6 was evaluated in a *Caenorhabditis elegans* model employing the GSH-depleting compound diethyl maleate. E18C6 demonstrated a marked reduction in the toxicity associated with diethyl maleate, providing compelling evidence for its efficacy in preventing ferroptosis in an *in vivo* context. In conclusion, our study identified E18C6 as a promising anti- ferroptotic lead that has the potential to prevent extensive pathological cell death in degenerative diseases and inhibit disease progression.

Keywords: ferroptosis, cell death, FSP1, FENIX assay, *C. elegans*, degenerative disease

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S3.P20 Chinese traditional medicine for preventing hair loss in androgenic alopecia mice

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Hair has a crucial role in protecting the skin from detrimental environmental elements, regulating body temperature, and contributing to an individual's personal identity in contemporary culture. The human hair follicle undergoes sequential stages, namely anagen, catagen, and telogen, throughout its lifespan. *Panax ginseng, Angelica sinensis*, and *Ligusticum chuanxiong* are frequently employed in Asia as a conventional botanical treatment for persons afflicted with alopecia. However, the effectiveness of this herbal ingredient has not been conclusively demonstrated. We aimed to ascertain the hair growth-stimulating impact of this botanical compound using an animal model. A cohort of male C57BL/6 mice, aged seven weeks, was randomly assigned to three groups: a blank group, a positive control group, and an experimental group. Each group consisted of five animals, except for the blank group. Each group was given Testosterone 0.05% by subcutaneous injection to induce male alopecia in the first seven days; the positive control group was given Finasteride 1%, the experimental group was given 1% of each extracts. The assessment of hair growth was conducted over a period of 21 days. The herbal mixture demonstrated hair growth-stimulating effects in C57BL/6 mice. The hair regrowth area was 3.04 (±0.6)% for the blank sample, 4.66 (±1.9)% negative control, and 5.98 (±3.8)% positive control, experimental group containing herbal extracts are 5.14 (±1.2) % for *Ligusticum chuanxiong*, 6.42 (±1.2) % *Angelica sinensis* and 7.18 (±2.3) % *Panax ginseng*. *Panax ginseng* has the best effect to improve the hair growth than control group and extracts.

Keywords: Androgenic Alopecia, *Panax ginseng, Angelica sinensis, Ligusticum chuanxiong*, hair growth

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S3.P21 Qualitative and semi-quantitative screening of triterpenes in *Vaccinium uliginosum* L. fruits using supercritical fluid chromatography

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Bog bilberry (*Vaccinium uliginosum* L.) is a small arctic-alpine bush that produces berries similar to commercial blueberries. Hydrophilic constituents of bog bilberries are well researched, but there is sparse information about the lipophilic substances (Vaneková et al. 2024).

Freeze-dried and ground berries originating from Alaska, Norway, Finland, Estonia, Austria, Czechia and Slovakia were defatted with n-hexane and extracted 3x with dichloromethane. These extracts were analyzed using environmentally friendly, ultra-high-performance supercritical fluid chromatography (SFC) device coupled with PDA, ELSD and QDa detectors using supercritical CO_2 and ethanol as mobile phase. A sufficient separation of constituents was achieved on a Waters Torus 1-Aminoanthracene 1.7 µm column in 10 minutes (Goels et al. 2022).

The main constituents of the dichloromethane extract of all berry samples were triterpenes oleanolic and ursolic acid in an approximate ratio of 1:6, respectively. In comparison, a dichloromethane extract of European bilberry (*Vaccinium myrtillus* L.) fruits contains these major triterpenes in a ratio of 1:1. The content of oleanolic and ursolic acid in lyophilized samples ranged from 0.22 ± 0.06 mg/g and

1.04 ± 0.34 mg/g (in Delta Junction, Alaska) to 1.64 ± 0.17 mg/g and 5.67 ± 0.25 mg/g DW (in Seefeld, Austria), respectively. All samples originating from Alaska also contain micromeric acid (20(30) dehydroursolic acid), which is absent in all samples of European origin, aside from a single location in Austrian Waldviertel. All European samples contain a set of plant sterols, likely isomers of stigmastanol and cholestanol, which are absent in the Alaskan samples.

Keywords: *Vaccinium uliginosum,* Ericaceae, triterpenes, SFC

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S3.P22 Matrix free laser desorption ion mobility mass spectrometry in chemometrics: A new approach to the rapid identification of activity markers in complex mixtures of natural products

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Matrix free laser desorption ionization mass spectrometry (LDI-MS) is a highly efficient tool for the quick chemical characterization of complex mixtures of Natural Products (Nps) (Islam et al. 2022). Showing very close structural similarities to compounds used as matrices in matrix assisted LDI-MS, many NPs can be easily ionized by simple laser irradiation. Compared to LC-MS the method yields instant results, works with any volatile solvent, and hardly requires sample preparation (Schinkovitz et al. 2018).

With this in mind, the current presentation highlights recent advances in LDI-MS and its integration into chemometric workflows. As a practical example, the correct prediction and consecutive isolation of three bioactive xanthones (rubraxanthone, isocowanol and parvixanthone) from a crude bark extract of *Garcinia parvolia* (Miq) (Clusiaceae) is presented (Meunier et. al. 2023). All isolated compounds exhibited moderate inhibitory effects on the formation of advanced glycation end products (IC_{50}: 123-170 mM). In addition, the hyphenation of LDI tandem Mass Spectrometry with Ion Mobility Spectrometry (LDI-IMS-MS^2) will be discussed. The latter facilitated the distinct differentiation of xanthone isomers without chromatographic separation and permitted the creation of the first molecular network that is entirely based on LDI-MS^2.

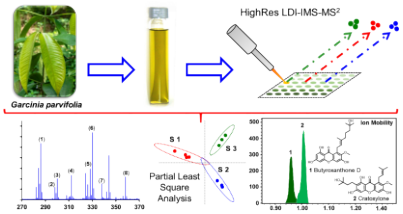

Fig. 1. The concept of high resolution LDI-IMS-MS^2

Keywords: Matrix free laser desorption mass spectrometry, chemometrics, xanthones, ion mobility, molecular networks

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S3.P23 Metabolomic study of tea tree oil and *Melaleuca* spp. using HPTLC, LC-MS and GC-MS

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Tea tree oil (TTO) is an essential oil obtained from the leaves and terminal branches of *Melaleuca alternifolia*, and is widely used in cosmetics, household products, and dermatology treatments (Yadav et al., 2017). The concentration range of the main components in TTO including 15 terpenes and terpenoids is defined by an international standard (International Organization of Standardization, 2017). This study aims to evaluate the quality of commercial TTOs and explore the commercial potential of oils from other *Melaleuca* species using metabolomic techniques for chemical profiling. TTOs purchased from the United States and Kenya, along with terminal branches of *M. salicina* and *M. citrina* were extracted, diluted, and analyzed using high-performance thin-layer chromatography (HPTLC), liquid chromatography-mass spectrometry (LC-MS), and gas chromatography-mass spectrometry (GC-MS). A total of 120 compounds were tentatively identified, including all 15 markers. The results showed that the commercial oils purchased from markets did not meet the international standard in comparison with the TTO provided by Kenyan Forestry Research Institute. The oils from two closely related species, *M. salicina* and *M. citrina*, are distinct from TTO based on their chemical profile. Three polar compounds, grandinol, multifidol, and 3,3-di-*O*-methylellagic acid--*O*-D-glucopyranoside were detected in *M. citrina* using LC-MS, while only the first two compounds were detected in *M. salicina*. None of the samples showed potent antioxidant activity through a DPPH assay on an HPTLC plate.

Keywords: tea tree oil, *Melaleuca spp.*, HPTLC, LC-MS, GC-MS

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S3.P24 Preclinical evaluation of oral nanoformulation based on natural extract as a potential treatment for type 2 diabetes mellitus

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An oral nanoformulation (ONF) based on natural extracts (OBE100), ursolic acid, oleanolic acid, and ursolic acid lactone obtained by hexane: methanol: water 4:3:1(v/v) extraction from *Eucalyptus tereticornis Sm. (Myrtaceae)* leaves reduce metabolic alterations in a diet-induced obese mouse (DIO) model (1)(2). The work aims to develop preclinical safety and biological efficacy tests for the ONF. Three groups were established: vehicle, empty nanoparticles, and ONF OBE100 encapsulated in nanoparticles; 214 mg/kg OBE100 for repeated dose oral toxicity for 28, 90 days and combinate chronic toxicity/carcinogenicity (3). The ophthalmological response to proprioception, threat reflex, and ocular evaluations were performed. At the endpoint, blood samples for hemogram and blood chemistry were taken. All mice were euthanised and necropsied, and samples of the internal organs were taken for histopathological analysis.

Results showed that ONF is biologically effective in decreasing metabolic abnormalities in a DIO model. No clinical abnormalities were recorded during the administration of repeated doses of oral toxicity for 28, 90 days and chronic toxicity. Body weight gain was stable, and all groups' hemograms and blood chemistry analyses were within normal ranges. No signs of neurological or ocular toxicity were recorded during the study. Macroscopically, no lesions were observed in any organ, and the weight of the internal organs was homogeneous for the different treatments.

The findings of acute oral toxicity, oral toxicokinetics, repeated doses of oral toxicity for 28 and 90 days, and chronic toxicity indicate that the compound ONF is safe based on clinical and paraclinical evaluation and preliminary histopathological analysis.

Keywords: *Eucalyptus tereticornis*, nanoformulation, preclinical studies, type 2 diabetes mellitus

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S3.P25 Employing Plant-Bacterial Interactions to Access the Metabolic Capacity of Bacteroidetes

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To improve the discovery of unusual bioactive natural products, it is necessary to explore untapped bacterial taxa. Bacteroidetes are often found interacting with eukaryotic hosts like plants, and several species have been shown to have plant-beneficial attributes (Pan et al., 2023). The metabolic capacity to produce bioactive natural products by this phylum is yet to be explored (Brinkmann et al., 2022). Previous studies prove metabolic impacts from a bacterial perception of plant signals (Adaikpoh et al., 2020). We aim to explore the metabolic and transcriptomic responses of our Bacteroidetes strains to various plant metabolites often found within the rhizosphere. We hypothesize that exposure to plant metabolites will result in the production of natural products not observed under traditional cultivation methods. Phenotypic changes observed in the lab in response to selected plant metabolites could be evidence of significant metabolic changes. Analysis of RNA sequence data in addition to LC-MS/MS datasets and antimicrobial assays of extracts from supplemented cultures will be presented. This project provides data for optimizing culture conditions for the discovery of novel bioactive natural products from Bacteroidetes.

Keywords: plant-bacteria interactions, bacteroidetes, natural products, transcriptomics, metabolomics

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S3.P26 *Epilobii* herba: Quality assessment using chromatographic techniques

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The genus *Epilobium* (willow herb) includes ca. 160 species distributed throughout the world. The most rudely used species are *E. angustifolium* L., *E. parviflorum* Schreb., and *E. hirsutum* L. as herbal teas, which have been reported to have prostate-protective and anti-inflammatory properties (1). However, despite their wide use, these plants are not included in key pharmacopoeias. Only the Herbal Medicines Committee (HMPC) has assessed the botanical drug (2, 3) regarding *E. parviflorum's* medical use, but not in terms of metabolites or analysis.

Here, we focus on a comparative qualitative analysis for *E. hirsutum* herb and the assessment of the homogeneity of samples during a growing season. From April until October 2023 *E. hirsutum* samples (n=78), including leaves and stems, were collected in the United Kingdom. Polyphenols, e.g., chlorogenic acid, gallic acid, caffeic acid, avicularin, guajaverin, isoquercitrin and hyperoside used as reference standards. The analysis was carried out in HPTLC plates Si 60 F_{254} (Merck) in mobile phase: ethyl acetate: formic acid: water (68:8:8) and 2-aminoethyldyphenylborinate and macrogol 400 for derivatization.

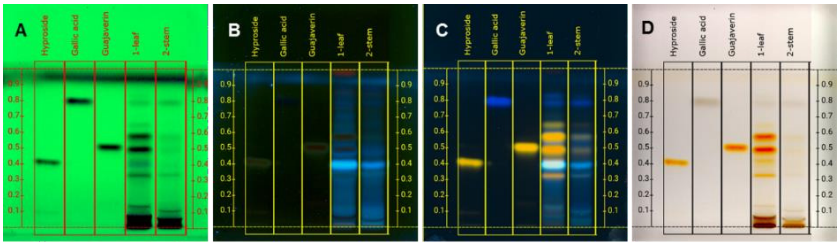

Fig. 1. HPTLC profile under UV 254 nm (A), UV 366 nm (B) prior to derivatization, and under UV 366 nm after derivatization (C), and white light after derivatization (D)

The HPTLC analysis showed all reference substances in *E. hirsutum* extracts but in different concentrations, e.g., yellow fluorescent zones (R_f=0.4; R_f=0.38; R_f=0.85; R_f=0.52) were in line with isoquercitrin, hyperoside, avicularin and guajaverin, and these were the dominant compounds in leaves. The light blue, fluorescent zone (R_f=0.7) was identified as gallic acid, and it accumulated more in the stems (Fig. **1**). The presented method can be used for assessing the *Epilobium* quality, including botanical drugs or finished commercial products.

Keywords: *Epilobium hirsutum,* Onagraceae, quality control, HPTLC

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S3.P27 Phytochemical analysis and quality control study of *Dendrobium fimbriatum* Hook. flowers

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Dendrobium fimbriatum Hook (Orchidaceae) is a renowned edible and medicinal plant in Chinese medicine used traditionally for the treatment of yin deficiency, clearing heat from the body, and nourishing the stomach. A literature review suggests that there has been no major study on pharmacognostic and phytochemical aspects of the flowers.

This study aims to develop an analytical framework for the chemical characterization of the *Dendrobium fimbriatum* flowers in combination with defining basic pharmacognostic parameters. We investigated different extracts/fractions including crude methanol and ethanol extracts sourced from a commercial production site in Taiwan to characterize the phytochemical profile using chromatography (HPTLC, LC and GC), spectroscopic (NMR) and spectrometric (MS) analysis. The samples were analyzed using proton-NMR and carbon-NMR to identify the metabolite composition and to assess similarities and differences between crude methanol and ethanol extracts/fractions. The samples were subsequently analyzed using HPTLC to assess if there were any benefits gained by employing a dual analysis strategy.

No significant difference was found in the chemical composition of both methanol and ethanol extracts/fractions by NMR. Nevertheless, HPTLC detected the existence of metabolites such as terpenes and flavonoid markers, including quercetin, rutin, ursolic acid, and lupeol. The combination of chromatographic, spectroscopic, and spectrometric techniques forms the basis for the authentication and quality control of *Dendrobium fimbriatum* flowers. Further research is ongoing to define the best marker compounds.

Using HPTLC technique to analyze the crude methanol and ethanol extracts of *Dendrobium fimbriatum* flowers showed the presence of terpenes when tested in comparison with markers lupeol and ursolic acid.

Fig. 1. HPTLC of crude MeOH and EtOH extracts of *Dendrobium fimbriatum* flowers.

Keywords: *Dendrobium fimbriatum* flowers, Standardization, NMR, Quality control study, HPTLC

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S3.P28 Identification of chemical defenses of a tropical tree *Sextonia rubra* using molecular networks from GC-MS, LC-MS/MS and MALDI-MS/MS imaging data

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Sextonia rubra is a tropical tree endemic to the Guiana Shield and the Brazilian Amazon. It is known for its heartwood natural durability that comprises numerous lactone derivatives (Rodrigues et al., 2010). However, its fruits have not been studied chemically. Here we propose analytical methods to explore the chemical diversity of these fruits using SPME-GC-MS, LC-HRMS/MS and MALDI-FT- ICR MS imaging. For the first time, GC-MS and MALDI-MS/MS (Levasseur et al., 2024) data were analyzed using molecular networks for efficient annotation using MetGem software (Olivon et al., 2018).

The most abundant volatile compounds identified by SPME-GC-MS in the hydrolate were eucalyptol (13.5%), α calamenene (7.4%), β caryophyllene (7.4%), α copaene (7.3%) and δ cadinene (5.8%). The antimicrobial activity of the hydrolate was tested against pathogens with a relative MIC equal to 5%.

In parallel of chemical analysis, wood decay resistance was assessed using long-term soil bed tests. Ethyl acetate extracts of different parts of the tree were tested against 6 Glutathione-S-Transferase (GST) of *Trametes versicolor*, corollary of their anti-fungal activity (Barbier et al., 2020).

Our results suggest that for pith and heartwood, the higher the concentration ratios of lactones towards alkaloids, the higher the reactivity of GSTs, and the more the natural durability against wood- decaying fungi is positively affected. Although the involvement of lactone derivatives in the natural durability of this species was known, the involvement of alkaloids is new and suggests that *S. rubra* specializes its chemical defenses according to the tissue.

Keywords: *Sextonia rubra,* metabolomic, tandem mass spectrometry, MALDI imaging, molecular network

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S3.P29 An ethnobotanical, pharmacological and phytochemical review on flowers of Shi-hu (*Dendrobium* spp.)

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The genus Dendrobium is a medicinal plant with nutraceutical importance. Dendrobium species have been used to nourish "Yin" and tonify the five viscera in traditional Chinese medicine. The Taiwan Herbal Pharmacopoeia (4th English Edition,2022) includes the fresh or dried stem of seven Dendrobium species (Shi-hu in Chinese) in the monograph. However, it is not clear how the flower part of these seven species could be used.

We reviewed various aspects of the chemical composition and pharmacological characteristics of Dendrobium flowers aiming at defining future research and development priorities. Additionally, traditional uses, conservation status and cultivation techniques relevant to ascertain a sustainable use of the species were summarized.

Web of Science, SciFinder, Scopus, PubMed, Google Scholar and CNKI were used to gather data on the phytochemistry and pharmacological studies of Dendrobium flower usage. The keywords for this study are Dendrobium, Pharmacology and Chemistry.

Open column chromatography, GC-MS, UPLC, HPLC and GC-FID are common separation and analytical techniques that have been utilized for the isolation, characterization and quantification of polysaccharides, alkaloids, essential oils, and flavonoids. They have prominent pharmacological/biological activities like pro-apoptotic, immune-modulatory, antioxidant and anti- microbial activities. The most investigated species of flowers are *D. officinale* Kimura & Migo. and *D. chrysanthum*Wall.ex Lindl.

This study sheds light on the historical uses of Dendrobium flowers, their current clinical significance and future potential**.** The study systematically assesses the link between chemical composition, pharmacological activity and current or potential uses. According to our findings, in-depth investigations are needed in areas where there is a paucity of knowledge on the potential of flowers, such as quality control, phytochemical characterization, and dosage form development.

Acknowledgment

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S3.P30 Patients' use of medicinal plants in the urology service of Ibn-Sina hospital in Rabat, Morocco: integrating ethnobotanical and pharmacovigilance approaches

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Phytotherapy is a very old medical discipline, based on the use of medicinal plants or traditional pharmacopoeia products to treat various pathologies. It depends on the socio-economic context, and given the wealth of medicinal plants in Morocco, the use of phytotherapy has only increased (Bouzouita, 2016). While popular belief assumes that herbal remedies are natural and therefore mistakenly considered harmless, scientific research has shown that these plants can contain powerful chemical compounds that can induce intoxication and serious, even fatal, side effects. The aim of this work is to carry out an ethnobotanical study on the use of medicinal and aromatic plants and tradiional Moroccan pharmacopoeia products among patients with disorders and diseases of the urinary system, in the urology department of the Ibn Sina hospital in Rabat. The Rabat-Salé-Kenitra region is one of the 12 regions of Morocco, created in 2015 following the new territorial division of the regions. It is limited to the north by the region of Tangier-Tetouant-Al Hoceima, to the southeast by the region of Fez-Meknes and to the south by the two regions of Casablanca-Settat and Beni-Mellal-Khénifra and to the west by The Atlantic Ocean. This region covers a total area of 17,569 km², or 2.5% of that of Morocco (3). Note that the study region was chosen for the traditional know-how of its local population (4) and for its floristic and ecological diversity (2). A semi-structured questionnaire was used, targeting patients in the urology department of the Ibn Sina Rabat hospital. Data was collected from 649 patients, in full respect of patient confidentiality and anonymity, and the questionnaire used consists of 44 questions and four items: patient identity, patient pathology, use of medicinal plants, and adverse effects (AEs) related to the use of plants. Although the questionnaire was written in French, oral interviews with patients were conducted in Moroccan dialect. In addition to completing the questionnaire designed for this purpose, additional information was recorded for each day of the survey, namely:

- the total number of patients hospitalized;
- the total number of consulting patients
- the total number of patients who refused to take part in the survey;
- the total number of patients who took part in the survey but did not use plants to treat their illness;
- the total number of patients who took part in the survey and used herbs to treat their illness, but did not experience any adverse effects as a result;
- the total number of patients who took part in the survey and who used plants to treat their illness and who experienced undesirable side-effects as a result.
- patient encountered in the various health facilities surveyed;
- patients who voluntarily agreed to take part in the survey;
- patients who use plants alone or in mixtures with or without prescribed conventional treatments.
- patients who have experienced undesirable effects as a result of using plants.

For socio-demographic data, the age ranges used in this study were established with reference to the age range classification provided by the WHO in its latest World Diabetes Report (WHO, 2016). Among 656 patients, 29,88 % used medicinal plants. Floristic analysis of the results obtained identified 37 species belonging to 23 plant families. 23 patients from the urology department reported the occurrence of one or more AEs following the use of medicinal plants, with a prevalence of 3.54%. 15 plants linked to these AEs were listed. *Eruca sativa* L. was associated with 3 (13,04 %) cases of AEs, and *Petroselinum crispum* L. with 2 (8,69 %).

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S3.P31 Unveiling the multifaceted efficacy of Gu Sui Bu in osteoporosis treatment: insights from a zebrafish model and advanced analytical approaches

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Osteoporosis significantly increases the risk of fractures and adversely affects the quality of life, particularly in the elderly. Existing osteoporosis treatments are often expensive and may lead to severe side effects with long-term use (Peng et al., 2021). In this scenario, herbal medicine, celebrated for its diverse bioactive components and multifaceted action, presents a promising alternative with potentially fewer side effects. Specifically, Gu Sui Bu (GSB), the dried rhizome of *Drynaria fortunei* J. Sm., is acclaimed in traditional Chinese medicine for its bone-healing capabilities. Our prior research using a zebrafish model has revealed GSB's dual role in reducing bone resorption and enhancing bone formation (Peng et al., 2022). Zebrafish, as an *in vivo* model, are instrumental in assessing both the direct and indirect effects of the drug, providing comprehensive insights into its mechanisms. To decipher GSB's complex therapeutic mechanisms, we implemented a sophisticated multidimensional analytical approach, including RNA-seq analysis, a network pharmacology approach, and molecular docking for the active ingredients of GSB on the zebrafish osteoporosis model. By comparing the gene expression profiles before and after the treatment with 1 mg/ml GSB in a glucocorticoid-induced osteoporosis zebrafish model, we identified 469 genes whose adverse expression changes due to glucocorticoid treatment were reversed by GSB. According to the results of Gene Ontology enrichment analysis, genes involved in osteoblast differentiation and ossification were identified. Among them, TMEM119 particularly piqued our interest; it could stimulate the expression of *runx2*, *sp7*, *collagen 1a1*, *osteopontin*, and *alkaline phosphatase*, crucial for osteoblast differentiation. The expression of *TMEM119* decreased 1.6-fold after dexamethasone treatment but returned to normal levels after GSB treatment. In addition to exploring GSB's effects using RNA-seq analysis, we also employed network pharmacological analysis to investigate the direct targets of GSB. We collected 117 chemical ingredients of GSB from TCM- ID, LTM-TCM, INPUT 2.0, and TCMSP databases, and predicted 1011 possible action targets using the Swiss Target Prediction and Similarity Ensemble Approach web servers. Interestingly, over-representation analysis revealed key signalling pathways, such as Hedgehog and FGF, as potential GSB targets to activate *runx2* expression for osteoblast differentiation. We further performed molecular docking to demonstrate that Naringin, an active ingredient in GSB, could bind to FGF receptors. Considering the multi-active component and multi-target nature of herbal drugs, adopting a multidimensional analytical approach could facilitate the understanding of the action mechanisms of herbal drugs and establish a foundation for the innovative development of cocktail therapy, combining prescription drugs with herbal medicine. This aims to improve treatment outcomes and reduce side effects in clinical practice.

Keywords: *Drynaria fortunei,* herbal medicine, osteoporosis, osteogenesis, zebrafish

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S3.P32 Phytochemical profiling and the wound healing potential of five *Bulbine* species using *in vitro* and *in vivo* assays

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Traditionally, the leaf exudates of *Bulbine* species are used to treat skin conditions (Bodede and Prinsloo, 2020), which has led to the development of bulbine-containing cosmetic products. However, scientific evidence to support the claimed benefits is still lacking, hence the need for evidence-based methods to investigate the medicinal properties of some indigenous *Bulbine* species. In the current study, the *in vitro* scratch assay and *in vivo* zebrafish caudal fin amputation assay were used to evaluate the wound healing properties of five ethnobotanically important species; *Bulbine abyssinica*, *B. asphodeloides*, *B. frutescens*, *B. latifolia* and *B. narcissifolia*. Phytochemical profiling was achieved using ultra-performance liquid chromatography coupled to mass spectrometry, and knipholone was identified as the marker compound which varied quantitatively across the five species. Pharmacologically, *B frutescens* and *B. abyssinica* (100 µg/mL) extracts accelerated HaCaT cell migration and subsequent wound closure in the scratch assay (96% and 94% acceleration after 24hrs, respectively). Similarly, the two species increased caudal fin regeneration by 90% and 83% after 72 hrs for *B. frutescens* and *B. abyssinica*, respectively. The results of the study align with the traditional use of *B. frutescens* and *B. abyssinica* in wound healing, which could inform further research for cosmetic and pharmaceutical product development involving these species.

Keywords: *Bulbine* species, wound healing, zebrafish larvae, scratch assay, knipholone

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S3.P33 Translating community-wide mass spectral libraries into actionable chemical knowledge: a proof of concept with monoterpene indole alkaloids

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Mass spectral libraries play a key role in annotating metabolite features in untargeted metabolomics profiles. Here, we will present the latest update of the MIADB (Fox Ramos et al., 2019), a public spectral library containing fragmentation spectra of more than 400 unique monoterpene indole alkaloids (MIAs) and the tools we developed to extract chemical knowledge from it.

MIAs represent one of the most complex and rich classes of natural products with more than 4000 representatives to date. This chemical class is also renowned for the valuable biological activities associated with some of its representatives such as vinblastine, quinine and camptothecin. One objective of this collaborative project was to chart the MS/MS spectral space of this chemically diverse library using several mass spectral similarity scores. We used molecular networking and heatmaps to map those similarities. MIAs were first classified according to their structural similarity, determined by Tanimoto scoring. As anticipated, compounds with the highest chemical similarities generally demonstrated greater MS/MS similarities, irrespective of the chosen spectral similarity score. For each MIA skeleton, the commonly shared mass fragments and neutral losses were then extracted and used as MassQL (Jarmusch et al., 2022) queries against a dataset of 71 MIAs-producing plants and 4 non-producing plants adding a further layer to the many available dereplication tools. Finally, this database was confronted to state-of-the-art MS/MS annotation tools. Overall, we believe that the MIADB is a great example highlighting a general strategy for extracting spectral and chemical information from a variety of metadata-informed spectral libraries.

Keywords: Metabolomics, spectral library, monoterpene indole alkaloids, chemo-informatics, MS/MS

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S3.P34 Non-targeted profiling analysis by GC/MS and LC/MS/MS of extracts derived from *Eucalyptus tereticornis* with antiobesogenic and antidiabetic activity

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Previous studies have shown that triterpene-enriched natural extracts (OBE100), ursolic acid, oleanolic acid, and ursolic acid lactone obtained from *Eucalyptus tereticornis Sm (Myrtaceae)* leaves, reduces metabolic alterations in a diet-induced obese mouse model (1). The present work aims to chemically characterize the additional molecules in the OBE100 to obtain a standardized extract.

The chromatography conditions for HPLC measurements were using a column for polycyclic aromatic hydrocarbons (PAHs, Agilent) of 5µm pore and readings at 210nm wavelength on diode array detector.

Gas chromatography (GC) and Liquid chromatography (LC) coupled with mass spectrometry (MS) were utilized to identify constituents present in OBE100. The GC–MS analysis involved a derivatization step using bis(trimethylsilyl)trifluoroacetamide (BSTFA) for polar compounds prior to MS analysis. The full Scan method was applied to screen and identify small molecular metabolites (<850 Daltons) by comparing spectral data with the NIST 14 Mass Spectral Library. GC–MS analysis was developed using a mass spectrometer (Agilent 7890/MSD 5975C) equipped with a capillary column (HP-5MS Agilent). The results revealed the presence of over 140 small acids, alcohols, hydroxy acids, sugars, fatty acids, sterols, and terpenoids. The LC–(ESI+)–MS/MS was developed using an HPLC-QTOF (Bruker Impact II). A tentative identification of principal constituents was achieved by analyzing the MS/MS data and exact mass. Compounds identified include germacrene, glycyrrhetaldehyde, loxanic acid, dehydroursolic acid lactone, sirenin, ophiopogonone B, 1,3,6- trihydroxy-8-(3-methylbutyl) anthraquinone, coumafuryl, 2-O-E-p-coumaroyl alphitolic acid, ursolic acid, ursolic acid lactone, and oleanolic acid. These compounds have also been reported previously in other *Eucalyptus* species.

Keywords: *Eucalyptus tereticornis,* antiobesogenic, antidiabetic, chemical profiling analysis

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S3.P35 Design, synthesis and screening of novel prenylated chalcones as optimised anti-cancer agents

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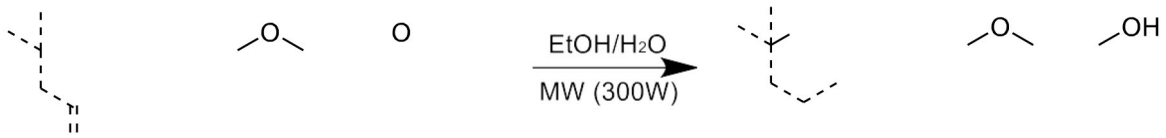
The anti-cancer potential of simple chalcones has been an intense area of research for some years, which has allowed structure-activity relationships to be well defined. Although many chalcones display cytotoxic properties, important predictors of activity are the presence and position of hydroxy and methoxy groups. An important example is the liquorice metabolite, licochalcone A (LA), a prenylated chalcone. LA has been shown to have interesting cytotoxic activity against glioma (U87) cells, at concentrations of >5 μM. LA and related prenylated chalcones thus represent potentially valuable lead compounds. To prepare novel chalcones derived from LA, we reacted *O*-prenylated aldehydes with various acetophenone derivatives, substituted at positions 3′ and 4′ of the phenyl ring. We also replaced the phenyl ring with heteroaromatic systems. Rearrangement of the novel *O*- prenylated chalcones via 3,3-sigmatropic reaction resulted in the novel licochalcone A analogues **(Scheme 1).**



Scheme 1. [3,3]-sigmatropic rearrangement reaction.

This poster presents the synthetic methods developed to prepare the aldehyde and ketone precursors of a family of LA and prenylated chalcone analogues, and to describe the reaction conditions necessary for the condensation of these precursors. Requisite prenylated aldehydes were condensed with either monocyclic acetophenone derivatives or various heterocycles (including chromans and chromanones), with a key subsequent reaction being Claisen rearrangement of the substituted alkenyls. The resultant products represent novel heteroaryl systems, some of which display interesting fluorescence properties. We further show the results of cytotoxicity testing of the compounds on brain cancer cell lines, including the U87 cell line, revealing initial structure-activity relationships.

Keywords: prenylated chalcones, licochalcone, condensation, cytotoxicity, glioblastoma

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S3.P36 Does the combined effect of natural abietane diterpenes with temozolomide have therapeutic potential against glioblastoma?

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Glioblastoma (GB) is the most aggressive and malignant glioma of the Central Nervous System. GB standard of care involves surgical resection followed by radiotherapy and chemotherapy (Temozolomide-TMZ). However, these treatments are often ineffective. Despite intensive efforts, less than 10% of patients survive beyond 5 years, due to late diagnosis, poor prognosis, and lack of effective therapies. Concerning this, there is a growing interest in exploring new molecules isolated from natural sources.

In this work, we investigated the potential of combined therapy using two abietane diterpenes with TMZ in U87 cells. We isolated the abietane diterpenes 7alfa-acetoxy-6beta-hydroxyroyleanone (Roy) and Parvifloron D (ParvD) from the acetonic extract of *Plectranthus hadiensis* Schweinf. and *Plectranthus ecklonii* Benth., respectively. After, we assessed the cytotoxic/antiproliferative activity of co-administration of Roy (16-64 μM) or ParvD (4-14 μM) with TMZ (100-500 μM) using Alamar Blue® assay. Cell death and cell cycle regulation was analyzed by flow cytometry. This work evidenced that treatment with these natural compounds alone is comparable with those observed in cells treated with the co-administration approach, with strong synergistic effects only emerging at higher concentrations of TMZ. Also, Roy and ParvD induced apoptosis and cell cycle arrest at S/G_2M phase, which was also observed after a co-administration at high concentrations of TMZ. Surprisingly, our results unveil that both natural compounds could be used in monotherapy, showing no advantages at co-administration with TMZ. Roy and ParvD emerge as promising drug leads for future therapeutic strategies against GB, rather than only being adjuvants to the existing chemotherapy.

Keywords: glioblastoma, *Plectranthus* L'Hér., abietane diterpenes, temozolomide, antitumor effect

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S3.P37 Assay-directed method development for natural product purification

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Natural products are an important source of lead compounds in drug discovery research, presenting such unique challenges to drug discovery as screening, purification, and identification. Purification can be streamlined using Assay-Directed Method Development, where small extractions and scouting gradients are used to determine an efficient purification strategy.

Extraction and dissolution solvents are used to select initial column media and solvents in liquid chromatography. The retention times of active fractions collected from scouting gradients are used to calculate efficient focused gradients using tools such as the Time-on-Target algorithm. Scouting gradient active-fraction retention times are used for assay-directed method development for liquid chromatography and supercritical fluid chromatography. Assay-directed method development allows rapid purification of active compounds from extracts by providing data that enables decision-making about extraction solvents, column and mobile phase selection, and efficient chromatography gradient methods.

Keywords: chromatography, purification, method development, extraction, *assay*

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S3.P38 Comparative analysis of the anti-inflammatory properties of nanoparticles derived from the medicinal plants *Zingiber officinale* (Ginger) and *Caralluma edulis* (Chunga), in the treatment of Inflammatory Bowel Disease

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Inflammatory Bowel Disease (IBD) is a chronic inflammatory condition affecting the gastrointestinal tract of patients (Baumgart and Carding, 2007). Diverse approaches have been proposed to develop novel treatments for IBD, including targeted delivery of therapeutics using nanomedicine and/or nanoparticles. Recently, plant-derived exosome-like nanoparticles (PENs), a group of multifunctional nanoparticles, have been explored for their potential as drug delivery vectors, in addition to having intrinsic biological activities (Kim et al., 2021). Ginger-derived nanoparticles (GDNPs) are one of the more investigated PENs, while Chunga nanoparticles (ChNPs) have recently been isolated by our group. The aim of this study was to evaluate and compare the anti-inflammatory properties of both PENs *in vitro*.

Both PENs were isolated using a top-down approach. An inflamed intestinal epithelial barrier of Caco-2 cells was established using a cocktail containing pro-inflammatory cytokines. Inflamed monolayers were treated with different sizes of GDNPs and ChNPs. Confocal Laser Microscopy, transepithelial electrical resistance (TEER) monitoring and cytokine quantification were used to compare the effects of these nanoparticles on the monolayer.

Nanoparticles of different average particle sizes were isolated from both parent plants. Inflamed monolayers treated with ChNPs had the highest increase in TEER value among the various nanoparticles compared. ChNP treated cells also showed a higher reduction in pro-inflammatory cytokines when compared to GDNP treated cells.

Overall, this study showed that ginger- and Chunga-derived PENs have anti-inflammatory effects on the inflamed intestinal Caco-2 epithelial barrier *in vitro*. The results of this study also suggest that ChNPs may exhibit stronger anti-inflammatory effect than GDNPs.

Keywords: *Zingiber officinale, Caralluma edulis*, multifunctional nanoparticles, Inflammatory Bowel Disease, intestinal epithelial barrier model

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S3.P39 Isolation, characterization, and bioactivity of compounds from the stem bark of *Prunus africana*

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Prunus africana is a forest tree that grows on hillsides and flourish in humid, semitropical, and tropical central areas (Komakech et al., 2017), and a wide range of therapeutic benefits have been reported (Ting et al., 2014). However, limited information on the bioactive compounds responsible for specific therapeutic effects is available. Thus, several antioxidant and cytotoxic compounds were isolated from the DCM and methanol extracts of the stem bark of *Prunus africana* via a bioassay- guided approach.

The crude DCM extract showed the highest cytotoxicity against MCF7, CEM, HeLa, and BJ cell lines, with IC_{50} values of 3.5 ± 0.4 µg/mL, 17.1 ± 2.0 µg/mL, 12.9 ± 3.8µg/mL and 23.7 ± 1.4 µg/mL, respectively. The MeOH crude extract with a total phenol content of 381 mg GAE/g dry weight and a total flavonoid content of 67 mg QE/g dry weight demonstrated the best antioxidant activity. The DPPH and FRAP antioxidant tests were used and IC_{50} values of 26.94 µg/mL and 77.28 µg/mL were obtained, respectively. Compounds **1**, **2** and **3** were isolated from the DCM extract, and compounds **4** and **5** from the MeOH extract (Fig. 1)

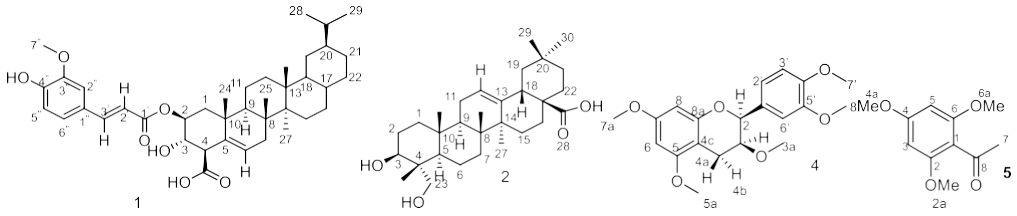

Figure 1: Structures of compound **1**, **2**, **3**, **4** and **5**

Against HeLa and MCF7 cells compound **1** had the best cytotoxic activity, showing percentage cell proliferation inhibition of 4% at the test concentration of 16.7 µg/mL for the HeLa cells, and cell proliferation inhibition of 15% against MCF7 at a concentration of 50 µg/mL. Computational docking calculations confirmed these results and suggested that the extract and some isolated compounds of *P. africana* has the potential to be developed as anti-cancer preparations or drugs.

Keywords: *Prunus africana*, antioxidant, cytotoxicity, anticancer activity

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S3.P40 Lanostane triterpenoids of *Inonotus obliquus* are modulators of inflammation regulators ROR_γ and GPBAR1

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The sclerotic conks of the birch-parasite mushroom *Inonotus obliquus* (Fr.) Pilát called chaga, have a rich history in traditional medicine across various cultures, with applications ranging from adaptogenic properties to cancer and inflammation treatment (Duru et al., 2019). In light of its historical use in traditional medicine, we included two *I. obliquus* extracts to screen for new natural modulators of GPBAR1 and RORγ, promising targets in anti-inflammatory research (Ladurner et al., 2021, Pols, 2014).

The two extracts, differing in lipophilicity, were obtained via supercritical liquid extraction. The lipophilic extract was acquired using CO_2 with 10% EtOH, while the more hydrophilic extract was obtained with CO_2 and 20% EtOH. Our screening revealed distinct activities: the lipophilic extract inhibited RORγ (0.6-fold at 10 µg/mL), while the hydrophilic extract activated GPBAR1 (8.8-fold at 30 µg/mL). To identify the different compounds contributing to these effects, we performed a dereplication with UPSFC-ESI-MS. The tentatively identified constituents were forwarded to molecular docking which indicated lanostane triterpenoids as active principles. Subsequently, we isolated seven lanostane triterpenoids along with four additional compounds and tested them for RORγ and GPBAR1 modulation. Notably, the lanostane triterpenoids inonotsutriol A and inonotsutriol epoxide demonstrated potent and concentration-dependent inverse agonistic activity with IC50s of 1.3 µM and 1.8 µM in a luciferase assay employing RORγ-Gal4. Additionally, trametenolic acid B was identified as a modest GPBAR1 activator.

These findings contribute to a better mechanistic understanding of the pharmacological profile of *I. obliquus*, shedding light on its potential mechanisms of action against inflammatory diseases.

Keywords: *Inonotus obliquus, RORγ*, GPBAR1, lanostane, supercritical fluid chromatography

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S3.P41 COSMO-RS as cutting-edge technology for design and pre-screening of Natural Deep Eutectic Solvents for selective extraction: Spirulina as case study

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The aim of this work is to demonstrate the selective extraction of high value compounds of nutraceutical or cosmetic interest from Spirulina using an advanced methodology combining experimental and simulation approaches. The SIMBA laboratory uses NaDES (Natural Deep Eutectic Solvents) to extract these compounds. NaDES are combinations of two or three primary plant metabolites that are considered biocompatible (Dai et al., 2013). NaDES are also known for their versatile selectivity, which can be modulated by the nature or ratio of components that enter into NaDES composition.

NaDES modulation was previously achieved experimentally, resulting in time-consuming and chemical-intensive procedures. However, this work introduces a dual approach that combines solvent restriction regulation with the utilization of simulation tools, such as COSMO-RS (Conductor-like Screening Model for Real Solvents) method, as an innovative pre-screening tool to achieve this modulation. By determining *ab-initio* key descriptors such as 3D shape, charge density, and sigma profiles onto the molecule surface for each pre-selected bio-compatible compound, we were able to formulate NaDES compatible with our application *in-silico*. This approach saves time and minimizes the ecological impact of our experimental trials.

Several NaDES based on octanoic acid were selected for experimental testing to validate this screening approach. Furthermore, experimental works were done to not only validate the simulated eutectic window but also to determine if the eutectic ratio was required to achieve optimum recovery. Additionally, various experimental conditions were tested to guide process selection and define the best spirulina extraction conditions for future scale-up.

Keywords: Simulation, COSMO-RS, Green Chemistry, NaDES, extraction

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S3.P42 Tetrahydrofuran sesquiterpenes and the bronchodilator effect of *Artemisia judaica*

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Bronchodilators are employed for both acute and ongoing treatment of respiratory ailments such as asthma and chronic obstructive pulmonary disease (Barnes, 2010). The genus *Artemisia*, belonging to the family Asteraceae, is a large diverse genus with approximately 500 species. *Artemisia judaica.* is an aromatic perennial herbaceous plant. In Saudi Arabia, *A. judaica* is commonly used for treating bronchitis and bronchial asthma. Other species of *Artemisia* have been found to have beneficial effects in treating asthma and epilepsy (Sapkota, 2008). The previously reported secondary metabolites identified in *Artemisia* species are sesquiterpene lactones, flavonoids and essential oil (Abou El Hamd, et al. 2010). The objective of the present study is to provide scientific proof supporting the bronchodilator effects of *A. judaica.* isolating, identifying its active ingredients and exploring the mechanism of the bronchodilator effect. The bronchodilator effect was assessed using isolated guinea-pig tracheal tissues. Bronchoconstriction was induced by carbachol (CCh,1 M), and the inhibitory effect of the plant extract and fractions was tested against these induced contractions. The findings demonstrated that the petroleum ether fraction had greater potency (0.5 mg/ml) in inhibiting CCh-induced contraction compared to the other fractions. Several tetrahydrofuran sesquiterpenes (**1-6**) (**Fig. 1**) were obtained by bioassay-guided fractionation employing various normal CC and reversed phase HPLC chromatographic techniques. The identification of the isolated compounds was achieved using spectroscopic techniques, such as HRESIMS, 1D and 2DNMR. Compounds **5-7** are reported here for the first time. An investigation was also conducted to investigate the mechanism of action of the isolated compounds. The bronchodilator effect involved the activation of multiple K^+ channels.

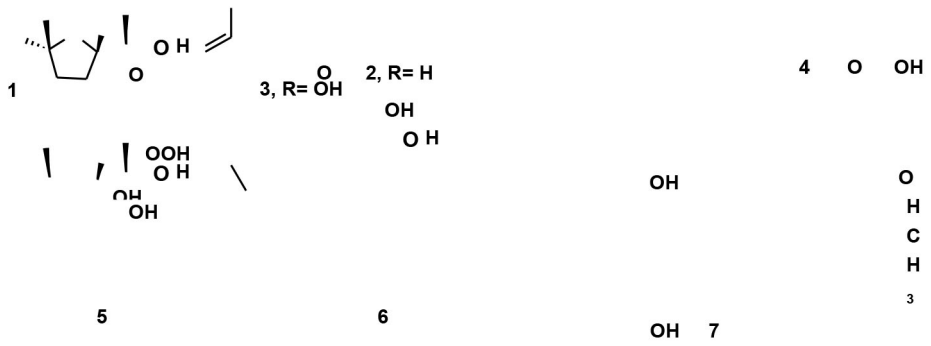

Fig. 1. Structures of isolated sesquiterpene

Keywords: *Artemisia judaica*, Asteraceae, bronchodilator effect

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S3.P43 Development of non-targeted metabolomic approaches for the screening of bioactive molecules in *Clematis* species

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Recent advancements in analytical techniques and data treatment have led to the development of new workflows for discovering natural bioactive compounds. UHPLC-HRMS/MS crude extract profiling allows rapid and sensitive analysis of small amounts of plants, but annotation of complex mixture, even using molecular networking (MN) (Wang et al., 2016) can be time-consuming. Furthermore, increasing annotation confidence is a challenge, requiring the integration of diverse approaches, such as implementing taxonomic information through filters, scoring (Tima-R) and comparison to experimental or predicted databases (Rutz et al., 2019).

Clematis genus, third largest of Ranunculaceae, is widespread throughout the northern hemisphere, used in traditional medicines or ornamental purposes. These different uses can be explained by a wide diversity of compounds leading to different bioactivities (Chawla et al., 2012). To highlight phytochemical differences and identify potential way of valorizing the huge biomass from these crops, 10 species of *Clematis* were analysed by UHPLC-HRMS/MS. Data were represented using MN, and annotation tools as SIRIUS4 (Dührkop et al., 2019) and Tima-R were employed to refine the compound identification.

Shared clusters of O-glycosylated flavonoids, triterpenoids and lignans had been determined between the 10 species. However, specific clusters had been also identifying for different species for example acetylated O-glycoside flavonoids for *C. chinensis* and C-glycoside flavonoids for *C. viticella* which may lead to a modulation of the bioactivity of plant extract (Xiao et al., 2016), while *C. koreana*, is the only species with a chemical profile rich in alkaloids directing to a different way of valorization.

Keywords: *Clematis* genus, metabolomics, mass spectrometry, molecular network, bioactivity

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S3.P44 Semisynthetic pyrazole ring containing hydrocurcumin derivatives as potential new antitumor agents

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Hydrocurcumins occur in the rhizomes and leaves of *Curcuma longa*, as well as some other species of the Zingiberaceae family. They are reduced metabolites of the deeply studied diarylheptanoid curcumin, the main bioactive compound of the well-known eastern spice and traditional medicine curcuma. Despite the promising effect of curcumin, it has not become a modern medicine due to its very poor pharmacokinetic properties. Hydrocurcumins possess much better bioavailability, and in many cases have similar bioactivities, so they have a higher drug discovery potential (Girst et al., 2021).

Tetrahydrocurcumin (4HC) inhibits tumour metastasis and tumour angiogenesis in mouse models. Synthetic modification of the diketo moiety of 4HC has led to pyrazole containing antiproliferative agents (Mahal et al., 2017).

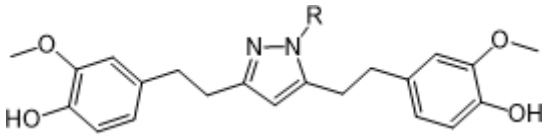

Fig. 1. General structure of pyrazole-containing hydrocurcumins

In this study, our aim was to synthesise new, more potent nitrogen-containing hydrocurcumin derivatives, as well as the most potent one from Mahal's work, and investigate *in vitro* their cytotoxic activities in different cancer cell lines (HeLa, A2780 and MCF-7).

Synthetic reactions were followed by chromatographic purifications (flash and HPLC) and the structures were confirmed by one- and two-dimensional NMR and HRMS measurements. Starting from curcumin, in this project 9 nitrogen-containing hydrocurcumin derivatives were synthesised, among which 6 are newly described. The A2780 ovarian cell line was found to be the most sensitive to them, with the best compounds having IC_{50} values of 11.2 and 11.4 μM.

Keywords: curcumin, hydrocurcumins, semisynthesis, pyrazole, ovarian cancer

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S3.P46 A decision theory-based aggregation tool for tandem mass spectrometry mutli-annotation outputs

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The accurate annotation of molecular structures in metabolomics is crucial across diverse chemistry fields, marked by explosive growth and ongoing improvements. Predictions of MS/MS spectra and chemical class using in silico approaches have emerged as powerful complementary tools for annotation. Breakthrough strategies include molecular networking (GNPS (Nothias et al., 2020)), fragmentation trees (SIRIUS (Dührkop et al., 2019)), artificial neural networks (ISDB (Wang et al., 2021)), and chemical ontology. However, the increasing number of outputs generated by these tools poses challenges in prioritization for natural product chemists. Thus, there's an urgent need for a tool that processes outputs from common annotation tools (e.g., GNPS, SIRIUS, ISDB) to compute a "knownness" score. This score would aid chemists in targeting new compounds effectively. This communication presents the development of a decision-support tool for the in-depth analysis of multi-annotation of tandem mass spectrometry data in natural product chemistry. We will utilize decision theory modeling and a decision-making approach to design a function that translates what the annotation tools say about the similarity of an MS/MS spectrum to known molecules. With this aggregated knownness score, natural product chemists can evaluate if a molecule warrants isolation and further structure elucidation efforts. Real-case scenarios, inspired by previously published phytochemical reports, will be further assessed from the angles of decision theory and targeted isolation.

Keywords: Annotation tools, multi-informative data, decision theory, aggregation

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S3.P47 *Plectranthus*: an important source of bioactive diterpenoids for therapeutic applications

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Plectranthus spp., commonly used in traditional medicine, offer a rich source of bioactive abietane diterpenoids, with diverse biological activities supported by recent scientific research. (Matias, 2016). Several approaches utilizing bioactive lead molecules from *Plectranthus* spp. for therapeutic applications are reported here. Examples include Parvifloron D (ParvD) from *P. ecklonii* and 7α- acetoxy-6β-hydroxyroyleanone (Roy) from *P. grandidentatus*. One notable instance involves the patented diterpenoid dibenzoylroyleanone (RoyBz; Bessa, 2016), derived from natural Roy. RoyBz has been identified as a PKC-selective activator, exhibiting potent anti-proliferative effects in colon cancer by triggering a PKC-dependent mitochondrial apoptotic pathway. Remarkably, the PKCδ- dependent anticancer activity of RoyBz was confirmed in vivo using xenograft mouse models of both control and PKCδ-knockdown human colon cancer cells (Bessa, 2018). Further investigation delves into the molecular mechanism underlying Roy-Bz's reported antitumor activity, particularly its impact on glucose metabolism in colon cancer cells (Bessa, 2023). This study sheds light on the role of PKC in tumor cell metabolism and underscores Roy-Bz's potential to target ATP-producing pathways of glucose metabolism in cancer cells, bolstering its candidacy as an anticancer agent.

Keywords: *Plectranthus,* Lamiaceae, diterpenoids, cancer, PKC

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S3.P48 Characterization of volatile and non-volatile compounds in *Larix decidua* (Pinaceae) oleoresin and its nanoemulsion development

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Chronic wounds continue to present a significant medical challenge (Falanga et al., 2022). Among natural products, *Larix decidua*'s oleoresin has long been recognized for its wound-healing properties and has traditionally been incorporated into ointment formulations (Batista et al., 2022). However, as modern pharmaceutical formulations increasingly favour emulsions for encapsulating lipophilic compounds, there is a growing interest in utilizing this technology to deliver the benefits of oleoresins. Thus, this study aimed to characterize the phytochemical profile of volatile and non-volatile compounds in the European Larch oleoresin, and to develop a nanoemulsion containing it as an innovative approach to delivering it.

Gas chromatography-mass spectrometry analysis revealed the volatile and non-volatile chemical composition of the oleoresin (Fig. 1). The monoterpenes alpha-pinene (**1**; >70%), beta-pinene (**2**; >8%), and limonene (**3**; >5%) were the major volatile compounds. The labdane-type diterpenes larixyl acetate (**4**; >23%) and larixol (**5**; >5%), and the pimarane diterpene isopimaric acid (**6**; >17%) were identified as the major non-volatile compounds.

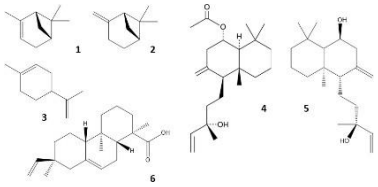

Figure 1. Structure of the main monoterpenes and diterpenes in the *Larix decidua* oleoresin.

A nanoemulsion comprising 10% oleoresin was developed. Two sterilization methods, membrane filtration and X-ray exposure, were employed, and the resulting nanoemulsions were evaluated over 14 weeks. The formulation subjected to sterile membrane filtration without X-ray exposure demonstrated optimal characteristics for further investigation. Throughout the evaluation period, the droplet size (~200 nm), PdI (<0.2), pH (~4.2), and the spherical morphology of the droplets were observed. The developed nanoemulsion effectively encapsulates the oleoresin from *L. decidua*, demonstrating potential for treating chronic wounds.

Keywords: *Larix decidua,* nanoemulsion, diterpenes, monoterpenes, GC-MS

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S3.P49 *In vitro* antihypertensive effects of *Viscum album* mother tinctures in kidney proximal tubule cells

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Alcoholic extracts (mother tinctures) from *Viscum album* (Santalaceae), a semiparasitic plant, have a centenary use in folk medicine to treat hypertension and cardiovascular diseases (Melo et al., 2023). However, the biological mechanisms related to this hypotensive property are fewer investigated. In this study, we evaluated the effects of *V. album* mother tincture (VAMT) in the Na^+/K^+ ATPase using the porcine proximal tubule kidney cells (LLC-PK1). The VAMTs were prepared using fresh European mistletoe from 3 different subspecies (*album*, *abietis*, *austriacum*), harvested in Summer and Winter, from five host trees (*M. domestica*, *Q. petrea*, *U. carpinifolia*, *P. sylvestris*, *A. alba*) (Holandino et al., 2020). The MTT assay indicated the cytotoxic effect triggered only by Summer VAMT from *Q. petrea* ($p<0.05$), after 24 h of incubation. Using the same incubation time, Summer VAMT from *A. alba* induced a significant decrease in the Na^+/K^+ ATPase of LLC-PK1 cells ($p<0.05$), highlighting the promising antihypertensive potential of this mother tincture. These interesting results support the traditional use of VAMT and suggesting, for the first time, one of its mechanism of action in hypertension. Besides, the influence of seasons and the host trees in LLC-PK1 points out the importance to investigate the biological influences regarding the mistletoe's origins. The effects in Na^+ pump expression are under investigation in order to evaluate if changes in Na^+/K^+ ATPase activity and expression are responsible for the hypotensive effects of VAMT on kidney cells.

Keywords: *Viscum album* mother tinctures, kidney enzymes, *in vitro* research

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S3.P50 ^{1}H NMR-based metabolomics on 30 east asian traditional herbal medicines for quality control

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East Asian traditional medicine typically consists of multi-herbal formulations consisting of up to 20 different herbs, each of which contributes to synergistic effects through complex interactions between their bioactive components. However, they possess chemical complexity issues, making it necessary to develop appropriate analytical methods for their identification and standardization (Jianping Zhao et al., 2022). Herein, we performed a comparative nuclear magnetic resonance (NMR)-based on metabolomics analysis to consider applicability of the method for quality control of multi-herbal formulations. A total of 86 samples composed of 30 formulation samples were analyzed by ^{1}H NMR spectroscopy, and chemical fingerprints were compared to each other in batch-to-batch and formulation-to-formulation manners. We could identify specialized metabolites with high content along with polar primary metabolites such as amino acids, sugars and some phenolic compounds. ^{1}H NMR offered comprehensive insights on the multiple formulations and has the potential to be applied for quality control.

Keywords: ^{1}H NMR spectroscopy, traditional herbal medicines, quality control, metabolomics

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S3.P51 Unveiling terpenoid diversity in *Sideritis* spp. through molecular networks

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The genus *Sideritis* L. (Lamiaceae family) is traditionally used for the treatment of various ailments. These plants are phytochemically rich and represent an inexhaustible source of natural products (e.g. flavonoids, phenylethanoid glycosides, and others) (González-Burgos et al., 2011). However, the terpenoids profile of *Sideritis* species has not yet been fully determined, and due to the complexity of these compounds, their characterization remains challenging.

As part of our ongoing research on *Sideritis* species (Tomou et al., 2023; Tomou et al., 2024), the main aim of the present study was to use the molecular network approach to investigate the terpenoid constituents of several *Sideritis* taxa (belonging to the *Empedoclia* section) and to highlight the relevance of molecular networks in exploring the chemotaxonomy of these plants. Therefore, GC/MS and the GNPS platform were used to analyze several different dichloromethane extracts.

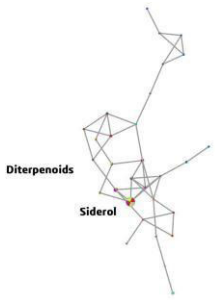

Fig. 1. Molecular network cluster showing diterpenoids of the dichloromethane extracts of different *Sideritis* species.

The generated molecular network shows differences among the investigated extracts. The characteristic *ent*-kaurene type diterpenoids (such as siderol) of these plants were identified (Fig.1) and our results revealed clusters common to all samples, as well as taxa-specific clusters. This work demonstrates the ability of *in silico* tools such as molecular networks to profile the terpenoids of *Sideritis* plants and to determine possible characteristic markers relevant to chemotaxonomy.

Keywords: *Sideritis* spp., Lamiaceae, terpenoids, GC-MS, molecular networks, GNPS

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S3.P52 Targeted discovery of sesquiterpene indole alkaloids from Gabonese *Greenwayodendron suaveolens*

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Annonaceous plants are of interest to natural product chemists due to their therapeutic value and to their isoquinoline alkaloids content. A few years ago, a MS/MS database of 322 isoquinolines and other annonaceous metabolites had been implemented to the GNPS repositories (*viz*. IQAMDB) (Agnès et al., 2022). We herein report on the dereplication of known alkaloids from stem barks of *Greenwayodendron suaveolens* (Engl. & Diels) Verdc. leveraging IQAMDB-informed feature-based molecular networking further refined by *in silico* annotation and taxonomic weighting based on Tima- R (Rutz et al., 2019). This strategy annotated over 30 compounds and streamlined the isolation of diverse sesquiterpene indole alkaloids (SIA) (**1-3**) (Fig. 1). Structure elucidation and absolute configuration assignment (by TDDFT-ECD and X-ray), determined these compounds to be greenwaylactam A (**1**), a recently reported Witkop-Winterfeldt oxidized SIA (Kemgni et al., 2021), and a newly reported diastereoisomer, namely greenwaylactam D (**2**). *N,O*-diacetylpolyveoline (**3**), is here first reported as a natural product (Kouam et al., 2014). The antibacterial activity of these isolates was assayed against *Staphylococcus aureus, S. epidermidis* and *Pseudomonas aeruginosa*.

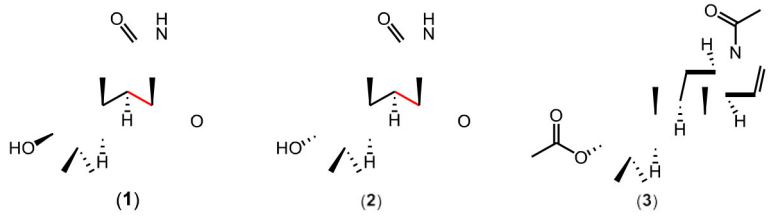

Fig. 1. Chemical structure of sesquiterpene indole alkaloids isolated from *G. suaveolens*

Keywords: *Greenwayodendron suaveolens,* Annonaceae, sesquiterpene indole alkaloid, molecular networking

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S3.P53 The benefit of classification machine learning in virtual drug discovery for beta-lactamase inhibitors identification based on an in-house library

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Machine learning (ML) is a cutting-edge computational technology that solves complex problems. Many studies utilized ML to improve virtual drug screening (Chan et al., 2023). However, few studies focused on identifying beta-lactamase inhibitors (Anant et al., 2022 and Papastergiou et al., 2022), even though beta-lactamase is the leading cause of antibiotic resistance and causes global medical problems. Therefore, the authors aim to fill this gap by proposing an alternative classification ML (random forest) integrated into a virtual screening to identify beta-lactamase inhibitors, validated by experimental data from an in-house chemical library. The authors performed a virtual screening via molecular docking using AutoDock Vina and DOCK6. A modified protocol from a previous report was used to evaluate beta-lactamase inhibitory activity (Everaert and Coenye, 2016). The results indicated that our proposed virtual screening with an ML-based quantitative structure-activity relationship model with a ROC AUC performance score of 0.67 outperformed a non-ML model, a score of 0.56, and better than some previously reported ML models with the best score of 0.51 in identifying bio-inhibitors against beta-lactamase (Anant et al., 2022). In addition, the anti-beta- lactamase activity of some aromatic bioactive molecules from the library was found for the first time. This hints at the potential of natural products in drug discovery. The authors' proposed alternative ML model integrated into a virtual screening initially proves to benefit the identification of beta- lactamase inhibitors based on the in-house chemical library and experiment. However, modification is needed to improve the ML model's performance.

Keywords: Machine learning, virtual drug screening, beta-lactamase inhibitors

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S3.P54 A bioactive chlorophyll-derived yellow phyllobilin from senescent leaves of *Carica papaya* (Caricaceae) and its potential role in the ethnopharmacological use of withered leaf extracts

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Extracts of *Carica papaya* leaves, especially withered ones, have been used traditionally by practitioners in Nigeria for the treatment of various diseases (Owoyele et al., 2008).

Phyllobilins are the degradation products of the green plant pigment chlorophyll and accumulate inside the vacuoles of the leaves during senescence of the plant. So far, over 70 different phyllobillins from more than 30 plant species have been identified.

These breakdown products have been completely overlooked as bioactive compounds. In our group, pharmacologically relevant effects of different phyllobilins from several plants could be demonstrated including anti-oxidative properties, anti-proliferative effects on cancer cells (Wang et al., 2021) as well as anti-inflammatory activities (Karg et al., 2021).

In this study we have isolated a yellow phyllobilin from senescent *Carica papaya* leaves, named *Cp*- PxB, and elucidated its structure with 1D- and 2D NMR experiments. Further we tested the anti- inflammatory potential of the compound in different assays studying the acute as well as the chronic phase of inflammation. The investigation on the senescence-related occurrence of phyllobilins and their bioactivities could provide a scientific rationale for the traditional use of senescent papaya leaves.

Keywords: *Carica papaya,* Caricaceae, phyllobilins, ethnopharmacology, anti-inflammatory

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S3.P55 Evaluating the use of Raman spectroscopy for the characterization of volatile compounds by direct measurement on the oregano leaf glands

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Essential oils (EOs) distilled from leaves and flowers of aromatic plants include volatile organic compounds such as terpenes and phenols. Owing to these compounds, EOs possess numerous and diverse biological properties, namely anti-inflammatory, antimicrobial, antioxidant and anticancer ones (Dhifi et al., 2016). In combination with their natural origin, they find extensive biomedical, cosmetic, food and domestic applications. Unfortunately, adulteration cases of EOs with synthetic products or cheaper EOs are not rare. Hence, the development of simple and reliable analytical techniques for their authentication is of great importance for the industry and commerce. Amongst them, Raman spectroscopy is a versatile, non-invasive method that requires minimal sample preparation, effectively utilized for identifying and quantifying EO constituents (Kampasakali et al., 2023).

In this work, the feasibility of using Raman spectroscopy in the identification of the main volatile components of oregano directly from the leaf glands has been explored for the cases of two μ-Raman systems, a standard benchtop spectrometer (LabRAM HR, Horiba) and a portable one (HE785, Horiba). Measurements on the glands have been compared to those of the respective EOs distilled from the leaves. For both instruments, the identification of volatile components is straightforward. On the other hand, the main volatile components can be identified from the oregano leaf glands with the standard system, but only the presence of carvacrol or thymol can be deduced using the portable one. Improvements in the spatial and spectral resolution of portable instruments may allow their use for quality control of raw oregano material.

Keywords: essential oils, Raman spectroscopy, *Origanum*, identification, adulteration

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S3.P56 New benzothiazines with pain-reliving activity from marine bacterium *Croceibacter atlanticus*

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Chronic pain is a debilitating and widespread disease, affecting approximately 20% of adults (Cohen et al., 2021). Current therapeutic approaches are either not able to completely relieve pain or have severe side effects such as dependence and addiction (Obeng et al., 2021). Microbial secondary metabolites possess a wide range of biological activities and are a highly promising source for novel drug leads. Here we explore the bioactivity of two *Croceibacter atlanticus* strains and the discovery of sulfur-containing natural products with pain relieving potential.

We employed a novel zebrafish behavior-based assay developed by the Strother Lab able to detect pain mediating activity even in complex extracts. 85 extracts from our collection of Arctic Ocean bacteria were screened for *in vivo* pain-relieving activity. 'Behavior-guided' isolation resulted in the discovery of metabolites with pain-relieving activity of the rare benzothiazine class of compounds. Subsequently, *in vivo* zebrafish whole-brain calcium imaging experiments were used to pinpoint targeted neuron populations to elucidate mode of action. Compounds are tested for receptor specificity *in vitro* using cell lines expressing ion channels and submitted to the NIMH's Psychoactive Drug Screening Program (PDSP) for receptor binding and functional studies.

Keywords: Pain, marine, Gram-negative, zebrafish

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S3.P57 3D printed polymeric scaffolds loaded with antibiotic and anti-inflammatory drugs for tissue engineering applications

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Critical skeletal defects and bone loss are common clinical scenarios in orthopedics and craniofacial surgery due to trauma, infection, and tumor resection. For the treatment of bone defects, engineered bone tissue can provide an alternative to conventional bone grafts. The use of innovative biomaterials, 3D bioprinting and autologous mesenchymal stem cells can provide 3D bioscaffolds with regenerative bone tissue properties (Ashammakhi et al., 2022). These 3D-bioscaffolds can further incorporate antimicrobial and/or anti-inflammatory active pharmaceutical ingredients, as modern drug delivery systems. The incorporation of antimicrobial agents is vital for preventing or treating existing infections upon implantation, which is critical for the success of tissue regeneration (Dos Santos et al., 2022).

In this work, tetracycline hydrochloride (TCH), a well-established and broad-spectrum antibiotic agent (produced by a fermentation process, involving microbes), and diclofenac sodium (DIC), a well-established anti-inflammatory drug, were incorporated into a poly(ε-caprolactone) (PCL) biomaterial. Drug-free and drug-loaded scaffolds (1 wt.% drug content based on polymer's weight) were manufactured using a bioprinter (BIO-X, Cellink, Sweden). The scaffolds were evaluated for their morphology by scanning electron microscopy (SEM). Their physicochemical properties were determined by means of differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Additionally, entrapment efficiency and *in vitro* drug release characteristics were evaluated, while cell biocompatibility was assessed using MC3T3-E1 pre-osteoblast cells. Both neat and drug- loaded scaffolds were tested for their antibacterial activity against *Staphylococcus aureus*.

Keywords: tissue engineering, bone remodelling, 3D-bioprinted scaffolds, antimicrobial activity

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S3.P58 ChromAnnot – a webserver for reproducible LC-HRMS/MS chromatogram annotation

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One main challenge in the exploration of organisms' chemical diversity remains the annotation of compounds detected within LC-HRMS/MS data, to be able to highlight known and/or unknown metabolites (Wolfender *et al.* 2019). Having information on all observed/detected peaks from a LC- HRMS/MS chromatogram remains challenging.

At ISOMer laboratory, we developed an entire workflow taking into consideration each step of the general annotation strategy that was particularly adapted for dereplication of specialized metabolites in LC-HRMS/MS profiles. This automated approach based on R (Cran), XCMS (Tautenhahn *et al.* 2008), IPO (Libiseller *et al.* 2015), CAMERA (Kuhl *et al.* 2012), SIRIUS (Böcker *et al.* 2009), database search, Taxize (Chamberlain *et al.* 2013) and CFMID (Wang *et al.* 2021). Biological origin and MS/MS *in silico* fragmentation comparison were added into the workflow to improve compound discrimination.

The workflow, which analyses directly raw LC-HRMS/MS data (in open format), was integrated into a web-based interface to ease exploring the annotation result. The ChromAnnot webserver is accessible at https://chromannot.univ-nantes.fr/.

This deep annotation of LC-HRMS/MS data strategy is currently applied to characterize and highlight known/unknown compounds produced various *Penicillium* strains.

Keywords: annotation, dereplication, LC-HRMS/MS

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S3.P59 Exploring the anticancer potential of *Polygonum barbatum* (L.) against colorectal cancer: *In vitro* and *in vivo* investigations

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Polygonum barbatum (L.), a medicinal herb, exhibits promising anticancer properties, yet its mode of action remains elusive. This study aimed to elucidate the anti-colorectal cancer (CRC) effects of *Polygonum barbatum* extract (PBE) both *in vitro*, utilizing HCT-116 ($KRAS^{G13D}$ mutation) and HT- 29 (BRAF mutation) human CRC cell lines, and *in vivo*, employing a BALB/c nude mice tumour xenograft model. Various analyses including RNA sequencing, western blotting, and co- immunoprecipitation assays were conducted. Additionally, assessments of cell viability, colony formation, migration, invasion abilities, and apoptosis were performed through Cell Counting Kit-8, colony formation, wound healing, Transwell invasion, and flow cytometry assays, respectively. Results demonstrated a significant reduction in CRC cell growth by PBE without impacting non- cancerous cells, accompanied by the downregulation of genes associated with extracellular matrix (ECM) organization, cell motility, and proliferation. Furthermore, PBE affected ECM interactions and focal adhesion pathways, modulating the expression of migration, invasion, and epithelial–mesenchymal transition markers. Notably, in the mouse xenograft model, PBE exhibited tumour growth inhibition, particularly synergizing with oxaliplatin. This study concludes that PBE impedes CRC cell motility and tumorigenicity via the YAP–β-catenin axis, suggesting its potential in addressing CRC resistant to epidermal growth factor receptor inhibitors.

Keywords: colorectal cancer, *Polygonum barbatum*, xenograft mouse model, cell-matrix adhesion

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S3.P60 Physical interactions between *Streptomyces* (Streptomycetaceae) and competing microbes trigger the production of natural products

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Streptomyces (Streptomycetaceae) are known for producing numerous natural products, including antimicrobial agents. However, antimicrobial resistance is rising, and the discovery of new antimicrobial agents has slowed down. While genome sequencing reveals a vast reservoir of secondary metabolites in *Streptomyces*, these often remain unexpressed in laboratory monocultures (Lewis, 2013). In our work, we have discovered predatory behavior by *Streptomyces* when encountering *Saccharomyces cerevisiae* (Saccharomycetaceae). Physical interactions with *S. cerevisiae* trigger *Streptomyces* to produce numerous lytic enzymes and antifungal compounds, such as pentamycin and filipin III, which are not produced in monoculture settings. RNA-seq data uncovered activation of 40 % of biosynthetic gene clusters of *Streptomyces lavendulae*, including unknown secondary metabolite biosynthetic gene clusters (Yamada et al., 2023).

To expand our research, we aimed to utilize cutting-edge technologies to study the interactions between *Streptomyces* and competing microbes. We have selected 50 *Streptomyces* strains and four non-pathogenic microbes that are related to important human pathogens: *Candida tropicalis*, *Aspergillus nidulans*, *Mycobacterium smegmatis*, and *Pseudomonas syringae*. Our preliminary results show that 21 of the selected *Streptomyces* strains react to the presence of the competing microbes by producing different compounds depending on the microbe. We utilized Global Natural Products Social Molecular Networking (Wang et al., 2016) and SIRIUS (Dührkop et al., 2019) to uncover the identity of the produced secondary metabolites, such as pamamycins, puromycins, and lydicamycins. We aim to further uncover the identity of all the unknown compounds by combining transcriptomics, metabolomics, and knock-outs.

Keywords: *Streptomyces,* drug discovery, microbial interactions

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S3.P61 Energy Saving Efficacy and Optimized condition of pulse energy fields technology on *Centella asiatica* extraction

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Eco-friendly technologies are focused worldwide to deal with climate crisis and resource depletion. As pulsed electric fields (PEF) treatment changes permeability of cell membrane is used as energy- saving green technology for sterilization in the food industry (Wei et al., 2023). To extend the application of PEF into the industrial realm, we attempted PEF on *Centella asiatica* (L.) Urban (Apiaceae).

C. asiatica, which is Southeast Asian native medicinal plant, is known to have several biological activities including antioxidation, anti-inflammation etc. (Orhan, 2012). *C. asiatica* has identical compounds, madecassoside, asiaticoside, madecassic acid, asiatic acid, which are reported to have wound healing activity and collagen stimulation in human fibroblast (Maquart et al.,1990).

To compare energy consumption and extracted contents of four well-known actives between PEF treated and non-treated extract of *C. asiatica* leaves, extract was sampled every five minutes. As the result, the maximum levels of active compounds were higher at PEF treated extract and found out the energy consumption to extract madecassic acid same level of the maximum extracted at non-treated extract showed about 20 % lower in PEF treated extract.

Next, optimization of PEF treatment was established with Box-Benhken design. Time, voltage, and frequency were selected as variables to maximize extraction of madecassoside, asiaticoside and yield. The optimized *C. asiatica* extract showed 18.39 ppm of madecassoside, 22.98 ppm of asiaticoside, 1.30 % of yield, similar to expected values. Therefore, PEF can be considered as new environmentally friendly solution for cosmetics with maximizing active compounds.

Keywords: pulsed electric fields, *Centella asiatica*, energy saving, optimization

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S3.P62 Identification of a leoligin-inspired synthetic lignan as a novel TGR5 agonist

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The G protein-coupled receptor TGR5 binds bile acids as endogenous ligands and emerged as an important regulator of metabolic and immunologic functions. It was shown to be an effective target in alleviating inflammatory diseases (Pols et al., 2011). The naturally occurring lignan leoligin and synthetic derivatives were shown to be effective anti-inflammatory compounds *in vitro* (Linder et al., 2021). However, the underlying molecular targets for these activities are still not fully understood and require further investigations. In this study we present the identification of a natural product- inspired synthetic derivative of leoligin, LT-188A, as a novel TGR5 agonist. Both, a CRE-Luciferase reporter gene assay and cAMP accumulation assay supported TGR5 agonism of LT-188A (3-30 µM). Specificity for TGR5 was shown by comparing activities in HEK293A cells expressing TGR5 *versus* non-expressing cells. In line with this agonistic activity, the transactivation activity of the pro- inflammatory transcription factor NFκB was inhibited by LT-188A concentration- (3-20 µM) and TGR5-dependently as shown by luciferase reporter assays. Functional consequences of these activities were studies in LPS-activated macrophages: the expression levels of pro-inflammatory cytokines (IL-1β, IL-6) and the release of nitric oxide were concentration-dependently (3-20 µM) reduced in response to LT-188A as shown by qPCR and the Griess Assay, respectively. Cytotoxicity was excluded by the Resazurin conversion assay. Taken together, LT-188A shows promising *in vitro* anti-inflammatory bioactivities in relevant cellular assays and provides a starting point for further optimization and development.

Keywords: *TGR5,* anti-inflammatory, macrophages, leoligin, lignans

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S3.P63 Nano-phytosome delivery system loaded with *Crataegus laciniata* (Rosaceae) flowers extract: whitening properties

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Hawthorn is a large genus of small trees belonging to Rosaceae family, including approximately 200 species. In recent years, hawthorn has been demonstrated to be an excellent source of natural bioactive molecules, which have promising benefits for human health (Venskutonis, 2018). In the present study, we focused on *Crataegus laciniata* Ucria, a species distributed through the western Mediterranean area, northern Algeria, and Italy (Puglia and Sicilia). Nowadays there is still a great interest for the research of novel non- toxic, effective and stabile tyrosinase inhibitory agents, from plant sources (Pillaiyar et al., 2017). Our previous study demonstrated the efficacy of *C. laciniata* flower's (CLF) ethanolic (70%) extract as new sources of antimelanogenic agents (Mirabile, et al., 2023). The chemical profile of extract showed the presence of both C-flavonoid and O-flavonoid derivatives and hyperoside and vitexin. Phytosome system could enhance skin penetration and bioavailability of phytoconstituent like polyphenol-rich extract. The objective of this research was to formulate phytosome containing CLF extract to enhance skin-whitening properties of the extract. In order to improve the physicochemical properties of the extract, the obtained CLF extract was developed into phytosome system by thin-layer method using soy lecithin. FT-IR and XRD diffraction confirmed the successful loading of CLF in nano-phytosomes. The phytosome complex containing CLF extract and lecithin (1:1) has good entrapment efficiency (94.8%) with spherical shape as confirmed by SEM. The CLF-loaded phytosome displayed particle size in the range of 338-534 nm and polydispersity index (PDI) values in the range of 0.39-0.47. This herbal preparation could be developed as tyrosinase inhibitory agent in skin pharmacology and cosmetics.

Keywords: tyrosinase, Crataegus, Phytosome, whitening properties, thin-layer method

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S3.P64 Leoligin derivatives as novel phosphodiesterase 4 inhibitors

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Phosphodiesterase 4 (PDE4) is the primary hydrolysing enzyme of the ubiquitous second messenger, cyclic adenosine monophosphate (cAMP). PDE4 plays a crucial role in physiological and metabolic functions, including pro-inflammatory pathways (Francis et al., 2011). Marketed PDE4 inhibitors, namely *Roflumilast* and *Apremilast*, have been used to treat chronic obstructive pulmonary disease and psoriasis, respectively (Baillie et al., 2019). Identification and characterisation of PDE4 inhibitors provides a promising strategy in targeting a variety of pathological processes.

In this study, leoligin, a natural lignan found in the roots of Edelweiss (*Leontopodium nivale ssp. alpinum*, Asteraceae) and 169 structural derivatives (Mihovilovic et al., 2015) were screened for their PDE inhibitory activity at 30 µM using a cAMP accumulation assay employing an EPAC-based FRET biosensor (Perhal, 2020). Seven compounds, including leoligin itself, were identified as causing a significant accumulation of cAMP and a structure-activity relationship was deduced. One derivative, designated LT-104A, showed a dose-dependent activity with the highest determined potency (EC_{50} = 2 µM) in the EPAC assay. LT-104A was further characterised using a CRE- Luciferase assay, showing comparable activity to known PDE4 inhibitors in inducing the cAMP- PKA-CREB pathway. Lastly, a functional study was carried out in LPS-activated macrophages in which LT-104A reduced nitric oxide release in the Griess assay, indicative of its anti-inflammatory properties.

In conclusion, extensive *in vitro* screening of leoligin derivatives led to the identification and characterisation of a novel PDE4 inhibitor, LT-104A, with promising *in vitro* anti-inflammatory properties.

Keywords: cyclic AMP, phosphodiesterase, PDE4, leoligin, lignans

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S3.P65 The search for nontraditional synergy: pairing *in vitro* omics and *in silico* machine learning methods to discover novel combinations of antimicrobial agents from natural products

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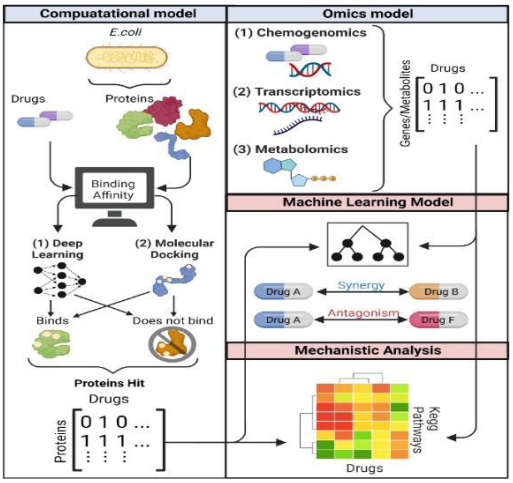
In response to lagging antibiotic development pipelines and to combat multi-drug resistance, antimicrobial combination therapy is a promising therapeutic option, and novel compounds that act synergistically are of clinical interest. However, combinations are often chosen empirically leading to suboptimal treatment outcomes and spread of resistance. Antibacterial adjuvants, such as natural products (NPs), have been understudied as most NP library campaigns screen individual compounds for bioactivity. The vast sample size of combinations make it impossible to systematically screen combinations *in vitro*. Recent methods to develop synergistic treatments depend on computational models to reduce the vast sample size of combinations available. These methods are limited by (1) requiring inaccessible, costly datasets for novel NP compounds and (2) black-box AI which provides little insight into mechanisms of synergy that govern drug interactions.

We hypothesized that by pairing computational drug – protein and NP – protein interactions with experimentally obtained chemogenomics via CRISPR interference (CRISPRi) screens, we could 1. predict synergy/antagonism of FDA approved antimicrobials with NPs, 2. undertake an unbiased exploration of the underlying biological mechanisms of synergy via profiling perturbed bacterial genetic networks. Our model uses a unique combination of computationally calculated drug – protein interactions, multi-omics studies, and machine learning (ML) to predict effective drug combination therapies.



(Fig. 1). The model was trained and tested on 648 two-way and three-way drug interactions in *E. coli*. To assess drug modes of action and mechanisms of synergy, we cross-referenced all five datasets to the Kegg database of 113 *E. coli* functional pathways and known drug targets.

Keywords: machine learning, antibiotics, synergy, target, CRISPRi-chemogenomics

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S3.P66 Electron diffraction-guided natural product discovery: identification and isolation of new scaffold class from *Podospora australis*

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This presentation will highlight the utility of ArrayED (Delgadillo et. al., 2024) as an effective screening platform to rapidly discover novel natural products. The presentation will also showcase how microED is employed in conjunction with high-resolution electron spray ionization mass spectrometry (HRESIMS) to elucidate the structures of novel scaffolds, as well as the use of nuclear magnetic resonance (NMR) spectroscopy for further supporting evidence.

As part of our continuing search for novel natural products, we identified several crystalline entities from a MeOH extract of *Podospora australis* utilizing ArrayED. Routine microED analysis provided the structural solution of a scaffold previously undescribed in the literature. HRESIMS of the well of interest revealed the presence of eight unique mass signatures, indicating that microED had produced a structural solution from a highly mixed fraction. Careful analysis of bond lengths and geometry, coupled with the observed *m/z* 477.1960 [M+H], enabled the elucidation of the structure of **1** (Fig. 1). Subsequent regrowth of the culture and isolation efforts yielded several analogs of **1,** which were characterized *via* 2D NMR to further support the structural solution provided by microED and HRESIMS.

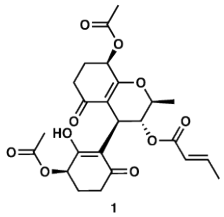

Fig. 1. Structure of the first novel natural product discovered via ArrayED **1**

Since the *Podospora australis* strain is known to produce anti-fungal compounds (Li et. al., 2016), the newly discovered compounds were submitted for anti-fungal activity screening. Two of the six compounds exhibited modest activity in a general screen against *S. cerevisiae*. These preliminary biological activity results are encouraging, and further biological evaluation is underway.

Keywords: microED, ArrayED, antifungal, *Podospora*, drug discovery

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S3.P67 Metaboseek 2.0: Deconvoluting the complexity of MS/MS Big Data through interactive data processing

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In the current era of technological advancements in mass spectrometry, we are now capable of acquiring thousands of high-quality features in a single analysis. This breakthrough signifies that the solution to a drug discovery or chemical ecology question could already be within our grasp. Significant efforts in the computational mass spectrometry field have been devoted to unlocking the full potential of this vast amount of recorded data (Nothias et al., 2020, Shah et al., 2023). In this context, Metaboseek (Helf et al., 2022) represents a notable step forward by integrating the primary advantages of these computational approaches in a user-friendly interface, and it additionally incorporates an effective data filter. Specifically, it offers an interactive workflow that seamlessly begins with data preprocessing using an integrated XCMS step (Tautenhahn et al., 2012). The processed data undergo comprehensive statistical analysis, culminating in the prediction of the prioritized features' identity through SIRIUS (Dührkop et al., 2019). Metaboseek's updated version pivotal characteristic lies in a sophisticated set of filters, including fold change and machine learning prioritization, designed to streamline the feature table. These filters ensure that only features meeting specific criteria are retained, thereby enhancing the efficiency and relevance of subsequent analyses. Here, we demonstrate the software's effectiveness in feature prioritization, elucidating metabolic pathways, and identifying novel targets.

Keywords: Metaboseek, computational mass spectrometry, metabolomics

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S3.P68 Cytotoxic activities of terpenoids from genera *Euphorbia rowlandii* and *Euphorbia grandocornis* (Euphorbiaceae) against the Triple-negative breast cancer (TNBC)

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Triple-negative breast cancer cells (TNBC) are a heterogeneous group of malignancies that affect women. This subtype accounts for approximately 10 to 15% of diagnosed breast cancers worldwide and in Africa, more than 50% of women diagnosed with (TNBC) succumb to the disease. The genus *Euphorbia* contains terpenoids with anticancer properties, (Damoun et al, 2015; Lebert et al., 2018; Chang-Qing et al., 2020; Yan et al., 2020; Anyawu 2008), hence exploring *Euphorbia rowlandii* R.A Dyer and *Euphorbia grandicornis L*, phytochemicals and evaluated their cytotoxic properties. Structures of the isolated terpenoids from the genera *E. grandocornis* and *E. rowlandii* were characterised using IR, NMR, and MS. *E. grandocornis* yielded, hexyl(E)-3-(4-hydroxy-3- methoxyphenyl)-2-propenoate (**1**), tirucalla-8,25-diene-3β,24R-diol (**2**), 24-methylenetirucalla-8-en- 3β-ol (**3**), β-glutinol, β-amyrin (**4**) whereas *E. rowlandii*, afforded phorbol ester, 16-Hydroxy-12- deoxy-phorbol (**5**), a tentative phorbol ester, 3β-hydroxy-5-glutinene (**6**). Extracts and compounds were tested for cytotoxicity using MTT against MCF-7, HCC70, and MCF-12A cells. Dichloromethane extract of *E. rowlandii* showed high toxicity against HCC70 and MCF-7 cells at 4.97 and 1.177 Mm, while *E. grandocornis* extract exhibited IC_{50} values of 1.03, 0.301 µg/mL. Compound **1** showed activity against HCC70 cells with an IC_{50} = 29.45 µM and MCF-7 with IC_{50} = 23.41 µM and compound **5** showed activity against HCC70 and MCF-7 cells with IC_{50} values of 0.592 and 1.003 µM respectively. Terpenoids from *Euphorbia* genus demonstrated cytotoxic activities against the breast cancer cells.

Keywords: *Euphorbia,* TNBC, HCC70, MCF-7, terpenoids

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S3.P69 Simultaneous extraction and synthesis of new cannabinoid acid esters and their evaluation on TRPA1, cell proliferation and in MDA-MB-231 xenograft model of cancer

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Natural phytocannabinoids have a privileged pharmacophore scaffold with antitumor properties (Hinz and Ramer, 2022). In this study we present the simultaneous extraction and synthesis of cannabinoid acid esters. The synthesis method was performed for esters of cannabidiolic acid (CBDA), cannabigerolic acid (CBGA) and cannabichromenic acid (CBCA) with C1-5 alcohols. Cannabinoid acid esters and their neutral forms (CBD, CBG and CBC) were evaluated for their effect on TRPA1 channels, which are implicated in several cancer forms (Takahashi et al., 2018), and for their cytotoxicity. Trichomes of *Cannabis sativa* containing either CBDA or CBGA, were used for the synthesis of each ester. Each time a different alcohol was used as solvent/reagent for esterification in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and 4-dimethylaminopyridine. The reaction with ultrasonication led to 90% yield of each ester. CBCA esters were synthesized by oxidizing CBGA esters with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone with 80% yield. Cannabinoid esters showed similar effect on TRPA1 activation, with their respective neutral cannabinoids in a single dose experiment (in 50 uM, 2-fold higher activation compared to AITC). The *in vitro* study of the cytotoxic activity with the MTT assay showed that CBG and its respective esters were the most potent with EC_{50} < 40 μM in MDA-MB-231 cancer cells. CBGA butyl ester (EC_{50} = 8.7 μM) was also evaluated in an *in vivo* MDA-MB-231 xenograft mouse model of cancer (oral administration, 50 mg/kg). Additionally, CBGA butyl ester was classified to "Category 5" of GHS (2000 mg/kg) in an oral acute toxicity assessment in mice (Fig.1).

Fig. 1. Structure of cannabigerolic acid butyl ester (CBGA butyl ester)

Keywords: cannabinoid acid esters, cytotoxicity, cannabinoids, antitumor, TRPA1 channels

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S3.P70 Exploring potential breast and melanoma cancer drug candidates from *Crocus sativus*: molecular docking insights

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Molecular docking can be particularly useful in identifying potential drug candidates from natural sources, exploring the binding mechanisms of natural products with therapeutic targets, and guiding the optimization of lead compounds derived from natural sources [1]. The current molecular docking aimed the analysis of descriptors with the greatest cytotoxic activity and the study of the possible mechanism of action of selected phenolic compounds of *Crocus sativus*. Previously [2], we identified the following compounds: chlorogenic acid, caffeic acid, mangiferin, isoorientin, ferulic acid, rutin, tectoridin, quercetin, t-cinnamic acid, genistin, apigenin, kaempferol, iristectorigenin B, nigricin and irigenin. The docking simulations were performed with the SCIGRESS software package (Fujitsu, Fukuoka, Japan (license 742F6852C191)). Among estrogen receptors that play an important role in cancer pathogenesis, we selected the human ERα-LBD (PDB ID 3ERT) complex with 4- hydroxytamoxifen, which is an active metabolite of tamoxifen. According to the docking studies, almost all natural compounds showed affinity to the active sites of the selected enzymes that were similar or even better than their native ligands. Such compounds as chlorogenic acid, isoorientin, ferulic acid, tectoridin, quercetin, cinnamic acid, genistin, apigenin, kaempferol, and irigenin showed, better affinity com-pared with the native ligands of breast cancer proteins 4RJ3, 2IOK, and 4XYF. Moreover, the affinity of many compounds (chlorogenic acid, ferulic acid, cinnamic acid, iristectorigenin B) to the estrogen receptor (3ERT) was comparable to hydroxytamoxifen. Subsequent *in vitro* assays of *Crocus* extracts and individual substances correlated with the results of docking.

Keywords: natural compounds, breast cancer, melanoma, docking studies, drug designing

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S3.P71 Metabolic fate of oleocanthal and oleacein after oral consumption: Novel biomarkers, human bioactivity insights and TRPA1 channel activation

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Oleocanthal and oleacein are secoiridoids found in olive oil, which have gained strong scientific interest due to their pharmacological properties. Although some efforts have been done to investigate their pharmacokinetics in mice or rats (Darakjian et al., 2021; López-Yerena et al., 2021), their metabolic fate in humans after oral consumption remains unknown. In this study, we present the results of a human-based protocol involving the oral administration of a commercially available olive oil extract enriched in oleocanthal and oleacein, in the form of a food supplement capsule (OLEOPROTECT®) in comparison with high-phenolic olive oil. UHPLC MS/MS analysis, for the first time succeeded to identify metabolites of oleocanthal and oleacein in human plasma: oleocysteine, oleotaurine and hydroxyoleocysteine, all making a peak at 30 min and being detectable even 4 h after oral consumption. Oleocanthal and oleacein were not detected in plasma, confirming the previously described spontaneous reactivity with amino acids. Interestingly, the administration of the encapsulated product showed higher rate of formation of the metabolites in comparison with the respective olive oil. Extensive studies investigated the spontaneous reactions with all plasma amino acids. Real-time structural elucidation through 1D and 2D NMR experiments revealed the biochemical transformation of oleocanthal and oleacein into a series of novel products. Furthermore, we investigated the activity of oleocanthal and some of the formed metabolites in TRPA1 channels. Notably, oleocysteine activated TRPA1 similarly with oleocanthal. This discovery provides a new perspective on understanding the well-documented bioactivities of olive oil in humans, offering valuable insights for future research.

Keywords: oleocanthal, oleacein, pharmacokinetics, biological fluids, TRPA1

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S3.P72 An innovative methodology for the production of a new food supplement enriched in oleocanthal and oleacein with multi target protective properties

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Olive oil aldehydic phenols (OOPs: oleocanthal, oleacein, oleuropein aglycone, ligstroside aglycone) are highly bioactive secoiridoids found in olive oil. Nutritional interventions in humans using olive oil with or without OOPs have provided strong evidence about their unique therapeutic role. The most important clinical trials have been performed in patients against chronic lymphocytic leukemia (Rojas et al., 2022), mild cognitive impairment (Tsolaki et al, 2020) and against platelet aggregation (Agrawal et al., 2017). The commercialization of OOPs as food supplements or active pharmaceutical ingredients has been hampered by the lack of economically viable methods for their large-scale isolation or synthesis. Towards this direction, we developed a new, fast and cheap method for their large-scale selective extraction from olive oil using polyethyleneglycol 400. PG400 is a bio- compatible solvent that is not miscible with olive oil and interestingly can react reversibly with olive oil aldehydic phenolics and transform them selectively to hydrosoluble hemiacetals (Fig.1). This unique extraction/reaction procedure has been applied to multi tons scale affording a commercially available extract of OOPs in PEG400 (OLEOPROTECT®). The extract has been formulated to solid or liquid food supplements with excellent stability. When the extract encounters aqueous biological fluids in the human digestive system it spontaneously reacts with water affording 1,1-diols that can further react with plasma or bile aminoacids and enter in the blood circulation. The extract has shown fascinating antitumor, neuroprotective and cardioprotective properties in animal models and currently several clinical trials are in progress.

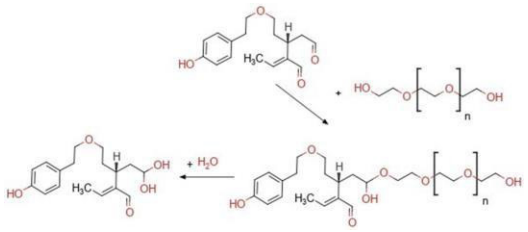

Fig. 1. Selective extraction/reaction of oleocanthal with PEG400

Keywords: oleocanthal, oleacein, olive oil, food supplement, Oleoprotect

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S3.P73 Online dried spot LC-Orbitrap analysis for the quality assessment of cannabis products

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Dried Blood Spot (DBS) is commonly employed in blood handling and analysis through microsampling on specialized paper cards form. It offers convenient sample collection, storage combined with increased analyte stability and easy transportation to testing laboratories (Tey et al., 2021). To our knowledge, there are no online applications to this day in natural products substrates, where it could confer significant advantages especially when combined with high-resolution MS platforms. This is particularly relevant to Cannabis sativa L. extracts and products based on non- psychoactive cannabinoids like CBD, which have recently inundated the market. A major concern regarding these products is inadequate or misleading labeling about the content of CBD and other cannabinoids, and absence of the psychoactive THC (EMCDDA, 2020). In view of potential risks for consumers, authorities have been prompted to issue regulations. These vary from requiring novel food authorization for CBD edibles in the EU (EU Regulation, 2021) to even prohibition of THC and CBD in food by the FDA (FD&C Act, 2023); however, in the latter, cannabis-derived ingredients without CBD or THC might be allowed in foods and supplements. Consequently, there is a necessity for high-capacity workflows that ensure accurate quantification of cannabinoids in cannabis extracts and end-products, while relieving limitations related to sample collection, delivery, and processing. This study presents an integrated protocol encompassing fully automated DBS-based extraction and LC-IT-Orbitrap MS analysis of cannabis products. The proposed strategy combines robust determination, speed, and cost-effective sample handling, which is particularly suitable for quality control of cannabis-derived materials.

Keywords: Cannabis sativa, DBS, CBD, THC, LC-IT-Orbitrap

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S3.P74 Anti-prostatic effects of *V. album* L extracts are influenced by season and the use of hydrogel delivery system

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Viscum album L. extracts (VA) are widely used in complementary cancer therapy because of its cytotoxicity, immunostimulating, and anti-inflammatory properties (Oei et al., 2019). The cytotoxic activity of a hydrogel containing VA from *Abies alba* host tree (VA-hydrogel) was previously reported by our group (Batista et al., 2022; Rocha et al., 2022). In the present work, the effects of these VA dry extracts from Summer (VAS) and Winter (VAW), as well as VA-hydrogels, were investigated for the first time, in *in vitro* human prostate cancer (PCa) cells.

The following analyses were performed using DU145, PC3, LNCaP prostate cells: cytotoxiciy; clonogenic growth; apoptosis and cell cycle progression. The chemical profile of extracts was performed by UV spectrophotometer and thin-layer chromatography.

VAS and VAS-hydrogel presented highest cytotoxic effects, reducing tumor cell growth and colony formation, in a dose dependent manner. The number of PCa cells in early and late apoptosis increased after VAS and VAS-hydrogel treatment. A cell cycle arrest in the S phase was observed in DU145 cells, but not for the other cell lines. The VAW and VAW-hydrogel did not presented significant biological effects. Both extracts (VAS and VAW) showed phytochemical contents in accordance with the pharmacopoeia specifications.

This study highlights the antitumor effect of VAS and VAS-hydrogel on PCa cells. In addition to the importance of harvesting period traceability, the different effects observed in the tested cell lines should be further investigated to elucidate the role of VA and VA-hydrogel in prostate cancer cells.

Keywords: *Viscum album* L., cytotoxic assays, mistletoe, prostate cancer, thermoresponsive hydrogel

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S3.P75 A "*head*-to-*tail*" approach towards foodomics advancement: LC-HRMS and NMR correlation in the case of olive products

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Olive derived products, i.e. olive oil, and table olives, comprise sources of high nutritional and economic value, especially for the Mediterranean basin area. Thus, they are prone to adulteration and mislabeling. Conventional approaches commonly adopted by official organizations for their quality assessment, such as the International Olive Council (IOC), are trait-specific and time-consuming. Therefore, there is a need for more holistic approaches, namely metabolite profiling, using advanced analytical instrumentation like NMR and HRMS. Hence, the aim of the present study was to employ both analytical platforms towards the quality and authentication evaluation of Greek EVOOs and table olives, while exploiting statistical tools in the biomarkers' identification process.

Statistical Total Correlation Spectroscopy (STOCSY) and Statistical Heterospectroscopy (SHY) were applied for the first time in the dereplication process of such samples helping with the lack of commercial standards and public databases, especially in the case of NMR spectroscopy. The former generates a pseudospectrum by correlating peaks with the same fluctuation across NMR spectra of the respective samples (Beteinakis et al., 2020, 2023), while the latter detects correlations between spectroscopic and spectrometric data, in this case NMR and HRMS (Crockford et al., 2006). Herein, more than 130 EVOO samples and 60 table olive samples were analyzed and subjected to MVA. Following discrimination based on botanical and geographical origin, tentative biomarkers were detected in each case. The employment of STOCSY and SHY aided in the identification of several biomarkers. Both statistical tools could find further application in foods' profiling approaches.

Keywords: NMR & LC-HRMS profiling, STOCSY, SHY, olive oil, table olives

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S3.P76 Systematic cheminformatics analysis of herbal plants in malawi: uncovering chemical scaffolds with anti-candidal potential

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Novel medicines for various diseases, including antimicrobial agents against infections have been discovered from herbal plants. However, antifungal drug discovery has received limited attention compared to its antibacterial counterparts (Perfect, 2017). Ethnobotanical surveys remain a conventional approach to collect traditional knowledge on the medicinal uses of herbal plants by local communities (Vogl et al., 2004).

In this study, a literature review on herbal plants species used in Malawi was conducted. A validated algorithm, based on reported uses' similarity to clinical symptoms of candidiasis, identified 133 plant species likely to have anticandidal activities. PubChem queries using identified plants species' names returned 1909 compounds. The 166 Molecular Access System (MACCS) fingerprints and the Murcko chemical scaffolds of the compounds were calculated using RDKit in knime analytics (Khalid et al., 2022). The three most frequent scaffolds were flavanone (19%), piperidine (7%) and (2*S*)-flavanone (7%) (Yu et al., 2023). Moreover, the 40 most common chemical scaffolds in the dataset of 1909 compounds were screened for their presence in compounds active against *C.albicans* (Fig. 1). This analysis revealed 355 active compounds, a finding which underscores the chemical scaffolds' anti- candidal potential.

Table 1: The three most frequent chemical scaffolds

Scaffold Name	Scaffold Structure	Frequency
flavanone		36 (19%)
Piperidine		28 (7%)
(2*S*)-Flavonone		28 (7%)

ut: 40 chemical scaffolds

Fig. 1: CHEMBL database searching workflow

Chemical Scaffolds screened on the bioactive compounds against C.albicans using the Data Warrior module that connects with Chembl database.

In conclusion, future research should focus on bio-guided fractionation, structural elucidation, synthesis, pharmacophore modelling, and toxicity studies on extracts from these plant species.

Keywords: Herbal medicines, antifungal drug discovery, cheminformatics, chemical scaffolds

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S3.P77 From nature's palette to drug candidates: exploring the chemodiversity of *Piper fimbriulatum* and expanding the bioactive potential for the development of novel drug leads

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Piperaceae plants are rich sources of bioactive alkaloids with anticancer properties. *Piper fimbriulatum* is a member of the Piperaceae family from Central America living in symbiosis with *Pheidole bicornis* ants. By exploring *P. fimbriulatum* alkaloid chemodiversity using high resolution mass spectrometry and molecular networking, we discovered that this plant produces piperlongumine, a potent alkaloid that exhibited anticancer activities in numerous preclinical studies. Piperlongumine inhibits the GSTP1 enzyme, causing accumulation of reactive oxygen species and consequently selectively inducing apoptotic and autophagic cell death in cancer cells. Recently, this molecule also demonstrated selective anti-COVID activity in a mouse model. Through the structural annotation of compounds within the piperlongumine cluster in the molecular network representing the plant's chemical diversity, we discovered a diverse array of piperlongumine analogues and cyclobutane dimers produced by the plant. Among those identified, piperlongumine cyclobutane dimer demonstrated *in vitro* activities in the micromolar range against melanoma and breast carcinoma. Afterwards, we performed the synthesis of naturally occurring piperamides for confirmation of their presence in the plant and to investigate UV-light catalyzed 2+2 cycloaddition of these molecules. Our findings revealed that piperamide compounds undergo UV-light induced dimerization forming cyclobutane dimers, which can be synthesized via a straightforward procedure using light irradiation. Furthermore, drawing inspiration from nature's pharmacological treasures, we aim to synthesize new-to-nature analogues and dimers to systematically evaluate their bioactivities, thereby advancing the exploration of plant natural products as potential drug candidates.

Keywords: *Piper fimbriulatum,* alkaloids, piperlongumine, dimers, drug leads

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S3.P78 Inhibition of autotaxin by quercetin and its derivatives

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Autotaxin (ATX), an extracellular enzyme catalyzes the hydrolysis of lysophosphatidylcholin (LPC) to lysophosphatidic acid (LPA), is associated with several disease conditions including inflammation, fibrosis, and cancer (Magkrioti et al., 2019). Quercetin is one of the most potent anti-oxidants of plant origin as a member of flavonoids (Ganbold et al., 2019). In this study, we investigated the ATX inhibitory activities of seven kinds of flavonoids. We selected six flavonoids as quercetin-derived flavonoids (QDF), such as apigenin, kaempferol, azaleatin, rhamnetin, isorhamnetin, and tamarixetin, measured the blockage of ATX secretion by QDF using ELISA. We also determined cellular ROS generation by QDF using DCFDA assay and ATX protein expression change by QDF in vitro. In addition, we performed a protein-ligand docking simulation and examined the hydrophobic interactions and hydrogen bonds formed at the interfaces between the flavonoids and ATX using AutoDock 4.2 software. Four flavonoids, including apigenin, rhamnetin, tamarixetin, and isorhamnetin, showed the inhibition of ATX secretion by more than 30% at maximum concentration (10 μM). Among them, rhamnetin demonstrated the highest inhibitory effects. In cells, rhamnetin also strongly inhibited ROS generation and ATX protein expression comparing those of quercetin. QDF were further identified for their interaction efficacy with ATX, which supported our results demonstrating the inhibition of QDF against ATX binding interactions. Collectively, our findings suggest that QDF including rhamnetin inhibit ATX activity at a cellular level, which may prove useful as part of an overall modulating the diseases related to ATX.

Keywords: autotaxin, quercetin, quercetin-derived flavonoids (QDF), molecular docking

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S3.P79 Exploring vibrational spectroscopic approaches for quantifying main phytocannabinoids in medicinal *Cannabis sativa* inflorescence

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The exponential increase of medical cannabis consumption has resulted in higher demand for its effective large-scale cultivation and manufacturing. Additionally, quality criteria must be continually assessed throughout the manufacturing process since the growing procedures have a significant impact on the variability of phytocannabinoid content in plant material. Rapid technological advancements in recent years have resulted in a notable surge in the application of vibrational spectroscopic techniques, including Raman, mid-infrared (MIR), and near-infrared (NIR) spectroscopy, in conjunction with chemometric analysis, as highly effective for industrial research, process monitoring, and quality control (Deidda et al., 2022).

Thus, the goal of our study was to evaluate and compare these techniques for quantitative measurement of the main phytocannabinoids, tetrahydrocannabinol (THC) and cannabidiol (CBD), in medical cannabis flowers in order to further explore their potential for application as process analytical technology (PAT) in the medical cannabis industry.

MIR, NIR, and Raman spectra were obtained for large sets of dried cannabis flower, accompanied with HPLC determined content of total THC (THC% + 0.877*THCA%) and total CBD (CBD% + 0.877*CBDA%). Partial least-squares (PLS) analysis was carried out on the collected spectra in order to create calibration models for the determination of THC and CBD content in cannabis flowers.

Based on the acquired results, vibrational spectroscopic methods should be considered as a promising PAT tool for rapid and non-destructive estimation of THC and CBD in the medical cannabis industry.

Keywords: THC, CBD, spectroscopy, medical cannabis, PAT

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S3.P80 Exploring the therapeutic synergy: comprehensive assays unveil the potential of sericin and *Chelidonium majus* L. in developing a possible wound dressing

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Termed as a "silent epidemic," wounds exert a significant social and economic toll, detrimentally affecting the quality of life for millions worldwide. Natural substances like Sericin and *Chelidonium majus* L. (C. *majus*) have shown potential in wound care (1–4). Sericin, a silk protein, accelerates wound healing by promoting cell proliferation, collagen synthesis, and angiogenesis (5–7). On the other hand, C. *majus*, a medicinal plant, has anti-inflammatory effects and supports tissue regeneration (8–11). Combining these substances in wound dressings could address multiple aspects of the healing process. This investigation aimed to assess the potential synergy between Sericin and C. *majus* in the context of wound healing. Cytotoxicity, anti-inflammatory, antimicrobial, and wound healing assays were conducted to observe the healing rates under distinct concentrations (1:1, 1:2, 2:2, and 2:1 ratios) of the two extracts combined. These extracts, in all ratios, included various combinations of C. *majus* and Sericin sourced from Bragança, Castelo Branco, and two commercial types. Through these assays, valuable insights were gained into the collaborative influence of Sericin and C. *majus* on the wound healing process. Across all assays, results varied among the different combinations tested. However, the combination that consistently stood out across multiple assays was the 2:2 ratio with Sericin sourced from Castelo Branco. The exploration of these natural substances as constituents of wound dressings presents a hopeful path for crafting sustainable, efficacious, and biocompatible materials, that could significantly impact the field of wound healing.

Keywords: wound healing, biomaterial, dressing, sericin, greater celandine

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S3.P81 Synergistic impact of natural stilbene-linked mono-substituted nitroarenes in hormone- dependent breast cancer: a greener approach

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Resveratrol, a natural stilbene, is a promising multi-target drug with diverse biological effects. However, its low bioavailability has led researchers to develop novel derivatives to address pharmacokinetic issues (Turcov et al., 2022). In the present study, a series of nitroarene substituted resveratrol analogues (NSRAs) were synthesized using resveratrol isolated from green grapes to study their anti-breast cancer properties on a panel of hormone-dependent (HR+) and hormone-independent (HR-) breast cancer (BC) cells. Structures of NSRAs were elucidated by ^{1}H-NMR, ^{13}C-NMR, FT-IR, and HPLC/MS (Krishnamurthy et al., 2023).

The cell viability of both HR+ T-47D and HR- MDA-MB-157 decreased in a dose and time- dependent manner when treated with isolated resveratrol and NSRAs, individually. Interestingly, NSRA-I demonstrated augmented and selective antiproliferative activity against HR+ T-47D cells (IC_{50} = 17.92±0.072 μM) compared to isolated resveratrol (IC_{50} = 27.26±0.087 μM). Further investigations unveiled that both the NSRAs induced apoptosis in T-47D cells, leading to cell cycle arrest at the G_0/G_1 phase after 48 hours of incubation.

The study emphasises the curative potential of the synthesised novel resveratrol analogues, particularly NSRA-I, establishing it as a potential scaffold to design and develop novel stilbene analogues with improved therapeutic effectiveness and selectivity towards HR+ breast cancer cells. Additionally, the compound's antiproliferative capabilities, combined with its ability to cause programmed cell death along with cell cycle arrest, nominate it as a promising therapeutic candidate. Advancing research and uncovering potent inhibitors, utilizing resveratrol as a precursor for HR+ breast cancer with enhanced efficacy, remains a viable direction. Ongoing investigations are currently underway to delve deeper into these possibilities.

Keywords: stilbene, resveratrol, nitroarenes, MTT, HR+ breast cancer

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S3.P82 The natural diversity of acyltransferases reveals versatility and specificity in the synthesis of gene-encoded lipopeptides

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Lipopeptides comprise biochemical compounds that can be naturally produced or chemically synthesized. They exhibit diverse bioactive functions and are widely used in health care (Hamley, 2015). Despite their versatility, the production and diversification of lipopeptide compounds continue to be a challenge for state-of-the-art biological and chemical synthesis. In contrast, we offer a more streamlined and adaptable route to lipopeptide natural products via ribosomally synthesized and post- translationally modified peptides (RiPPs). Their primary structure is determined by a genetically encoded precursor protein, which is modified by maturases within the same biosynthetic gene cluster (Montalbán-López et al., 2021). The crucial lipidation reaction is catalyzed by a novel class of maturases of the GCN5-related *N*-acetyltransferase (GNAT) family, which introduce medium-chain fatty acids onto the sidechain amino group of lysines or arginine-derived ornithines (Hubrich et al., 2022) (Fig. 1).

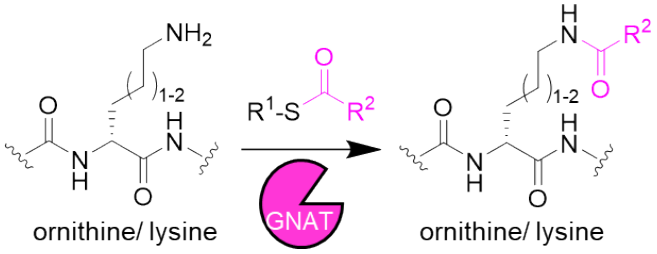

Fig. 1. Transfer of fatty acids to the sidechain of ornithines and lysines by RiPP GNATs.

Here we present the natural diversity of this GNAT family and their corresponding gene-encoded lipopeptides by characterizing the substrate scope of selected GNATs with their cognate precursor peptides. We could show that a variety of fatty acids can be introduced including chain lengths from C10 to C18. We are currently exploring this enzyme class to enable custom peptide engineering efforts towards generating bioactive lipopeptide compounds. We envision this platform to be used to facilitate the production of crucial lipopeptide compounds for human health and to quickly screen for antimicrobial lipopeptide compounds and non-ribosomal lipopeptide mimics.

Keywords: GNAT, RiPPs, lipopeptides, synthetic biology

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S3.P83 Exploring new dimensions: analytical method development for enhanced quality control of natural products

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Natural products are substances produced by living organisms. They serve as a valuable source for new leads in drug discovery and development and are widely used in medicine and phytotherapy as isolated plant compounds or as crude extracts.(Rodrigues et al., 2016) Natural products contain secondary metabolites that are not essential for basic life but play crucial roles, such as deterring predators and attracting pollinators. Natural products can occur as complex mixtures that vary in composition depending on the species, growing location, genetics and/or harvest time. Terpenes are the largest class of phytochemicals. They consist of isoprene units, can be linear or cyclic and can be oxygenated. The small subtleties in the carbon backbone give rise to the diversity of terpenes.(Raeber and Steuer, 2023; Hüsnü et al., 2007) However, these subtleties pose a challenge for analysis and structure determination. Terpenes may exist as enantiomers, exhibit further isomer structures, share identical masses depending on the terpene class, and have similar fragmentation patterns when analysed by mass spectrometry.

Consequently, the exact composition of terpenes can influence the pharmacological efficacy of plants extracts. In this study, we applied three strategies to enhance the current analysis of terpene patterns in plants. These strategies range from the analysis of enantiomeric ratios, to the training and testing of classification algorithms using machine learning, and the examination of adduct patterns using ambient ionisation methods followed by mass spectrometric analysis.(Raeber et al., 2023; Raeber and Steuer, 2023) These strategies are designed to overcome the existing limitations of terpene analysis and enable in-depth characterisation of plant extracts.

Keywords: chemometrics, terpenes, ambient ionization, classification, quality control

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S3.P84 Simultaneous quantification of terpenes and cannabinoids in *Cannabis sativa* by reversed phase LC-APCI-MS/MS

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In recent years, *Cannabis sativa L.* has undergone a significant paradigm shift, transitioning from a prohibited drug to being recognized as a medicinal candidate for a wide range of diseases. This change has also led to changes in legal regulations in Europe and other countries (Pattnaik *et al.*, 2022). As a consequence, the plant was included in various pharmacopoeias, such as the Pharmacopeia Europea 11.5. (Ph. Eur.). The Ph. Eur. distinguishes between different chemotypes of *C. sativa* based on their ratio of THC to CBD (Council of Europe, 2023). While widely accepted, this classification method is overly simplistic and does not do justice to the complex chemical composition of *C. sativa*. Other minor cannabinoids are of increasing interest, and interactions between cannabinoids (terpenophenols) and terpenes have been proposed, which may influence pharmacological effects (Russo, 2011).

Current state of the art analytical techniques favour liquid chromatography (LC) for cannabinoid analysis, as the cannabinoid acid forms are preserved. Gas chromatography (GC) is preferred for volatile compounds such as terpenes and can also be used for simultaneous cannabinoid analysis. However, this approach requires prior derivatization of cannabinoid acids, a timely process suffering from incomplete reactions (Zivovinovic *et al.*, 2018).

In this study, we present the first method to our knowledge that combines terpene and cannabinoid analysis in a single LC-MS/MS method. This method allows the quantification of both terpenes and cannabinoids without the need for prior derivatization of their acids. This technique offers a fast, reliable and holistic approach that allows a deeper understanding of chemotype classification.

Keywords: chemotype, classification, LC-MS/MS, ambient ionization, *Cannabis sativa*

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S3.P85 LumiNose: a novel instrument to apply the receptomics biosensor technology for identifying and quantifying medicinal bioactivities in plant extracts

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G-protein coupled receptors (GPCRs) are important targets for various plant and food components. The members of this large family of membrane proteins are involved in virtually every physiological process. By analysing the interactions between food components and receptors in the body, food scientists can determine the health potential of a particular food product. LumiNose, a powerful novel biosensor platform based on the Receptomics technology developed at Wageningen Research, mimics the exceptional sensing mechanism of insects for detection purposes by using insect olfactory receptors. Previous research by Wageningen Research has already demonstrated the technology to work with various human taste and health-related GPCRs and ion channels. This allows for the analysis of plant extract interactions with receptors in the human body, and the identification and quantification of the specific bioactive compounds. The implementation of LumiNose holds the potential to revolutionize the industry, offering more efficient and effective methods of discovery and product monitoring. In this regard, plant extract bioactivity has been detected for a range of different receptors, including satiety (GLP-1), dopamine, serotonin, and free fatty acid receptors. This information can also be used to optimize product formulations and improve bioavailability.

Keywords: GPCR, receptomics, bioactivity, health, food

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S3.P86 pH-Dependent degradation of andrographolide; kinetics, product identification and anti- inflammatory and cytotoxic activity assessment

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Andrographolide, the abundant labdane-type diterpene lactone in the leaves of *Andrographis paniculata* (Burm. f.) Wall. (Acanthaceae), is increasingly under investigation for its biological properties. Consequently, the kinetics of the chemical reactions of andrographolide under acid and basic condition, the identification of the degradation products, and their preliminary biological assessment, are of interest. The thermal degradation kinetics of andrographolide at pH 2 (70, 77, and 85 °C), 6 (60, 70, and 80 °C), and 8 (50, 60, and 70 °C) were determined. The calculated values of the activation energies (E_a), shelf-life ($t_{90\%}$), and rate constant (k) were determined. The effect of pH on andrographolide was assessed using the Arrhenius equation. First-order reaction kinetics were fitted to the data using linear regression analysis to determine the reaction rate constant at each pH and temperature combination which increased with increasing temperature and pH. The optimum pH for andrographolide stability was between pH 2 and 4.

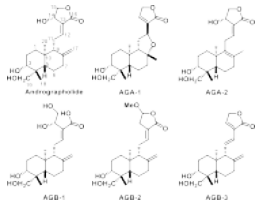

Fig. 1. Structures of andrographolide and its degradation products formed under acidic (**AGA-1**-**2**) and basic (**AGB-1**-**3**) conditions

The major degradation products in pH 2 and 6 solutions were isolated and characterized by NMR spectral analysis. Two degradation products, isoandrographolide (**AGA**-**1**) (Lui et al., 2019) and 8,9-didehydroandrographolide (**AGA**-**2**) were formed under acidic conditions, whereas the three degradation products generated under basic conditions (pH 8) were 15-*seco*-andrographolide (**AGB**- **1**), 14-deoxy-15-methoxy-andrographolide (**AGB**-**2**) (Gui-Yang, et al., 2017) and 14-deoxy-11,12- didehydroandrographolide (**AGB**-**3**) (Fig. 1) (Phattanawasin et al., 2018). The anti-inflammatory and cytotoxic activities of the degradation products were also evaluated.

Keywords: Andrographolide, pH-dependency, degradation products, structure determination, formation kinetics

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S3.P87 Immunohistochemical evaluation of the effect of *Kigelia africana* stem-bark extracts on benign prostatic hyperplasia induced by testosterone and estradiol in albino Wistar rats

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Stem-bark extract of *Kigelia africana* (KA) is traditionally used for treating various health conditions including benign prostatic hyperplasia (BPH). However, its use for treating BPH has not been validated.

Male rats weighing above 200g body weight (bw) had exogenous administration of testosterone and estradiol in staggered doses (three times weekly) for three weeks. Induced animals were divided into five groups of 3 per group. Group 1 was positive control while the induced animals were distributed between groups 2 to 6. Group 2 was untreated (negative control). Group 3 (prophylactic study) received by gavages the extract (200 mg/kg bw) simultaneously with the steroid administration for thirty days. Groups 4 and 5 received extract at 100 and 200 mg/kg respectively by gavages for thirty days while group 6 received finasteride (0.1 mg/kg). The assays conducted include dihydrotestosterone level (DHT) and prostate-specific antigen (PSA). Immunohistochemical (IHC) techniques were performed on the sections using primary antibodies TNF-a, a potent pro- inflammatory cytokine involved in wide spectrum of activity including cell proliferation.

The extract exerted marked decrease in the prostate weight (0.23 g) compared to the untreated with average weight of 0.70 g. Dihydrotestosterone level showed marked decrease in the extract treated (1352 ng/L) compared to the untreated with 1913 ng/L. IHC staining showed high intensity of the marker expression in the negative control but decreased very significantly with dose in the extract treated group.

The extract treated showed decrease in DHT and prostate weight with the IHC staining indicating less intensity.

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S3.P88 Styryl lactone derivatives and aristolactam alkaloids from *Goniothalamus tapis* Miq. and their α-glucosidase inhibitory activity

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Two recently discovered styryl lactone derivatives, namely goniothapic acids A (**1**) and B (**2**), along with 18 previously identified compounds, were extracted from the twig and leaf samples of *Goniothalamus tapis* Miq. The new compounds' structures were determined through spectroscopic methods and high resolution electrospray ionization time-of-flight mass spectrometry (HRESITOFMS), and their absolute configuration was confirmed by comparing experimental and calculated electronic circular dichroism (ECD) spectra. The α-glucosidase inhibitory activity of eleven compounds was assessed, revealing that (−)- goniothalamin (**5**) and oldhamactam (**16**) exhibited the most potent inhibition with IC_{50} values of 54.8 and 57.9 μM, respectively (Phukhatmuen et al., 2023).

Fig. 1. Structure of goniothapic acids A (**1**) and B (**2**)

Keywords: *Goniothalamus tapis*, Annonaceae, styryl lactones, aristolactam alkaloids, α-glucosidase inhibitory activity

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S3.P89 Exploring synergies between hemp cannabinoids and β-caryophyllene on triple-negative breast cancer cell line

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Hemp, also known as *Cannabis sativa*, contains over 500 compounds that include cannabinoids and terpenes. Some noteworthy studies have highlighted that cannabis inflorescence extracts were more active than the single purified cannabinoids on breast cancer cell lines (Blasco-Benito et al., 2018). Furthermore, the interplay between cannabinoids and terpenes has been emphasised, with β- caryophyllene, the major sesquiterpene, emerging as an agonist of the CB2 receptor, which is part of the endocannabinoid system.

Our study aims to determine the anticancer properties of combinations of hemp components and contributes to the understanding of the synergies in hemp. Neutral/acidic cannabinoids and β- caryophyllene were combined and tested in triplicate against a human triple-negative breast cancer cell line (MDA-MB-231). Cell proliferation was quantified kinetically for 3 days using an Incucyte SX-5 live-cell imaging system (Sartorius) and at a single time point (72 hours) with a resazurin-based metabolic assay. The SynergyFinder Plus web application was used for analysing and visualising multi-drug combination response data (Zheng et al., 2022) (Fig. 1).

The highest synergy score was obtained with cannabidiol and β-caryophyllene, highlighting the potential of a cannabis extract rich in these compounds. Nonetheless, the presence of cannabinoid acids in the extract is undesirable, as our results showed the lowest synergy score, indicative of an antagonistic interaction. These findings not only contribute to the understanding of the entourage effect in hemp but also guide the formulation of extracts that harness the full synergistic power of *Cannabis sativa* for enhanced therapeutic efficacy.

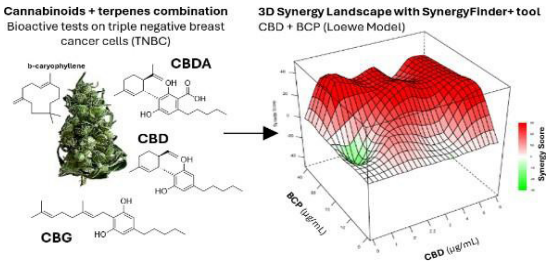

Fig. 1. Experimental flowchart

Keywords: *Cannabis sativa,* synergy, breast cancer, cannabinoids, β-caryophyllene

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S3.P90 Response Surface Methodology as a tool to obtain a potential satiating bitter enriched extract from *Gentiana lutea* L.

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In recent years, there has been a growing interest in bitter compounds due to their ability to regulate energy intake and hunger (Rezaie et al., 2021). Obesity is becoming a real challenge as current treatments are very limited with associated adverse effects. Nature offers several bitter compounds and in particular, *Gentiana lutea* L. roots represent a rich source of bitter secoiridoid glycosides like gentiopicrin or amarogentin, the most bitter natural compound (Ponticelli et al., 2023). Bitter compounds are able to interact with bitter taste receptors (*Tas2rs*), highly related to body weight regulation (Mennella et al., 2016).

Therefore, the aim of this work is to optimize the extraction parameters to recover bitter compounds from gentian roots and identify the molecular mechanism in the secretion of hormones that reduce the feeling of hunger in STC-1 cells. To obtain an extract rich in bitter compounds, three different parameters (time, solvent, and temperature) were considered using the Response Surface Methodology.

The optimal extractive conditions were 85 min, 95% EtOH, and 75°C. The main bitter compounds have been identified and quantified by HPLC-DAD. Treatment of STC-1 cells with optimized gentian extract increased the release of the incretins GLP-1 and CCK. These effects are mediated by the variation in intracellular calcium concentrations following the upregulation of the *Tas2r138* receptor or of other genes involved in the bitter taste transduction mechanism including *Plcβ2*, *Ip3r3* or *Trpm5*. Furthermore, molecular docking studies have allowed us to define the interactions between the main bitter compounds and the *Tas2r138* receptor.

Keywords: *Gentiana lutea* L., bitter compounds, Response Surface Methodology, *Tas2r138,* obesity

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S3.P91 The Botanical Safety Consortium: collaborative effort to improve botanical safety methods

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The use of natural health products, including botanical dietary supplements, is increasing, making their safety a crucial issue for public health. The Botanical Safety Consortium (BSC), has been formed through a Memorandum of Understanding as a collaborative effort between the US FDA, the National Institute of Environmental Health Sciences, and the Health and Environmental Sciences Institute. It aims to improve safety evaluation methods for botanicals, operating as an international platform where experts from various sectors, including government, academia, industry, and non-profits, collaborate to advance and apply new approach methodologies (NAMs) for the safety assessment of botanical products (1, 2).

The BSC is 1) collaborating with global stakeholders to advance scientific safety methods; 2) determining the right level of chemical characterization for complex botanical mixtures; 3) finding practical, suitable NAMs for safety evaluation; 4) testing these methods against existing safety data on certain botanicals; 5) incorporating these methods into a systematic framework for assessing botanicals.

Results representing the past year's work include a set of in vitro studies for selected herbs, supporting safety evaluations and exemplifying BSC's structure, ambitions, and methodologies.

Initially focusing on oral intake from dietary supplements, the BSC may broaden its scope in future work phases.

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S3.P92 Natural digestion mimicking curcuminoid formulation enhances oral bioavailability of curcuminoids

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Curcumin, the fat-soluble, active ingredient of the curcuma root, is known for its low absorption and bioavailability and therefore various compositions and galenic technologies like addition of cyclodextrin or pepper extracts are on the market. The galenic formulation tested in this study was intended to increase bioavailability of curcuminoids and to mimic the natural digestion process for fat-soluble substances additionally. Thus, the curcuminoids were provided in an oily matrix mixed with phospholipids without integration of new technology or artificial excipients. This study evaluated the oral bioavailability of curcuminoids in this natural galenic formulation compared to the unformulated native curcuminoid powder. A randomized, cross-over, single oral dose study (200 mg curcuminoids) in 12 healthy subjects was performed under fasting conditions. Pharmacokinetic parameters were analysed from individual concentration-time curves of total curcuminoids as well as curcumin. Data showed significantly higher AUC0-4h levels after the intake of the new phospholipid curcuminoid formulation for total curcuminoids (106.7 vs. 43.17 ng/mL*h, p=0.0001) as well as for Curcumin (77.8 vs. 20.66 ng/mL*h, p<0.0001) in comparison to the native curcuminoid powder. C_{max} was also significantly higher for both parameters analysed (total curcuminoids: 47.54 vs. 21.16 ng/mL, p=0.0001; Curcumin: 25.33 vs. 6.98 ng/mL, p<0.0001). In addition, the uptake kinetic of total curcuminoids was significantly faster with the new phospholipid curcuminoid formulation than that of the unformulated native curcuminoid powder (total curcuminoids: 2.21 vs. 5.17 h, p<0.05). In conclusion, the study confirms good tolerability of the new phospholipid curcuminoid formulation and its enhancing impact on curcuminoid uptake.

Keywords: Curcumin, bioavailability, phospholipid matrix, uptake kinetik

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S3.P93 Reveal the structure of plants by X-ray imaging and biomedical beamline at SSRF

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X-ray Imaging and Biomedical Applications Beamline (BL13W) at Shanghai Synchrotron Radiation Facility (SSRF) has been opened to users since 2009. It is a powerful tool for non-destructive, high- resolution, and three-dimensional imaging research on samples from biology, materials, archaeology, geophysics, and other fields (Xie et al., 2015). With the construction of the phase II project of SSRF, BL13W underwent relocation to BL13HB in 2021 (Ji et al., 2023). The multilayer monochromator significantly enhanced the photon flux at the experimental station by over an order of magnitude, making it more suitable for dynamic X-ray imaging. The high resolution of 0.8μm has greatly assisted in the identification of traditional Chinese medicinal materials (Ye et al., 2013) and the study of drug structures in pharmaceuticals. Additionally, dynamic X-ray imaging has provided valuable insights into the transport of water in plant roots and stems (Xu et al., 2023).

Projection images of Saw Palm was collected at X-ray Imaging and biomedical Beamline. The tomographic image (Fig.1) was calculated from the projections. The internal structure of the seed is clearly visible (Jaiswal et al., 2019).

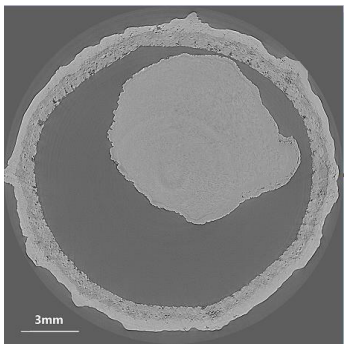

Fig. 1. Tomography which shows the structure of the seed

BL13HB is like a high-resolution X-ray microscope that can help us reveal the internal structure of plants and many other types of samples.

Keywords: *X-ray Imaging,* multilayer monochromator, tomography

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S3.P94 A cyclodextrin based tertiary complex of curcuminoids to improve bioavailability -its basic characteristics and immunomodulatory properties

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The work aims to develop a water-soluble formulation from the rhizomes of *Curcuma longa* L. with immunomodulatory properties. Curcuminoids (CUR) were extracted using ethanol, and the total curcumin content was quantified by high-performance liquid chromatography (HPLC) utilizing the Agilent Technologies 1260 Infinity Series (Pawar et al., 2013). CUR (Curcumin equivalent 0.02 mmol) were complexed with beta-cyclodextrin (Poly-β-CD) at a 2:1 ratio. Polysaccharides (PS) isolated by hot water extraction from the rhizome (Yue et al., 2010), along with ascorbic acid (A), were included. Complexation was achieved through freeze-drying technique (Mohan et al., 2012). The CUR-A-Poly-β-CD complexes exhibited an average particle size of 517.9 nanometers, determined by laser light scattering using Horiba, Partica LA-950V2, with a solubility of 13.65 mg/L and stability over 45 days. The complexation process was analyzed using X-ray powder diffraction (XRPD). FTIR spectroscopy (Spectrum GX, Perkin Elmer) was utilized to confirm the presence of CUR, PS, A, and CD by comparing the FTIR spectrum of a sample containing the CUR-A-Poly-β- CD complex with reference spectra of standard compounds. The release kinetics of curcuminoids from the CUR-A-Poly-β-CD complex were assessed *in vitro* (Monmai et al., 2018 and Tang et al., 2019). Different ratios of CUR and PS within the CUR-A-Poly-β-CD complex were evaluated using biological and immunomodulatory activity assays (viability, phagocytosis, CD86/CD69 determination) in THP1, Jurkat T, and fibroblast cells. None of the tested ratios exhibited cytotoxic effects, although they differentially influenced immune cell functions.

Keywords: *Curcuma longa* L., water-soluble formulation, immunomodulatory activity, CUR-A- Poly-ß-CD complex

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S3.P95 Phytochemical analysis of artichoke (*Cynara scolymus*) herb: Implications for *in vitro* neuroprotective and anti-obesity potential

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The artichoke (*Cynara scolymus*) is recognized for its positive impact on health, supported by experimental and clinical research. Its traditional application, renowned for addressing gastrointestinal issues, stems from its choleretic and cholagogic effects. Beyond this, the artichoke herb, enriched with active compounds like phenolic acids and flavonoids, holds potential in addressing neurodegenerative diseases and obesity (Nourhan et al., 2021, Rondanelli et al., 2020). Hence, the aim of this study was to assess the inhibitory effects on acetylcholinesterase and lipase, along with examining the antioxidant activity of a dry, spirit extract derived from artichoke herb.

The methodology involved analyzing the tested extract using HPLC to determine the percentage content of chlorogenic acid (Carneiro et al., 2017). The total polyphenol content in the extract was quantified. The antioxidant activity of the extract was measured using DPPH, CUPRAC, and ABTS methods. Furthermore, the extract's ability to inhibit lipase and acetylcholinesterase was determined (Stasiłowicz-Krzemień et al., 2023).

The obtained results show that the tested extract contains 1.11% of the chlorogenic acid, meeting the European Medicines Agency requirement of at least 0.8% of this compound in artichoke herb extracts. The extract demonstrated antioxidant activity, as assessed including the use of DPPH, ABTS, and CUPRAC assays. It also showed inhibition of lipase (EC_{50} = 53.144 µg/mL) and acetylcholinesterase (EC_{50} = 1.501 mg/mL), albeit less potent than standard substances. The average polyphenolic compound content was found to be 10.052 mg GAE/g, suggesting these compounds contribute to antioxidant and enzyme inhibitory activities.

Keywords: *Cynara scolymus,* phytochemical analysis, in vitro biological activity screening

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S3.P96 A preliminary study investigating the wound healing potential of *Cedrus brevifolia* extracts in mice

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Wound healing stands as a paramount therapeutic pursuit, imposing significant challenges on healthcare, particularly for vulnerable populations such as diabetic patients and the elderly. Notably, wounds in diabetic individuals may escalate to the point of limb excision, while bedsores (pressure ulcers) among the elderly can culminate tragically and may lead to death. In this context, our study aims to investigate the potential of resin, leaves and bark extracts from *Cedrus brevifolia* (Hook.f.) Elwes & A. Henry in promoting wound healing in mice model. *C. brevifolia* is an endemic species of Cyprus, grown in Tripylos region, commonly known as Cedar Valley, within the Paphos forest. Despite its endemism, this species exhibits negligible genetic divergence from its Mediterranean related species. Previous studies revealed the chemical profile of extracts of different plant parts, i.e. needles, sapwood, heartwood and resin, focusing on potential active constituents, namely phenolic acids and flavonoids from polar bark extracts, while resin was rich in lignans and phenolic acids. These investigations were followed by assessments of the antioxidant and anti-inflammatory potential of all extracts and derived isolates (Douros et al., 2018; id., 2019). The findings underscore the need for further exploration in an *in vivo* setting, as indicated by Charalambous et al. (2022). Our experimental design employed 40 male SKH-hr2 black and brown mice aged 2-4 months. Wounds measuring 1 cm^2 were meticulously induced in anesthetized mice and the potential healing effect of the extracts was evaluated. The healing potential of *C. brevifolia* resin and extracts was then rigorously assessed through daily application of gel formulations containing resin concentrations of 5% and 10% w/w, alongside bark methanol-water extracts at concentrations of 0.5% and 1% w/w. These formulations, meticulously prepared with 10% propylene glycol and 3% carbomer 940, were subjected to comparative analysis. Evaluation of the treatments encompassed a multifaceted approach, incorporating clinical observations, skin biophysical parameter assessments utilizing the Antera 3D camera, and FT-IR spectroscopy, in addition to histopathological examination. Through this comprehensive evaluation, we aimed to elucidate the effects of *C. brevifolia* extracts on wound healing dynamics. Our study bridging the gap between *in vitro* observations and *in vivo* outcomes attempts to shed light on the potential therapeutic benefits of *C. brevifolia* in wound care.

Keywords: *Cedrus brevifolia,* Pinaceae, resin, bark methanol-water extract, wound healing

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S3.P97 Preformulation studies of lycopene-rich tomato extract

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The most common sources of lycopene in diets are tomatoes and products containing tomatoes. More than 85% of this ingredient in our diet comes from these sources. Tomatoes are also the cheapest source for lycopene production. The tomato-based products are a better source of this compound than raw tomatoes. Different varieties of tomatoes, as well as other fruits and vegetables, contain different amounts of this ingredient (Kulawik et al., 2023).

The methodology involved analyzing the tested tomato extract using HPLC to determine the content of lycopene (1.5%) (Olives Barba et al., 2006). Holt-melt extrusion technique was used to obtain solid dispersions of tomato extract with polyvinylpyrrolidon and Soluplus with following extract concentrations 10%, 20%, 30% and 40%. The prepared extrudates were further studied by XRPD to confirm their amorphous state. The intermolecular interactions were investigated by FT-IR/ATR. To determine the impact on biopharmaceutical properties, solubility as well as dissolution studies of lycopene were performed. The antioxidant activity of the extract was measured using the DPPH method.

The obtained results show the improvement of pharmaceutically important parameters. Formulations showed great potential for further development.

Keywords: Tomato extract, lycopene, hot-melt extrusion, amorphous state, solubility

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S3.P98 Investigating the mechanism of action of the anti-aging ability of geraniin nano-phytosome

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This study explores geraniin, an ellagitannin from fruit waste, as a sustainable anti-aging solution in cosmetics. To overcome absorption challenges, a topical approach leveraging geraniin's potent antioxidant properties is pioneered. By unraveling its anti-aging mechanisms and introducing an innovative geraniin nano-phytosome, this study aligns with sustainability goals and addresses bioavailability issues, aiming to redefine skincare standards with eco-conscious formulations.

Geraniin from Rambutan rind was purified and assessed for its anti-aging effects on key skin enzymes. Nano-phytosome formulations containing geraniin, soy phosphatidylcholine, squalene, and edge activators (Tween 80, Span 80, sodium cholate hydrate) were developed. The optimized formulation was characterized for stability, entrapment efficiency, morphology, particle size, and permeability through ex vivo permeation studies.

Geraniin displayed significant anti-collagenase, anti-elastase, anti-hyaluronidase, and anti-tyrosinase effects compared to the positive control. The geraniin nano-phytosome with Tween 80 showed stability, elliptical morphology, entrapment efficiency of 67.61%, average particle size of 95.60 nm, and polydispersity index of 0.167. Although geraniin did not penetrate the skin, it accumulated within the membrane (53.87%), suggesting suitability for topical application.

This study underscores geraniin's promising anti-aging properties, demonstrating significant effects on key skin enzymes. Utilizing a nano-phytosome formulation with Tween 80 enhances its characteristics for topical application, indicating its potential in eco-conscious skincare formulations despite limited skin penetration.

Keywords: nanophytosome, geraniin, skin anti-aging, mechanism of action, permeability

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S3.P99 QbD approach for the development of CBD-loaded polymeric micelles for oral delivery

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As part of our studies dedicated to the development of new drug delivery systems (Vanti, 2021) of cannabidiol, (CBD) a main constituent of *Cannabis sativa*, we report on QbD approach for preparation and optimisation of polymeric micelles for oral delivery. To date, only one prescription medicine has been approved. Oral administration of cannabidiol has been reported to have two major drawbacks: very low water solubility (12 mg/L) and extensive first-pass metabolism (Grifoni, 2022). To overcome these limitations, the present study developed micelles made of Poloxamer 407 (P407) and D-α-tocoferolo polietilenglicole 1000 succinato (TPGS). P407 is a GRAS excipient, while TPGS has been approved by FDA as a safe adjuvant and widely used in drug delivery systems. It has multiple advantages including high biocompatibility, enhancement of drug solubility, and improvement of drug permeation and inhibition of the activity of ATP dependent P-glycoprotein. The experimental design was carried out using D-optimal design; mixture process-variable (MPV) approach and mixture design were used for screening and optimization, respectively. Micelles loaded with CBD showed a size of 21.14±0.30nm, polydispersity index (PdI) of 0.040±0.004, recovery % of 98.24±2.52% and encapsulation efficiency % of 83.00±2.52%. Micelles loaded with CBD were evaluated for the stability in simulated gastrointestinal fluids in the presence of enzymes; a slight increase in PdI (0.280) was registered after intestinal dilution, while the size remained unchanged. A loss of up to 10% of CBD was observed in the simulated intestinal environment.

Keywords: design of experiments (DoE), cannabidiol, polymeric micelles, simulated gastrointestinal fluids

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S3.P100 Native metabolomics for the discovery of new small molecule gαi protein modulators

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The cost- and time-consuming process of finding a single bioactive molecule for a target protein from multiple complex crude extracts hampers natural product discovery. To accelerate and facilitate this iterative process of fractionating and bioactivity testing, we present a scalable native metabolomics approach, which combines non-targeted liquid chromatography-tandem mass spectrometry with the detection of protein binding by native mass spectrometry (Reher et al., 2022).

Heterotrimeric G proteins represent an interesting, but so far "undruggable" pharmacological target despite being involved in the regulation of a wide range of physiological and pathophysiological processes in living organisms. Currently, selective small molecule modulators specifically target Gαq and more recently Gαs (Dai et al., 2022), leaving the remaining Gα subfamilies without such modulators (Li et al., 2020). To date, only two highly selective Gαq inhibitors of natural origin have been identified: FR900359 (FR) and YM-254890 (YM). FR is derived from the endophytic *Candidatus* Burkholderia crenata, which is produced in the leaf nodes of the Traditional Chinese Medicine plant *Ardisia crenata* and underscores the remarkable diversity of interactions between organisms (Reher et al., 2018).

We use the native metabolomics approach to screen Gai against a diverse library of complex extracts derived from marine fungi. This led to the discovery, isolation and structure elucidation of the first selective small molecule inhibitor of Gαi protein that paves the way for fundamental studies as well as new therapeutic strategies towards Gαi-mediated diseases.

Keywords: native metabolomics, G protein, marine fungi

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S3.P101 NMR-HetCA uncovers bioactive secondary metabolites from propolis extracts prior to isolation

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Propolis is known for its antioxidant, antimicrobial and anti-inflammatory properties and it has been used traditionally to treat cuts, burns, wounds, and other skin conditions such as eczema and psoriasis. In our previous study (Stavropoulou et al., 2021), several propolis samples originating from Greece showed strong tyrosinase and high collagenase inhibition, respectively. In the current study, the methanolic extract of a sample from Mainland was fractionated by FCPC and the obtained fractions were evaluated *in-vitro* for their ability to inhibit DPPH free radical and collagenase enzyme. In parallel, the ^{1}H-NMR spectra of the fractions were recorded and the biological activity was statistically correlated with spectral data through the HeteroCovariance Approach (NMR-HetCA). NMR-HetCA is a MATLAB based toolbox that correlates NMR resonances and activity values through generated NMR pseudospectra, the HetCA plots. Each point (NMR peak) that is highly correlated is positive and color coded according to the respective correlation values (ranging from blue for those that show low correlation to deep red for those that show high correlation). HetCA plots revealed prior to isolation, high correlation of phenolic substances for DPPH and collagenase inhibition. In particular, the analysis suggested that polyphenols contribute to the DPPH free radical scavenging activity, while caffeic acid derivatives (e.g. CAPEs) seemed to be responsible for the anti- collagenase activity. The direct identification of the bioactive constituents was achieved in the complex propolis extract by the NMR-HetCA approach, a promising tool that can be used to accelerate the discovery of bioactive natural compounds prior to isolation.

Keywords: HeteroCovariance approach, propolis, antioxidant, anti-collagenase, caffeic acid derivatives

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S3.P102 Discovery of new bioactive compounds: cloning, activation and engineering of specialized metabolites biosynthetic gene clusters

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Genomes of bacteria are considered an inexhaustible source of new structurally diverse specialized metabolites. However, most of this chemical diversity is hidden within bacterial genomes, since corresponding genes are rarely expressed efficiently under laboratory conditions. Genomes of soil- dwelling *Actinomycetes* (in first turn *Streptomyces*), each harboring approximately 30 SM-BGCs, are the richest and validated source of secondary metabolites that are still awaiting to be discovered (Lee et al., 2019).

Heterologous expression is one of the most efficient approaches for the discovery of new specialized metabolites. However, it is limited by the access to the biosynthetic gene clusters (BGCs) to be expressed. We developed two different but complementary approaches for cloning the bacterial natural products BGCs. The first approach is based on optimized TAR (transformation-associated recombination) cloning. The procedure was modified by introducing the direct selection of clones carrying chromosomal DNA inserts. As a showcase, chelocardin (35 kbp), kanamycin (42 kbp), and daptomycin (67 kbp) BGCs were cloned and expressed in *Streptomyces albus* and *Streptomyces lividans* hosts. Another approach is based on massive BGCs cloning by cosmid library construction, sequencing, and mapping. This way multiple BGCs were recovered including eight aminoglycoside BGCs expressed in the aforementioned hosts.

In conclusion, the approaches presented here significantly increase the chances of discovering novel specialized metabolites and could be efficient for cloning large bacterial genome fragments. The details of both approaches will be presented.

Keywords: specialized metabolites, TAR cloning, heterologous expression

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S3.P103 *Origanum dictamnus* infusion characterization by HPLC-PDA-MS and nanoformulation development

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Origanum dictamnus L. (Lamiaceae) is a Greek species endemic to the island of Crete, commonly known as dittany. In 2014, dittany was authorized by EMA (EMA, 2014) under the category of Traditional Use Herbal Medicinal Products in the form of herbal teas for the treatment of skin inflammations and bruises, as a wound healing agent, for mild gastrointestinal disorders and relief of cough associated with cold.

In the present work, dittany infusion was prepared according to EMA and characterized by HPLC- PDA-MS. Identification of the constituents was assisted by chromatographic isolations (Paloukopoulou et al., 2023). Rosmarinic acid, salvianolic acid P, and other depsides were the main constituents, whereas apigenin and luteolin glycosides, as well as neolignans, were also present. The infusion was quantified based on its rosmarinic acid content. In continuation of our investigation on this important medicinal plant and given the EMA indications, preliminary studies were carried out with dittany infusion, which showed a relaxing effect on the rabbit intestine. Since infusions need to be consumed instantly to maintain their nutritional features, the inclusion of aqueous herbal extracts in advanced technological forms was important to improve their chemical stability (Bilia et al., 2019). Specifically, this study focused on developing a nanoformulation for oral infusion delivery. The infusion was quantified in terms of rosmarinic and salvianolic acids by HPLC-DAD. The physical characterization of the nanoformulation was performed by dynamic light scattering and transmission electron microscopy. Both techniques showed nanoparticles of about 130 nm and optimal homogeneity in dimension distribution. The infusion encapsulation efficiency was evaluated by the dialysis method and showed a percentage of 93 0.41 for rosmarinic acid and 92 1.2 for salvianolic acid. Chemical and physical stability studies on the storage and incubation of the nanoformulation in simulated biological fluids (saliva, gastric, intestinal) are ongoing. Preliminary findings showed a good stability of the nanoformulation during the first two weeks of storage.

Keywords: *Origanum dictamnus,* Lamiaceae, infusion, HPLC-PDA-MS, nanoformulation

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S3.P104 Extract from the seedlings of *Brassica oleracea var. italica* (Brassicaceae) reduces tissue damage and alters inflammatory markers over time: development of new medications

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This presentation outlines the utility of a combinatorial of natural broccoli chemicals processed by liver microsomal S9 fraction to reduce direct tissue injury and produce a more favorable cytokine and inflammation profile. Pain is a global issue needing more effective, affordable, and non-addictive and non-opiate therapies. Many chemicals derived from phenylalanine have antioxidant and antinflammatory properties, yet many structures are as yet unstudied (Perkowski & Warpeha, 2019). Using the edible plant broccoli, a growth protocol was developed to induce a concentrated combinatorial of potential phenylpropanoid anti-inflammatories extracted from seedlings, then treated with liver microsomal S9 fraction (Gurgul et al. 2023). A mouse model of pain behavior was used for an in vivo test of early and late pain responses in a paw. The vehicle control mice demonstrated tissue damage, and cytokine and NOS2 levels indicating negative aspects of inflammation. Pre-treatment of mice with S9 broccoli extract (ip injection 30 min prior to pain stimulus) indicated improved tissue integrity in early and late responses examined at the tissue level in the paw. In addition, cytokine levels showed changes right after pain stimulus, but also positive affects 35 min later. Preliminary data suggest that this non-opiate inflammation and pain-reducing treatment could have multiple systemic affects to reduce pain.

Keywords: phenylpropanoid, broccoli, pain, anti-inflammatory, opiate

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S3.P105 Identification of naturally-derived lipids as GPR84 agonists

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G Protein-Coupled Receptor 84 (GPR84) has emerged as a promising target for therapeutic interventions in metabolic dysfunction, inflammation-related diseases, cancer, and bacterial infections (Luscombe et al. 2023, Wang et al., 2024) due to its predominant expression on immune cells (Lucy et al., 2019). However, understanding the pathophysiological role of GPR84 is hindered by the absence of a verified endogenous ligand. The suggested endogenous ligands of GPR84 (C_{10}- C_{14} Medium Chain Fatty Acids) show low potency *in vitro cAMP assay* (Marsango et al., 2022). This study aimed to develop suitable methods to identify potent endogenous or exogenous GPR84 ligands from natural resources.

As a test system, we focused on Goat Milk (GM) to discover novel GPR84-activating lipids through bioactivity-guided isolation and a biochemometric approach. Polar lipid fractions (S30_a and S30_b) from GM exhibited over 10-fold higher potency in cAMP assays compared to the most active reported medium chain fatty acid (capric acid C10), suggesting the presence of previously unreported GPR84 agonist(s). Component analysis and bioactivity-NMR correlations tentatively identified sphingolipid- like ligands.

Subsequently, five sphingolipid analogues with various alkyl chain lengths (R:C_6 to C_{10}) were synthesized and tested. The findings revealed that the Sphinganine analogue (R:C_9) showed better activity (EC_{50}:215 nM) than capric acid EC_{50}: 4.5 μM). Moreover, this discovery unveils the previously unreported potency of sphinganine analogues on GPR84 activation. Further efforts will involve additional separation and analysis of bioactive compound in the GM fraction, and development of omic-based platforms to robustly identify bioactive compound(s) in the natural product mixture.

Keywords: goat milk, GPR84, lipid, sphingolipids

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S3.P106 Quantitative analysis of endogenous steroids by UPLC-MS/MS in various medicinal plant species

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Traditionally, mammalian steroids were thought to be a diverse group of bioactive compounds found only in animal tissues. However, recent research has confirmed that mammalian steroids are also present in several plant species. These steroids, including pregnenolone, progesterone, testosterone and estrogens, have been identified in different parts of plants such as leaves, stems, roots and reproductive organs. Their localisation in specific tissues suggests a potential role in plant development and reproductive processes (Tarkowská 2019).

Plant steroids are known to modulate a wide range of physiological responses in plants, leading to improvements in both the quality and yield of food crops. Treatment of plants with steroids or their precursors affects plant development: cell division, root and shoot growth, embryo growth and flowering. These compounds also have many interesting medicinal and pharmaceutical effects, such as anti-cancer, anti-angiogenic, antiviral and antibacterial bioactivities (Janeczko 2021).

The aim of this work was to determine the profile of mammalin sex hormones (progestagens, androgens and estrogens) in medicinal plants (in their flowers, leaves, buds). A combination of SPE and ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC- MS/MS) was used for the determination of steroid compounds. The antiproliferative activity of selected plant extracts was also tested on cancer cell lines.

Keywords: steroids, UHPLC-MS/MS, anti-cancer activity, medicinal plants

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S3.P107 Enhancing drug discovery efficiency in plant extracts through polyphenol removal

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Polyphenols, such as flavonoids, tannins, and phenolic acids, are common compounds found in plant extracts. In the drug discovery process polyphenols in plant extracts are often promiscuous hitting compounds due to assay interference, non-specific binding, and redox cycling resulting in false positives. Moreover, their low *in vivo* oral bioavailability makes most polyphenols unsuitable as drug leads. In addition, the high abundance of these nuisance compounds can make the discovery of more attractive drug leads difficult. This study presents a method using polyvinylpolypyrrolidone (PVPP) resin in solid-phase extraction to reduce polyphenol content, thereby improving the detection of minor bioactive compounds. Evaluation of the process via HPLC-QTOF and MS/MS using Compound Discovery in Agilent MassHunter confirmed that approximately 80% polyphenols have been removed, and many previously obscured compounds have been identified. This method enhances confidence and quality in drug discovery screening outcomes and provide true hits that were previously suppressed due to excess polyphenols.

Keywords: polyphenols, polyvinylpolypyrrolidone, drug discovery, HPLC-QTOF, solid-phase extraction

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S3.P108 Bioactive *Lycopodium* alkaloids from clubmoss cultivars and transformation to enhance anti- acetylcholinesterase activity

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The *Phlegmariurus* genus (Lycopodiaceae) is cultivated as ornamental plants in Thailand, with extracts serving as a rich source of *Lycopodium* alkaloids (Ngernnak et al., 2021). This presentation encompasses the isolation, structure elucidation, transformation, and biological evaluation of isolated *Lycopodium* alkaloids from Thai clubmoss cultivars. Eleven unprecedented *Lycopodium* alkaloids, namely phlegcarines A-C and nummulines A-H, were isolated from *Phlegmariurus carinatus* (Desv. ex Poir.) Ching. (Thamnarak et al., 2023) and *P. nummulariifolius* (Blume) Ching, respectively. Additionally, an acetylcholinesterase (AChE) inhibitor, huperzine A, was identified. The structural assignments were established through comprehensive spectroscopic techniques, including HR-ESI- MS, NMR techniques, chemical correlations, and further confirmed by X-ray analysis specifically for nummuline D. While the isolated *Lycopodium* alkaloids were evaluated for anti- acetylcholinesterase activity, none demonstrated efficacy surpassing that of huperzine A (IC_{50} *ee*AChE = 0.03 μM). Huperzine A derivatives were further synthesized to enhance potency (Anukanon et al., 2021).

Fig. 1. Structure of new *Lycopodium* alkaloids

Keywords: Lycopodiaceae, *Lycopodium* alkaloid, *Phlegmariurus*, huperzine A, acetylcholinesterase

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S3.P109 Depicting the chemical diversity of bioactive meroterpenoids produced by the largest organism on earth

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We have explored the diversity of melleolide-type meroterpenoids produced by *Armillaria ostoyae*, one of the largest and oldest organisms on Earth, using extracts from liquid and solid fermentation media. The study unveiled three unprecedented dimeric bismelleolides (1-3) and three novel fatty acid-substituted congeners (4-6), along with 11 new (7-17) and 21 known (18-38) derivatives. Structure elucidation was done by 1D- and 2D-NMR spectroscopy, HRESI-MS data, and ROESY spectral analysis for relative configurations. Absolute configurations were determined through crystal structures and ECD spectra comparison. A compound library of melleolide-type meroterpenoids facilitated metabolomics-wide associations, revealing production patterns under different culture conditions. The library enabled assessments of antimicrobial and cytotoxic activities, unveiling that the Δ2,4 double bond is not crucial for antifungal activity. Cytotoxicity was linked to the presence of an aldehyde at C-1, but lost with a hydroxylation at C-13. Chemoinformatic analyses demonstrated the intricate interplay of chemical modifications on biological properties. This study marks the first systematic exploration of Armillaria spp. meroterpenoid diversity via MS-based untargeted metabolomics, offering insights into structure-activity relationships through innovative chemoinformatics.

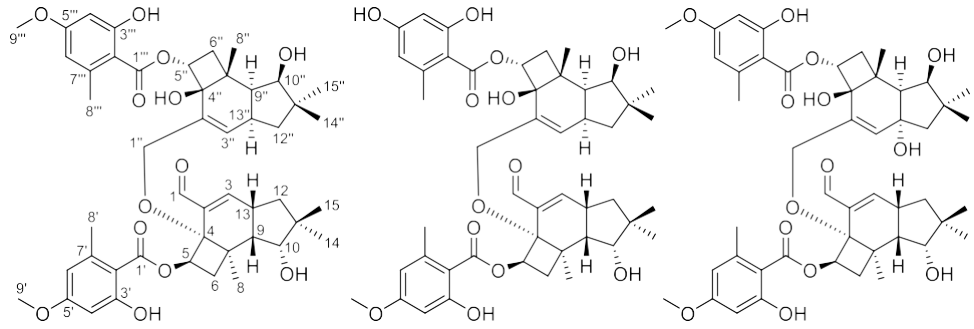

Fig. 1. Some chemical structures of newly identified metabolites from cultures of A. ostoyae.

Keywords: Basidiomycota, metabolic diversity, metabolomics, cheminformatics

Reference

Pfütze, S., Charria-Girón, E., Schulzke, E. et al.. 2024. Depicting the chemical diversity of bioactive meroterpenoids produced by the largest organism on Earth. Angew Chem Int Ed, in press. DOI:10.1002/ange.202318505

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S3.P110 Comparative *In Vitro* enzyme inhibitory activities of different plant parts of five *Albizia* species (Fabaceae)

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The number of diabetic patients in Africa is expected to rise to 41 million in 2045. Management of diabetes without any side effects is still a challenge to the medical community. The study aims to evaluate the comparative *in vitro* enzymatic inhibitory activities (a-amylase and a-glucosidase) of different plant parts of five *Albizia* species (*Albizia lebbeck, Albizia ferruginea, Albizia saman, Albizia coriaria Albizia zygia*).

The plants were selected based on previous studies, and were further authenticated at Forest Herbarium Ibadan, where voucher specimens were deposited. The plant parts were macerated for 72 hours at room temperature using methanol. The extract was filtered and concentrated to dryness and stored in a refrigerator at 4 ℃. The phytochemical screening of the crude extract was done following a standard procedure (Trease and Evans, 2002). Ethyl acetate fractions from partitioned extracts showing the best activity in α −amylase and α −glucosidase inhibitory assays were subjected to vacuum liquid chromatography (VLC). Data were analyzed using Graph Pad Prism 5.0.

The phytochemical screening of the crude extract revealed the presence of secondary metabolites in some species, and some are absent or present in little quantities. *Albizia lebbeck* bark had the lowest α- glucosidase and α- amylase inhibitory values (IC_{50} <31.20 µg/mL and 42.33 µg/mL, respectively) when compared with the standard acarbose (IC_{50} 121.50 µg/mL) and was subjected to solvent-solvent partitioning. Ethyl acetate fraction which showed the best activity in both α- glucosidase and α- amylase inhibitory assay was subjected to VLC.

Albizia lebbeck bark extracts could be regarded as a source of phytocompounds with potential use in the management of postprandial hyperglycemia.

Keywords: *Albizia lebbeck* (L.) Benth, Diabetes, Maceration, Vacuum liquid chromatography, α-glucosidase inhibitory assay

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S3.P111 Study and nutritional screening of some notanicals used as herbal remedies in managing eye and ear ailments in Ado-Odo Ota Local Government Areas, Ogun State

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Traditional medicine is one of the most well-established and widely used types of healthcare. It has long been known that phytochemicals can be used to treat infectious disorders. Several ailments, including those concerning vision and auditory senses, are treated with a variety of natural products. This research aims to source the natural products used in the management of ear and eye ailments.

An ethnobotanical survey was conducted in Ado-Odo, Ota Local Government. A total of hundred structured and validated questionnaires were administered to a hundred respondents by purposeful sampling method. Also, nutritional, phytochemical, and antinutrient constituents of the most mentioned plants were investigated. Ethnobotanical data were analyzed by ANOVA and DMRT at $p<0.05$.

Results revealed that 37 plant species belonging to 26 families being used to treat eye and ear ailments were collated. The most frequently mentioned plants were *Cucumeropsis mannii, Allium cepa,* and *Allium sativum*. Leaves (37.0%) seeds (30.0%), roots (10.0%), flowers value (7.0%) and fruits (7.0%), stems (5.0%) and barks (4.0%) were the most exploited plant part used for the diseases. The preparations of these plants were administered by dropping the preparations of the plants into the affected eye and ear, bathing with the leaf preparations, and oral application. Anti-nutrient, proximate values, and Vitamins were documented in *Cucumeropsis mannii. Allium cepa* and *Allium sativum.*

The effectiveness of plants like *Cucumeropsis mannii*, *Allium cepa,* and *Allium sativum* for the treatment of ear and eye diseases could be due to the presence of alkaloids, flavonoids, saponin, and Vitamin C.

Keywords: nutritional, conjunctivitis, phytochemicals, glaucoma, deafness

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S3.P112 New data about chemical composition of *Cynanchum acutum L.*

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This presentation will cover the isolation and structure elucidation of a new data about chemical composition from *Cynanchum acutum* (L.). This genus comprises of about 200 species in *Asclepiadaceae* family and is distributed worldwide. *C. acutum* is quite poisonous with few medical applications.

The roots of *C. acutum* were powdered and extracted three times with MeOH at room temperature. The collected extracts were dried under reduced pressure, and the concentrate was partitioned between $CHCl_3$ and H_2O. A part of the aqueous extract was subjected to column chromatography.

Column chromatography was carried out on Diaion HP-20, Sephadex LH-20 and silica gel yielding 7 known compounds: 3 cardenolide glycosides - lanatoside A, lanatoside C (Youchu Fujii et al., 1990) and corchorusoside C (Nathan P Mirtallo Ezzone et al., 2022), 2 flavonoids - kaempferol 3-O-β-D- rutinoside and quercetin 7-O-β-D-glucopyranoside (Mona A. Mohamed et al., 2008) and 2 pregnans – atratosides A and atratoside D (Zhuang-Xin Zhang et al., 1988) The full chemical structures were determined by using one- and two-dimensional nuclear magnetic resonance spectroscopy (1H, C13, HSQC, HMBC, COSY) and mass spectrometry.

It is significant that cardenolide glycosides in plants of the genus *Cynanchum* were described for the first time by us.

Keywords: Cynanchum acutum, cardenolide glycosides, flavonoids, pregnans

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S3.P113 A comparative study between CPC and flash chromatography for natural product purification

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Centrifugal Partition Chromatography (CPC) and flash chromatography are both chromatography- based techniques that are widely used in the pharmaceutical and cosmetic industries for drug discovery or enrichment of active ingredients. Further, CPC and flash chromatography are easily applicable to scale up to meet industrial applications (Sutherland, 2007) (Fernando et al., 2022). They differ from each other in the nature of their stationary phase. The CPC is liquid-liquid chromatography using two immiscible liquid phases, collectively referred to as the solvent system. Whilst flash chromatography requires the use of a cartridge filled with a solid stationary phase (e.g. silica, C_{18}). Due to its liquid nature, CPC is often seen as a solvent-consuming technique compared to other preparative strategies.

Based on these findings, the main objective of this study was to compare CPC and flash chromatography for the purification of piperine (**1,** Fig. 1), an alkaloid that confers the pugent taste to black pepper (*Piper nigrum*). Piperine can be easily purified in one step from crude extract of black pepper using CPC or flash chromatography. Both purification techniques were compared at laboratory and pilot scales in terms of purity, yield, solvent consumption, and injected quantity.

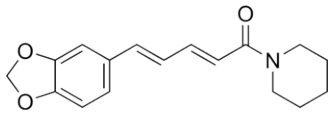

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Fig. 1. Structure of Piperine **1**

Results highlight CPC at laboratory and pilot scale for piperine purification. A larger quantity of sample can be injected in CPC, with lower solvent consumption and no solid waste to treat (i.e. silica cartridge). Furthermore, the purities obtained by CPC were always higher than those obtained by flash chromatography. Therefore, this work addresses the issue of solvent consumption and recommends CPC for purification of natural products.

Keywords: piperine, purification, CPC, flash chromatography

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S3.P114 Molecular docking and dynamics simulations study of Cathecin from *Mentha cordifolia* as potential HIV-1 protease pnhibitor and Tat1-Cyclin competitor

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There has been a 411% increase in daily incidence of Human Immunodeficiency Virus (HIV) cases in the Philippines from 2012 to 2023 (Gangciangco and Eustaquio, 2023). There are antiretroviral drugs that are available which increases a patient's survivability and prolonging their lifespan. However, the emergence of drug-resistant HIV strains and discovery of detrimental side effects of these drugs threatens the effectiveness of it.

A study showed that *Mentha cordifolia*, a plant included in the Philippines' Department of Health's Ten Medicinal Plants, can reduce the p24 production in HIV-1 latently infected cells (OM10.1) *in vitro* (dePaz-Silava et al., 2022). The same group was able to identify that cathecin, one of the known compounds found in the plant extract, can strongly bind to HIV-1 protease and Tat-Cyclin, and potentially inhibit their functions, through *in silico* analysis.

Researchers performed 100 ns of Molecular Dynamics simulation for both HIV-1 Protease and Tat- Cyclin to obtain and capture the movement of the proteins in the said timeframe. The conformations obtained will then undergo k-means clustering using CPPTRAJ-Amber. This is done to cluster the conformations into 99 cluster and obtain one representative conformation from each cluster. The conformations were then used for Molecular Docking Analysis of the proteins and cathecin. In the following weeks, the top five (5) cathecin-protein interactions that had the highest binding energy will be identified and further analyzed. This will be done to determine the important interactions present between the proteins and cathecin. The results that will be obtained from this will give insights on how cathecin can potentially inhibit both HIV-1 Protease and Tat-Cyclin. This will give insights on how *Mentha cordifolia* plant extract can potentially prevent the viral replication in the virus' life cycle at a molecular level.

Keywords: HIV, *Mentha cordifolia*, molecular docking, molecular dynamics simulations

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S3.P115 Phytochemical profiling of *Acalypha australis* and screening for its most potent anti-inflammatory compounds with bio-affinity ultrafiltration LC-MS

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Acalypha australis, commonly known as Copperleaf or Tiexian Cai, has served as an expectorant and for managing bacterial diseases and skin disorders. (Seebaluck et al., 2015). However, its phytochemical profiles along with its anti-inflammatory compounds have not yet been explored (Kim et al., 2020; Ma et al., 2017). In order to identify its potential anti-inflammatory compounds, an in vitro activity-guided fractionation followed by bio-affinity ultrafiltration LC-MS(UF/LC-MS) screening with the COX-2 enzyme, was successfully developed. By UPLC-QTOF/MS, 95 chemical compounds were characterized from *A. australis*, out of which 49 were reported for the first time in this species. Bioactivity-guided fractionation revealed that AAEA and AANB exerted similar COX- 2 inhibition with IC_{50} values of 0.08 ± 0.01 μg/mL and 0.20 ± 0.02 μg/mL respectively, which showed better activity than the reference drug indomethacin with an IC_{50} value of 0.47 ± 0.07 μg/mL. To further screening out its most potent compounds, the AAEA-3 fraction was subjected to UF/LC-MS targeting COX-2, and aurantiamide was then screened out and identified as the most promising COX- 2 ligand. Thereafter, the most potent ligand candidates were isolated and subjected to docking simulation and activity substantiation towards COX-2 to comprehend their probable mode of action. The docking interaction pf aurantiamide within the COX-2 active pocket exhibited a binding energy (BE) value of -9.56 Kcal/mol. The substantiation of aurantiamide activity towards COX-2 indicated a moderate inhibitory effect, with an IC_{50} calculated to be 512.11 ± 21.80 μM.

Keywords: *Acalypha australis,* anti-inflammatory compounds, UF/LC-MS, COX-2

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S3.P116 The natural products magnetic resonance database (NP-MRD): comprehensive resource for natural products NMR data enabling discovery and understanding

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The Natural Products Magnetic Resonance Database (NP-MRD, np-mrd.org) is a database and repository for natural products and specialized metabolites NMR data. NP-MRD contains raw (time domain) data, derived data (chemical shift assignments, coupling constants) curated from the scientific literature or raw data, predicted data from density functional theory (DFT) calculations or machine learning, and simulated spectra. The database also provides structures, synonyms, search and other tools, links to other databases, and deposition interfaces. NP-MRD is an open database complying with FAIR (findable, accessible, interoperable, reusable) database principles. The NP- MRD mission is to create an enduring research resource to facilitate discovery and understanding in natural products and secondary metabolites research.

NP-MRD accepts raw data collected in support of novel structure elucidations and characterization of mixtures, through an intuitive deposition interface; deposition is now required by some journals and funding agencies. NP-MRD also accepts legacy data still residing on computers in individual laboratories. Curation of derived data (*e.g.* chemical shifts) published in the literature is problematic due to ambiguous or erroneous assignments, incorrect referencing, or typesetting mistakes. Curation itself, particularly from older literature in pdf format, is slow and may introduce additional errors. These limitations are less problematic as chemical shift predictions *via* both AI/ML and DFT calculations are inreasingly accurate and can be used to validate published chemical shifts and locate and correct outliers. NP-MRD has predicted chemical shifts and simulated spectra for most natural products.

Insofar as this abstract describes a scientific database resource, rather than a research investigation, and in the interest of brevity, we do not have a conclusion. I have added an acknowledgment of the major funding source. References can be found on the database web site which appears at the beginning of the abstract.

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S3.P117 Exploring the impact of moderate water stress on anthocyanin, flavonol glycosides and terpene dynamics across fruit development and ripening in *Pistacia lentiscus* L.

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Pistacia lentiscus L. (Anacardiaceae) is a Mediterranean shrub whose fruits can be a source of new nutraceuticals (Milia et al., 2021). Our experiment evaluated the effects of water stress on the biochemical composition of *P. lentiscus* fruits at two ripening stages (November, T1, and December, T2). The experiment involved twelve potted plants, six of which (WW, well-watered) received adequate watering to pot capacity. The remaining plants underwent moderate water-stress treatment (WS, water-stressed), providing 70% of the Fraction of Transpirable Soil Water. At T1 and T2, Total Terpenes Content (TTC) was analyzed by GC-MS in the oil extracted from fruits. In the remaining oil cake, Anthocyanins and Flavonol Contents (TAC and TFC, respectively) were quantified through HPLC-DAD. Plants physiological status was monitored through measurements of stomatal conductance, chlorophyll fluorescence, leaf Relative Water Content, and leaf water potential, alongside leaf flavonol and chlorophyll indexes.

Results of oil cake biochemical analyses revealed that the best ripening time was T1, with TAC and TFC being 5.6 mg/g DW and 1.2 mg/g DW, respectively. In contrast, T2 was the best ripening stage for TTC (0.25 mg/g DW). Overall, TAC, TFC, and TTC were negatively influenced by the deficit- irrigation treatment at both ripening stages. However, a significant increase in β-myrcene in the oil collected from WS plants at T1 was observed. This emphasizes the potential use of a moderate water deficit treatment to enhance specific metabolite pathways. These findings offer valuable insights into strategic agricultural practices aimed at optimizing the nutraceutical potential of *P. lentiscus* oil and oil cake extracts.

Keywords: *Pistacia lentiscus,* fruits, ripening stages, oil, polyphenols

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S3.P118 Isolation and Characterization of novel compound from *Anaphalis triplinervis,* its HPTLC quantification and antioxidant potential

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The genus *Anaphalis* (Asteraceae), comprised of herbaceous, annual and perennial flowering plants comprise of 110 species *Anaphalis* genus belongs to family Asteraceae, which is well reported for its medicinal importance. Traditionally many species of genus *Anaphalis* used as antiasthmatic, anticoughing, expectorant, sedative, antiepileptic, as anti-inflammatory agents and to cure many disorders (Murugan et al., 2021). The aim of the present study was Isolation and Characterization of novel compound from *Anaphalis triplinervis*, its HPTLC quantification and antioxidant potential. *Anaphalis triplinervis* (aerial part) was taken for extraction (successive solvent extraction scheme) and further ethyl acetate extract was taken for isolation by column chromatography. For isolation, the column was packed with silica gel (60-120) and eluted in toluene and ethyl acetate in gradient manner. Each fraction of was collected, concentrated pooled together according RF values and left for crystallization. Purification of the isolated compound was carried out by repeated recrystallization technique. The isolated compound was characterized by using various spectroscopic techniques, including UV, IR, NMR and MASS. Further qualitative and quantitative estimation (Murai et al., 2021) of isolated compound was done by HPTLC and antioxidant potential was done by DPPH assay (Akter et al., 2019). In summary, the isolated compound shows significant antioxidant efficacy, supported by comprehensive characterization efforts. This finding underscores its potential as a therapeutic agent against oxidative stress-related ailments.

Keywords: anaphalis triplinervis, characterization, isolation, antioxidant, chromatography

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S3.P119 Trapping and detecting electrophilic metabolites of botanical natural products as glutathione conjugates

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Hepatic metabolism of drugs and natural products is usually a detoxification process that inactivates and increases polarity of xenobiotic compounds for excretion. However, oxidation by the cytochromes P450 sometimes forms electrophilic products that can react with cellular nucleophiles and cause toxicity. In the liver, the endogenous nucleophile glutathione (GSH) serves as a natural scavenger that deactivates reactive metabolites such as epoxides, arene oxides, alkyl halides, and quinones. Trapping of these short-lived metabolites with GSH can form stable products that may be analyzed using ultrahigh-pressure liquid chromatography-tandem mass spectrometry (UHPLC- MS/MS). Originally developed to detect reactive drug metabolites (Nikolic et al., 1999), we pioneered the application of this assay to test for the formation of electrophilic metabolites in botanical extracts. UHPLC-MS/MS (Huang, et al., 2015) was used to screen ethanolic extracts of 16 botanicals for hepatic cytochrome 450 formation of electrophiles that could be trapped as GSH conjugates. Botanical authentication and standardization of the test materials were carried out by the Botanical Safety Consortium, which is an international group of experts working to identify assays for the evaluation of the safety of botanicals used as dietary supplements. Botanicals included those with a safe history of human use as well as some with known toxic effects. Botanicals such as *Panax ginseng* and milk thistle were negative in this assay, whereas botanicals known to form toxic metabolites such as Aristolochia and kava produced GSH conjugates. Some toxic plants such as blue cohosh did not form GSH conjugates, indicating they have different mechanisms of toxicity.

Keywords: Metabolism, toxicity, safety, botanicals, glutathione

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S3.P120 Validation of artificial intelligence tools for expedited innovation and globalization of medicinal plants in the dietary supplement industry

The aim of this study was to identify a valid and reliable AI tool for fostering Innovation and globalization of medicinal plants in the dietary supplement industry with artificial intelligence.

We have surveyed several available tools. These include Google DeepMind, MIT Media Research Lab, Aradeepopsis, PlantCV, AyurAI and NutrifyGenie. Two of these tools were selected for this study - AyurAI and NutrifyGenie. These tools are not open access and require a paid subscription for their use. The AyurAI is available as mobile version while NutrifyGenie offers both desktop and mobile versions. The study is divided in three key steps. The first step involved the establishment of the theoretical background, outlining the essential standards for quantitative and qualitative effectiveness in AI tools for medicinal plants in the dietary supplement industry. The second step included a quantitative study to assess the performance of the identified tools. Specifically, the tools were evaluated against established standards for data management, accuracy, presentation, consistency, validation, and adaptability. The third step involved a qualitative study, comprehensively exploring the capabilities and features of each tool, with a particular focus on evidence verification, data exploration, regulatory compliance, feature analysis, automated label claims, integration of the supply chain, and more.

The experiment on NutrifyGenie yielded significant outcomes, identifying 320 potential ingredients for prevalent health conditions like diabetes, polycystic ovary syndrome, and osteoporosis backed by sound published clinical, mechanistic, safety, and efficacy data. The data included PubMed and peer- reviewed human clinical trials with validated findings. We found an average of three supporting pieces of evidence per ingredient. The NutrifyGenie tool also produced some systematic reviews and intellectual property data for evaluation. The NutrifyGenie contains regulatory compliance in eleven countries, offering overview of ingredient compliance. There are some other notable features available, including personalized label creation and integrated supply chain information for manufacturing a commercial product. These results underscore Nutrify Genie's transformative potential in facilitating evidence-based herbal ingredient formulation for self-care and eventually a fast-track option for product development. The AyurAI on the other hand is based more on the principals of Ayurveda with focus on 5 areas - personalized, preventive, predictive, participatory, and promotive aspects of the Ayurvedic therapies. This tool uses digital biomarkers, blood biochemistry, and genomics markers to reach a recommended product.

AyurAI and NutrifyGenie emerge as transformative AI-powered solutions for medicinal plants in the dietary supplement industry's global expansion. NutrifyGenie, operating as a hybrid model, caters to both B2B and partial B2C segments and is a regulatory complaint. AyurAI offers personalized wellness experiences directly to consumers through the B2C segment. These tools address challenges, enhance insights, and streamline industry processes, supported by accurate data sourcing and user- friendly interfaces.

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S3.P121 Modified proanthocyanidin DESIGNER materials with dentin biomodification activity

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Proanthocyanidins (PACs, *syn.* condensed tannins) are oligomers to polymers of flavan-3-ols, a group of plant constituents that impact humans via their health, nutritional, and agronomic value. To date, quantitative analysis of PAC-rich materials depend on colorimetric assays or phloroglucinolysis/thiolysis combined with UV-HPLC analysis - all methods with limited accuracy, extended sample preparation, degradation, and/or need for identical reference standards. In the past decade, high-field and low-temperature NMR as well as via precise assignments using computer- aided ^{1}H full spin analysis (HifSA) have jointly advanced the analysis of intact, genuine PACs. Towards our broader goal of developing new plant-sourced biomaterials that modulate the mechanical properties of dental tissue for clinical interventions, we have characterized 12 new PAC DESIGNER (Depletion and Enrichment of Select Ingredients Generating Normalized Extract Resources) materials. The DESIGNER approach involves centrifugal partition chromatography (CPC) or size-exclusion chromatography (SEC) for the selective enrichment of trimeric and tetrameric PACs. Moreover, the rare but biologically interesting all-A-type PAC DESIGNER materials were successfully produced via conversion of their natural AB-type precursors via an improved method using a free radical oxidative reagent. Quantification of the total PAC content by quantitative NMR (qNMR) utilized an internal calibrant and diol-HPLC to ensure quality and stability of the PAC DESIGNER materials. Diol-HPLC deduced the polymerization profiles, and qNMR determined the total PAC content to range from 61% to 95%. This highlights the complementarity of diol-HPLC and qNMR to accurately assess the amount of PACs across a range of concentrations as well as PAC stability in the DESIGNER materials. Presented dental bioactivity data will demonstrate the unique ability of these materials to enhance dental biomechanical properties comparable to isolated PACs. The newly available quantitative methodology paves the way to standardized DESIGNER materials via rigorous quality control of PAC-based dental (pre-)clinical interventions.

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S3.P122 Antimicrobial activity of 2'-hydroxychalcones with a chlorine atom and their glycosides

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Chalcones are intermediate products in the biosynthesis of flavonoids, which possess a wide range of biological properties, including antimicrobial activity. The introduction of a chlorine atom and the glucosyl moiety into their structure may increase their bioavailability and bioactivity (Lin et al. 2002, Guvenalp et al. 2015). To receive such flavonoids we combined chemical and biotechnological methods (Krawczyk-Łebek et al. 2021). Firstly, we obtained four flavonoid aglycones in the Claisen- Schmidt condensation reaction, i.e., 2-chloro-2'-hydroxychalcone, 3-chloro-2'-hydroxychalcone, 4- chloro-2'-hydroxychalcone, and 5'-chloro-2'-hydroxychalcone. Secondly, we biotransformed them in the cultures of two entomopathogenic filamentous fungi strains, i.e., *Isaria fumosorosea* KCH J2 and *Beauveria bassiana* KCH J1.5 in order to obtain their glycosides. The structures of the resulting compounds were determined by NMR spectroscopy and confirmed by MS spsectroscopy. The received compounds along with 2'-hydroxychalcone were used for the tests of their antimicrobial activity against three bacteria strains *Escherichia coli* 10536 (Gram-), *Pseudomonas aeruginosa* DSM 939(Gram-), *Staphylococcus aureus* DSM 799 (Gram+), one strain of yeast *Candida albicans* DSM 1386, and three strains of lactic acid bacteria *Lactococcus acidophilus* KBiMZ 01 (Gram+), *Lactococcus rhamnosus* GG (Gram+), *Streptococcus thermophilus* KBM – 1 (Gram+). In general, chlorinated chalcones were more effective in the inhibition of the tested microbial strains than their unchlorinated counterparts and aglycones were similarly or a little more effective than their glycosides. The highest antibacterial potential among the tested compounds was demonstrated by 5'- chlorodihydrochalcone 2'-*O*-β-D-(4"-*O*-methyl)-glucopyranoside.

Keywords: chalcones with a chlorine atom, flavonoid glycosylation, *Isaria fumosorosea, Beauveria bassiana,* antimicrobial activity

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S3.P123 Novel ardisiphenol D derivatives 1279P1 and 1279P2: promising anti-colorectal cancer agents targeting the PI3K/Akt pathway and lipogenesis enzymes

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This study investigates the potential efficacy of two novel derivatives of ardisiphenol D (**1**) as anti- colorectal cancer agents with improved therapeutic outcomes and decreased toxicity profiles in comparison to the parent compound. The efficacy of the novel ardisiphenol D derivatives, 1279P1 (2) and 1279P2 (**3**) (Fig.1), has been validated in anti-colorectal cancer treatment through comprehensive *in vitro* and *in vivo* experiments. Both derivatives have been shown to effectively reduce phosphorylated PI3K and AKT protein expression, inhibiting the critical PI3K/AKT signaling pathway associated with cell transformation, tumor progression, and drug resistance (Bahrami et al., 2018).

Furthermore, both compounds exhibit significant inhibitory effects on key lipogenic enzymes ACCA and FASN, essential for *de novo* lipogenesis (Röhrig et al., 2016), which could disrupt cell energy supply, particularly in cancer cells (Krauß et al., 2022). This dual mechanism of action highlights their promising role as potent inhibitors of cancer-promoting pathways. Notably, the cytotoxic effects of **2** and **3** on colorectal cancer cells underscore their therapeutic potential. The synergistic blockade of the PI3K/Akt pathway and suppression of lipogenesis enzymes present a novel mechanism for their anti-cancer activity.

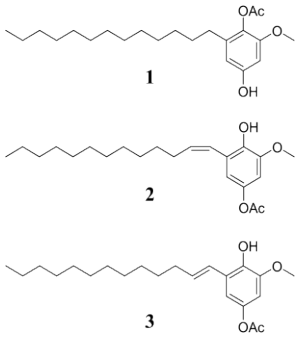

Fig. 1. Structures of ardisiphenol D (**1**), 1279P1 (**2**), and 1279P2 (**3**)

These findings underscore the synergy between PI3K/Akt pathway blockade and lipogenesis enzyme suppression delineates a novel mechanism for the anti-cancer potential of ardisiphenol D derivatives. These results warrant further exploration and clinical translation of **2** and **3** as potential agents for innovative colorectal cancer therapy. The work was financially supported by Hong Kong Baptist University, Research Committee, Initiation Grant – Faculty Niche Research Areas (IG-FNRA) 2021/22, Hong Kong (RC-IG-FNRA/21-22/SCM/01).

Keywords: Ardisiphenol D derivatives, PI3K/AKT pathway, de novo lipogenesis, anti-colorectal cancer

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S3.P124 Antifibrotic activity study of natural stilbenoids from *Dendrobium nobile* Lindl

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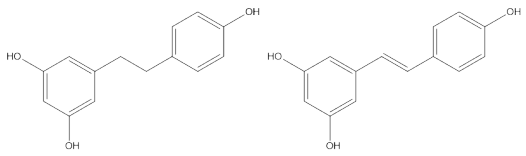
This presentation will cover the biological evaluation and mechanism of action study of dihydro- resveratrol (**1**), a natural stilbenoid from *Dendrobium nobile* Lindl. (Orchidaceae). The compound can be found in many different plant sources, while it is also regarded as a microbial metabolite of resveratrol (**2**) by gut microbiota. Dihydro-resveratrol possesses higher solubility and bioavailability than resveratrol (Li et al., 2022), suggesting that **2** possesses better potential in developing as an antifibrotic agent than **1**.

We compared the cytotoxicity of **1** and **2** in different pancreatic cell lines, suggesting that **1** possesses lower cytotoxicity than **2** in primary murine acini cells (Tsang et al., 2022), both human and rat pancreatic stellate cells (HPSC, LTC-14) and murine pancreatic beta cells (NIT-1) (Fig.1).



Dihydro-resveratrol **(1)** Resveratrol **(2)**

Compounds / Cell lines	Cytotoxicity: IC_{50} (μM)			
	LTC-14	HPSC	NIT-1	Primary acini cells[1]
1	384.6	>200	~125	>250
2	63.4	>200	~50	<250

Fig. 1. Structures and cytotoxicity of dihydro-resveratrol **(1)** and resveratrol **(2)**

Our *in vitro* studies revealed that **1** is a potent antifibrotic agent against pancreatic fibrosis and chronic pancreatitis by inhibiting the activation of pancreatic stellate cells. Our *in vivo* findings indicated that **1** could significantly ameliorate pancreatic fibrosis and macrophage infiltration and protect the islets of the pancreas in a murine chronic pancreatitis model. Moreover, **1** is shown to have an anti- inflammatory effect on macrophage cell lines and a protective effect on pancreatic beta-cell and pancreatic acini cell lines against apoptosis and ferroptosis, respectively. Preliminary mechanistic studies and HuProt human proteome array suggested that **1** modulates PI3K/AKT, NFκB, and MAPK cell signaling pathways. The work described in this study was financially supported by the Food and Health Bureau, the Government of the Hong Kong Special Administrative Region (grant number COVID190214).

Keywords: *Dendrobium nobile,* dihydro-resveratrol, resveratrol, pancreatic fibrosis, chronic pancreatitis

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S3.P125 Planar chromatography method development for the herbal metabolomic study on *Achillea millefolium* (Asteraceae)

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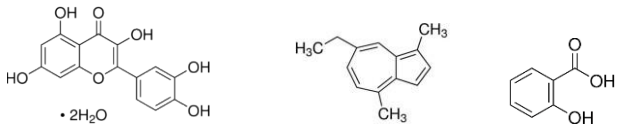
This study covers both quantitative and qualitative analysis of extracts of *Achillea millefolium,* using planar chromatography. Comparing and contrasting extracts of biomass grown regeneratively in the EROC[b], with organic, and wild harvested commercial samples. The isolation and structure elucidation will be undertaken separately, but this study will focus on the known reference standards, Chamazulene (**1**), Salicylic acid (**2**), Quercetin (**3**) and using these marker compounds for qualitative analysis.

Achillea as a source of phytochemicals for the cosmetic Industry has gained increased interest (Becker et al, 2016). This study focuses on flowering tops of the Achillea plants and comparative samples were obtained commercially and grown regeneratively at EROC. Comparing and contrasting monographs published in the British Herbal Pharmacopeia (BHP 1996) and monographs from Herbal drugs and phytopharmaceuticals (Bisset et al 1994), and the European Pharmacopeia. We determined a simple method to identify qualitative parameters for commercial batch sampling of extracts. Working with Shannon ABC[c] using CAMAG HPTLC apparatus, we developed a solvent system of Toluene: Ethyl Acetate: Formic Acid (70:30:1) using a stationary phase of Merck HPTLC Plates, silica gel 60F 254 and using Methanol as a sample solvent and pre dosage volume of 0.2 µl visualising with VisionCats software, white light, and UV 254nm and 366 nm.

As part of our ongoing phytochemical research project developing simple monographs for commercially interesting herbal extracts the aim is to use these results in producing open access best practice for laboratory guidance documentation.



1 2 3

Fig. 1. Structure of Chamazulene **1 Fig. 2.** Structure of Salicylic acid **2. Fig. 3.** Structure of Quercetin **3**

Keywords: *Achillea millefolium,* Asteraceae, HPTLC, planar chromatography, Chamazulene, Salicylic acid, Quercetin

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S3.P126 Metabolomics and microbiomics analysis reveals the dynamic changes during *Citrus depressa* Hayata fermentation

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In recent years, biotransformation has emerged as a new source for small molecule natural drugs, expanding chemical diversity. Many traditional Chinese medicines are produced through biotransformation to generate active ingredients. Representative examples include Chen-Pi, which undergoes microbial transformation through aging to produce more flavonoids and volatile components (Wang et al., 2015). *Citrus depressa* Hayata is renowned for its high content of polymethoxyflavones (PMFs) and used as raw materials of Chen-Pi in early Taiwan (Wu et al.,1983). Therefore, this study aims to explore the interaction between metabolites produced during the fermentation process of *C. depressa* and microorganisms, and to develop a more stable fermentation method. The changes in microbiota within *C. depressa* endophyte fermentation products at each time point were analyzed using 16S rRNA, and analyzed for differential metabolites using non-targeted LC-MS/MS. Additionally, *Bacillus amyloliquefaciens* was isolated from endophyte fermentation samples, and the same method was employed for metabolomic analysis as with endophyte fermentation. In metabolomic analysis, carbohydrates, amino acids, and PMFs were the three most abundant categories among differential metabolites, and these compounds were subjected to quantitative analysis and metabolic pathway analysis. In the end, we found that *B. amyloliquefaciens* can produce a large amount of PMFs more rapidly and steadily, including nobiletin, 5- demethylnobiletin, and tangeretin, reaching concentrations of 8, 0.8, and 3 μg/mg, respectively, in 12 weeks.

Keywords: *Citrus depressa* Hayata, metabolomics, microbiomics, LC-MS/MS

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S3.P127 LC-MS analysis-based molecular networking for identifying anthelmintic compounds in *Terminalia leiocarpa*, a traditional plant used in livestock farming in West Africa

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Terminalia leiocarpa (Combretaceae) is a medicinal plant widely used in ethnoveterinary medicine to treat digestive parasitosis (Tchetan et al., 2021). Its extracts were shown to be active against gastrointestinal nematodes of domestic ruminants (Ndjonka et al., 2012; Tchetan et al., 2022). The objective of our study was to identify compounds responsible for this activity. Open column fractionation was performed, and the activity of the fractions was assessed *in vitro* on *Haemonchus contortus* and *Caenorhabditis elegans* as well as their cytotoxicity on WI38 fibroblasts. Two fractions were the most active on both nematode models and less cytotoxic. LC-MS/MS analysis and manual dereplication coupled to molecular networking allowed identification of the main compounds: ellagic acid and derivatives, gallic acid, astragalin, rutin, quinic acid, and fructose. Other potentially identified compounds such as shikimic acid, 2,3-(*S*)-hexahydroxydiphenoyl-D-glucose or an isomer, quercetin-3-*O*-(6-*O*-galloyl)-β-D-galactopyranoside or an isomer, and rosamultin or an isomer are reported in this plant for the first time. Evaluation of the anthelmintic activity of the major compounds available showed that ellagic and gallic acids were the most effective in inhibiting the viability of *C. elegans*. Ellagic acid and its derivatives may have additive or synergistic effects when combined, but other unidentified compounds could also be implicated in the observed activity.

Keywords: anthelminthic activity, molecular networking, *Terminalia leiocarpa*, *Haemonchus contortus*, *Caenorhabditis elegans*

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S3.P128 Integration of Wnt-inhibitory activity and metabolomics structural novelty results to discovery novel bioactive natural products: new bicyclo[3.3.1]non-3-ene-2,9-diones from the leaves of *Hymenocardia punctata*

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In natural products (NP) research, efficiently prioritizing samples in natural plant extract (NEs) libraries is crucial to accelerate the discovery of original bioactive NPs. In this study a biodiverse collection of 1600 NEs, previously analyzed by UHPLC-HRMS^2 metabolite profiling (Allard *et al.* 2023) was screened for Wnt pathway regulation (**Fig. 1**). The results of the biological screening initially drove the selection of NEs. Only non-toxic NEs with an inhibitory $IC_{50} \leq 5$ µg/mL were considered, resulting in a subset of 30 NEs. To increase the chance of finding structurally novel bioactive NPs, *Inventa*, a computational tool for automated scoring of NEs based on structural novelty was used to mine the HRMS^2 analysis and dereplication results (Quiros-Guerrero *et al.* 2022). This procedure, which is aimed at targeting NE with novel structures, resulted in a 2nd step selection of 4 NEs out of the 1st step post-screening selection of 30 bioactive NEs. The most promising candidate was *Hymenocardia punctata* (Phyllantaceae). Further phytochemical investigations of this species resulted in the targeted isolation of three known prenylated flavones and ten novel bicyclo[3.3.1]non-3-ene-2,9- diones, named *Hymenotamayonins*. Assessment of the Wnt inhibitory activity of these compounds revealed that two prenylated flavones and three novel bicyclic compounds showed interesting activity without apparent cytotoxicity. This study highlights the potential of combining *Inventa*'s structural novelty scores with biological screening results to effectively discover novel bioactive NPs in large NE collections (Quiros-Guerrero *et al.* 2024).

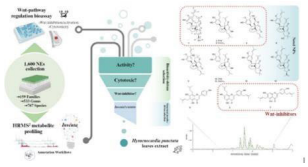

Fig. 1. Overview of the general strategy for the selection of promising NEs for the discovery of structurally novel bioactive NPs in collections of NEs.

Keywords: Natural products, Wnt-pathway modulators, structural novelty discovery, '*Inventa*' scoring, Hymenocardia punctata

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S3.P129 Enhancing prioritization strategies of natural extracts: integrating heterogeneous data from metabolomics datasets and biological screenings into knowledge graphs

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Integrating heterogeneous data in natural products (NPs) research, like metabolite profiling (UHPLC-HRMS^2), annotations and biological screening results is challenging. Typically, data is processed in an *aligned* manner to create feature tables across samples for explorative techniques like Molecular Networking. This facilitates precise comparisons within homogeneous sets. However, integration of new samples and comparisons across batches are hindered by experimental variations. To address these limitations, novel *sample-centric* approaches have been developed to explore diverse datasets of Natural Extracts (NEs) over time (Gaudry *et al.* 2022). Additionally, the need for a more comprehensive *knowledge-driven* framework integrating all type of data led to the implementation of Knowledge Graphs (KG) in metabolomic projects (Gaudry et al. 2023). KG provides structured representations of complex datasets through RDF semantic web data standardization as subject-predicate-object triples (RDF-SemanticWebStandards 2014). This facilitates exploration and interconnects information (Caufield *et al.* 2023). For example, it enables to link sample's taxonomy, spectral annotations, bioactivity with prior information. In this context a multidimensional KG containing over 200 million triples was generated in the frame of a collaborative project incorporating UHPLC-HRMS^2 data of over 3,000 NEs, fractions and pure compounds; taxonomical information, chemo-informatics results, and bioassay outcomes for tuberculosis, obesity, anticancer, and antiviral models. We will describe key element for the constitution of the KG based on NEs experimental data. Additionally, several examples will showcase the potential of using SPARQL queries (SPARQLQueryLanguageforRDF 2018) to explore this KG, demonstrating their efficacy in guiding sample selection and accelerating NP discovery.

Keywords: knowledge graph, RDF, sample-centric, SPARQL query, drug discovery

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S3.P130 The impact of various technological parameters and a place of origin of *Nigella sativa*. L seeds on the thymoquinone content in black cumin oil

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A black cumin oil has a great therapeutical potential and is commonly used as a food supplement. Very important feature of black cumin oil is content of thymoquinone (**1**) (Fig. 1), which can vary from 0,45% to 4,57% depending on a genotype of *Nigella sativa* (Telci et al., 2023). Thymoquinone exerts very broad spectrum of effects among others: antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, antineoplastic, antidiabetic, hepatoprotective and neuroprotective activity (Darakhshan et al., 2015).

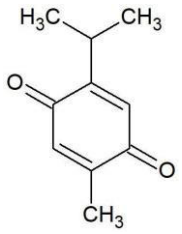

Fig. 1. Structure of thymoquinone **1**

The aim of this study was to evaluate the impact of various technological features of oil making procedure and a place of seeds origin on the content of thymoquinone. For this purpose, the validated HPLC/PDA method was used. The validation procedure included such elements as repeatability, intermediate precision, linearity, accuracy, LOD and LOQ assessment according to ICH guidelines. The biggest impact on the **1** content has the seeds humidity. The greatest difference was observed between 10 and 15/16% of water content in the seeds. There was almost no difference between sedimentation and centrifuging procedure. The type of press machine had smaller influence than seeds humidity. Increase in pressing temperature from 40 to 48°C had small impact on the **1** amount in oil. The place of origin was the most important factor. In conclusion the biggest impact on the **1** content in the oil had the black cumin origin. Nevertheless, the technological parameters especially the seeds humidity can have significant influence on the quality of the *Nigella sativa* oil represented by thymoquinone level.

Keywords: *Nigella sativa,* black cumin oil, thymoquinone, validation, HPLC

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S3.P131 Unravelling enzymatic hydrolysis inhibitors in lignocellulosic biomass samples: a combined LC-HRMS/MS and NMR multiblock modelling approach.

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Considering the current environmental challenges, transformation of lignocellulosic biomass into bioethanol offers a sustainable alternative to petroleum fuels (Wyman, 1999). The pretreatment step, applied in some industrial processes, generates aqueous phases called hydrolysates. These complex mixtures contain a variety of oxygenated compounds which may interfere with the enzymatic hydrolysis (EH) step and hinder the conversion of cellulosic feedstock into glucose (Kim, 2018). The confident identification of these EH "inhibitors" is therefore crucial for the optimization of the bioconversion dynamics.

LC-HRMS and NMR spectroscopy are complementary techniques for the molecular characterization of complex samples (Bhinderwala *et al.,* 2018). Indeed, HRMS sensitivity allows detection of a large number of compounds while NMR provides crucial information about species' abundance. Moreover, trend determination and biomarker identification from these large analytical datasets have been markedly improved by the use multivariate analyses (Deng *et al.,* 2016). In this work, we propose the simultaneous exploration of LC-ESI(±)-HRMS, ^{13}C & ^{1}H NMR and enzymatic reactivity datasets, through a multiblock modelling strategy (Beniddir *et al.,* 2022), for the determination of candidate inhibitory species in biomass hydrolysates.

This study focuses on the comparison of data from multi-informative analytical techniques to pinpoint descriptors of EH inhibition in biomass samples. The first part of the presentation will focus on sample preparation and applied analytical data preprocessing approach. The core "Data fusion and Multiblock modelling" strategy will then be thoroughly covered. Ultimately, the potential of this methodology to assist biomarker discovery in complex NP matrices will be highlighted.

Keywords: Biomass conversion, inhibitors, mass spectrometry, nuclear magnetic resonance, Multiblock modelling

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S3.P132 Obtaining new [6]-gingerol derivatives with potential antiparasitary activity

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Some parasitic infections are recognized as affecting predominantly the most vulnerable population's parcel due to the state of need and difficulty of access to health services (da Silva et al., 2013). Leishmaniosis is a prevalent pathology in Brazil, and the current treatment options are faced with many obstacles, such as long therapy duration and high toxicity (Troncoso et al., 2022). To mitigate these problems, it becomes necessary to develop new pharmaceutical inputs that are accessible and effective, such as natural product derivatives. [6]-Gingerol is a strong contender to meet such a need, due to the vast range of positive biological effects already visualized, including against parasitic diseases. A form of achieving a better biological activity is though chemical reactions aiming to result in modified products. In this way of thinking, its chemical structure lets different compounds be made by replacing two molecules of nucleophiles at its phenolic hydroxyl. Given that, the goal of this study was to submit [6]-gingerol to modifications using structurally diverse reagents, resulting in seven different and new products. Once synthesized and characterized, these new compounds were tested against the parasitic species *Leishmania amazonensis* to verify the possibility of the enhancement of the [6]-gingerol's activity, something that was achieved even in a moderate form. This work seeks to find new, promising inputs to be used in leishmaniosis treatment.

Keywords: *Leishmania amazonensis,* biological activity, semisynthesis

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S3.P133 Identification of the C2-sulfonamidyl chromone scaffold for developing anti-inflammatory agents targeting hyperactivation of human neutrophils

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Neutrophil hyperactivation is implicated in a spectrum of inflammatory pathologies, which implies the potential for targeting neutrophils as a pharmacological management to treat inflammatory disorders (van der Linden and Meyaard, 2016). In continue of the previous study that C2-ether bridge of capillarisin was replaced by a more active C2-thioether linkage (Chang et al., 2021), herein, a structure-activity relationship study focusing on the different C-2 bridging moiety was leveraged with the bioisostere-replacing and scaffold- hopping approaches. Among various chemotypes, the C2-sulfonamidyl chromone derivatives emerged as the potential inhibitors against superoxide anion generation and elastase release from fMLF-activated human neutrophils. The most active compound exhibited IC50 value of single digit micromolar range against both neutrophilic superoxide anion production and elastase release. Pharmacophore model predicted the most active compound to be a p38a inhibitor, which is validated by direct p38a inhibition assay, p38a cascade assay as well as western blot analyses. This study provides a successful example of natural product-derived new p38a inhibitor ameliorating neutrophilic inflammation.

Keywords: capillarisin, C2-sulfonamidyl chromone, p38a, neutrophilic inflammation

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S3.P134 New xanthone analogues with potent intracellular reactive oxygen species inhibition effects

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An elevated level of reactive oxygen species (ROS) often results in oxidative stress, subsequently leading to the initiation and progression of inflammation. As a consequence, compounds that could suppress the ROS levels by upregulating intrinsic antioxidant Nrf2/ARE signaling pathway are needed. The polyketides xanthones are potential candidate due to their reported antioxidant, anti- inflammatory and anticancer activities (Minami et al. 1994; Gunter et al. 2023). Their biological activities rely on the types and position of substituent group present on the molecular scaffold of xanthone (Loh et al. 2021). Thus, this study aimed to synthesize new xanthone analogues starting from 3-hydroxyxanthone to provide further insight on their structure-activity relationship on intracellular ROS inhibition level. The analogues obtained were purified by chromatography techniques and structurally characterized via NMR, MS and FTIR spectroscopy. Moreover, the xanthone analogues were evaluated for their inhibitory effects towards intracellular levels of ROAs a consequence through the DCFH-DA assay in H_2O_2-induced oxidative damage model using SNU-1 cells. Furthermore, the modulation of the gene and protein expression of Nrf2 and downstream proteins were assessed. Remarkably, the xanthone analogues inhibited the ROS by restoring the levels to that of negative control. Their activities were found to be comparable to an established Nrf2 activator, sulforaphane. Molecular investigations showed concentration-dependent increases in the mRNA expression of Nrf2 and downstream antioxidant enzymes HO-1, NQO1, SOD, and CAT. Similar results were obtained for the protein expression of these enzymes even though no marked increase was observed for Nrf2. In summary, xanthone analogues are potential lead compounds for anti-inflammatory agents and thus further *in vivo* studies are highly recommended.

Keywords: antioxidant, Nrf2/ARE signaling pathway, structure-activity relationship

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S3.P135 A new complementary process toward functionalized dihydropyridinones: application in total synthesis of phenanthroindolizidine alkaloids

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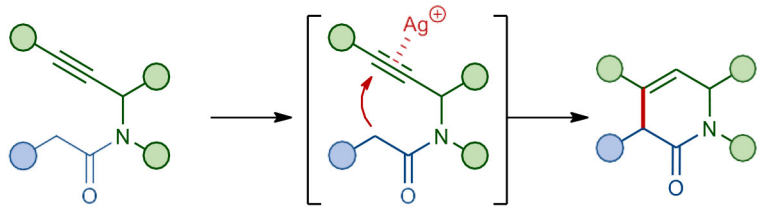
Due to their interesting biological properties, functionalized dihydropyridinones have attracted considerable interest from drug research fields. Although several synthetic strategies have been developed for preparing these structural motifs, only a limited number of these procedures can be applied for the direct and selective synthesis of polysubstituted dihydropyridinones. Thus, the development of new synthetic strategies that enable an efficient and selective synthesis of highly substituted dihydropyridinones are highly desirable. Herein, we report our research on a new process toward functionalized dihydropyridinones through the intramolecular cyclization of amides with a tethered alkyne moiety and its application. An unexplored reaction between amide and alkyne was realized through an O-silyl N,O-ketene acetal to give functionalized dihydropyridinones in high yields. Applications of this reaction for the synthesis of various functional dihydropyridinones and investigation of its extension to the total synthesis of phenanthroindolizidine and phenanthroquinolizidine alkaloids were achieved. Our strategy could be extended to realize the related heterocyclic substrate series.



Keywords: dihydropyridinones, total synthesis, phenanthroindolizidine, phenanthroquinolizidine, alkaloids

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S3.P136 Divergent synthesis of prenylated xanthones as novel fungal IRE1 inhibitors

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The development of fungal infections is a major threat to people's health and agriculture. Over the past decades, with profound environmental changes, fungal pathogens infecting plants and humans have evolved resistance to several antifungal drugs (Fisher et al., 2022). The unfolded protein response (UPR) is essential in controlling the folding and maturation of endoplasmic reticulum (ER) proteins in eukaryotes. UPR has been shown to be involved in plant pathogenic fungal virulence (Joubert et al., 2011), whereas IRE1 protein appears as its only effector in fungi. Therefore, we considered the IRE1 protein as a promising target for the development of new antifungal drugs. The evaluation of a small but high-diversity library of 76 natural products (terpenoids, alkaloids, and polyphenols) through a cell-based screening assay allowed us to identify seven potential inhibitors of IRE1, including polyhydroxylated and prenylated xanthones. Antifungal activity of the latter was finally assessed in planta on cabbage leaves infected by *Alternaria brassicicola* (Charpentier et al., 2023).

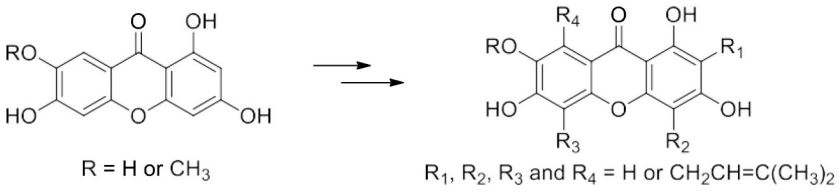

Fig. 1. Structure of synthesized xanthones.

This study found that the inhibitory potential of xanthone derivatives against IRE1 depends on the number and location of prenylated side chains on the xanthone backbone. To broaden the structure- activity relationship (SAR), different xanthone derivatives with varying degrees of prenylation were synthesized using a divergent strategy (Fig. 1). Inhibition of IRE1 was then assessed in a cell-based assay, and a molecular docking study was performed.

Keywords: antifungal, xanthone, total synthesis, IRE1 inhibitors

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S3.P137 Cytotoxic effects of xanthone derivatives towards leukemia cells

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Xanthones derivatives are a group of natural products with interesting pharmaceutical properties including antioxidant, anti-inflammatory, anti-bacterial and anti-cancer, activities (Gunter et al. 2023; Wong et al. 2020). Many previous studies have been focused on the anticancer effects but limited on their anti-leukemia effects with detailed mechanisms of action. Thus, this study aimed to evaluate the cytotoxic effects of synthesized xanthone derivatives towards leukemia by using A20, HL20 and Jurkat cells through the MTT assay. A total nineteen xanthone derivatives were evaluated at a concentration of 10 μM and four of the derivatives were found to be show strong cytotoxic effects. These xanthone derivatives exhibited at least 90% of cytotoxicity towards all the cell lines. Thus, a range of concentrations of these derivatives were evaluated to obtain their IC_{50} values. The results have shown that their IC_{50} values are less than 3 μM, indicating their potent cytotoxicity. The structure-activity relationship revealed that the halides are nitrogen containing substituent groups are the main contributors to the cytotoxic effects of xanthones. In summary, xanthone derivatives are potential lead compounds for leukemia cancer drugs. Thus, studies on the molecular mechanism pathways for the cytotoxic xanthone derivatives are under progress.

Keywords: xanthones derivatives, anticancer, cytotoxicity, MTT assay, structure-activity relationship

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S3.P138 Bimatoprost potentiates hair growth via Akt/Wnt/β-catenin signaling by targeting the K_{ATP} channels

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Various prostaglandins (PGs) and PG synthases are involved in hair growth or hair loss. Male pattern hair loss is caused by increased PGD2 (Garza, 2012), but the mechanism by which PGE2 or PGF2α promotes hair growth is unclear. Here, we investigated the effect and mechanisms of bimatoprost, an ethyl amide derivative of 17-phenyl trinor PGF2α, on the hair growth. When C57BL/6 mice were applied topically with bimatoprost, not only rapidly progressive skin color changes but also large- sized hair structures were observed. To elucidate the mechanism by which bimatoprost promotes hair growth, we evaluated whether bimatoprost activate the Akt and Wnt/β-catenin pathways. Bimatoprost induced cell proliferation through activation of the Akt and wnt/β-catenin pathways in rat dermal papilla cells (rDPC), which are hair follicle cells, as well as in NIH3T3 cells, and similar results were observed in the minoxidil-treated group. Moreover, all effects of bimatoprost were completely inhibited in rDPC and NIH3T3 cells using glibenclamide, a selective KATP channel blocker. Bimatoprost significantly increased the level of cPGES, which is a PGE2 synthesis enzyme, in both 2D rDPC and 3D spheroids. In conclusion, these results indicate that bimatoprost induces the proliferation of rDPC by the activation of the KATP channel/Akt/Wnt/β-catenin signaling cascade and increases cPGES level, which ultimately exerts a superior hair growth effect.

Acknowledgments: This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education(RS-2023-00270936) and by the Korea government(MSIT) (2023R1A2C1007234)].

Keywords: bimatoprost, hair growth, wnt/β-catenin, cPGES, K_{ATP} channel

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S3.P139 Large scale synthesis, anticonvulsant effect and toxicity assessment of cannabigerol and cannabinol

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Cannabigerol (CBG) and cannabinol (CBN) are prominent cannabinoids with diverse molecular targets. This study presents a novel, environmentally friendly approach to the total and large-scale synthesis of CBG and an improved method for the scaled-up synthesis of CBN. For CBG, we utilized Amberlyst 15 resin in the condensation of olivetol with geraniol. This method offered a more manageable and less toxic alternative to the $BF_3{\cdot}OEt_2$ approach cited in literature (Nguyen et al., 2022) with 55% yield. Regarding CBN, we optimized the iodine-promoted process (Pollastro et al., 2018) by replacing toluene with heptane and by applying an air stream inside a chemical reactor, removing effectively hydrogen iodide, achieving 97% conversion of cannabidiol (CBD) to CBN in a Kg scale reaction. Our pharmacological investigation revealed that both CBG and CBN exhibit potent anti-seizure effects. Following induction of seizure activity in hippocampal mouse brain slices using the *ex vivo* high potassium model, we recorded decreased spontaneous activity (>30%) in a dose- dependent manner (1-40 uM). Their anticonvulsant effect was similar to the one of CBD, an FDA approved anti-epileptic drug branded as Epidiolex. Furthermore, their safety profiles were assessed through acute oral toxicity experiments in mice, with doses up to 2000 mg/kg being nontoxic. Our findings highlight the anticonvulsant properties of CBG and CBN and suggest further *in vivo* investigation to assess their potential as candidates for new or supplementary oral anti-epileptic treatments. Their scalable synthesis, anticonvulsant potential, and favorable toxicity profiles highlight their significance in the ongoing research for effective epilepsy therapies.

Keywords: cannabinoids, anticonvulsants, cannabinol, cannabigerol, cannabidiol

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S3.P140 Thymol-based heterocyclic derivatives: synthesis and SARS-CoV-2 M_{pro} molecular docking studies

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SARS-CoV-2 main protease (SARS-CoV-2 M^{pro}) enzyme is involved in the replication of the SARS- CoV-2 virus, and is therefore an important target in the development of drug treatments for COVID- 19 disease. Plant based natural products of the monoterpene class have been explored as inhibitors of the enzyme, including thymol, a major component of thyme essential oil. In this study, twelve heterocyclic derivatives of thymol containing various heterocyclic moieties were synthesized. Molecular docking studies were performed with SARS-CoV-2 M^{pro} (PDB ID; 6lu7) to determine the influence of the heterocyclic unit on docking affinity. All of the derivatives showed interaction with His-41 and Cys-145, which are important in the catalytic mechanism of the SARS-CoV-2 M^{pro} enzyme. Docking scores ranged from -6.4 kcal to -8.0 kcal/mol, with evidence of structure-docking affinity correlations among the heterocyclic units. These scores are in a similar range to that of ML- 188 (-7.5 kcal/mol), a known SARS-CoV-2 M^{pro} inhibitor.

Keywords: thymol, heterocycles, SARS-CoV-2 M^{pro} docking affinity

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S3.P141 Expanding chemical space around structures from *Strychnos variabilis*: late-stage functionalization strategies using photochemistry

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Strychnos variabilis De Wild. is a small deciduous tree native to the regions in and around Brazzaville and Kinshasa, situated on both banks of the Congo River in Africa. Despite its geographical prevalence, *S. variabilis* leaves were found to contain only trace amounts of indoline alkaloids, particularly from the retuline series. However, our laboratory, over the past three decades, isolated a plethora of rare flavonoids, including flavonol glycosides, from these leaves (Brasseur and Angenot, 1987). Flavonoids, renowned for their antiviral properties, particularly in glycosidic form, have garnered interest due to their enhanced solubility and efficacy. Therefore, our study sought to explore the potential antiviral activity of *S. variabilis* leaves against SARS-CoV-2. Initial findings indicated significant antiviral activity in the total extract, with an IC_{50} range of 12.5 to 6.25 µg/mL. Encouragingly, our investigation into two specific flavonoids, variabilosides C and D, revealed even greater potency, with an IC_{50} range of 6.25 to 3.12 µg/mL. Building upon these promising results, we adopted late-stage functionalization (LSF) strategy through photochemistry (Pitre and Overman, 2022) to chemodiversify *S. variabilis* extracts. LSF may alter functions already present in the structure, but more importantly it is able to target the C–H bonds of drug leads as points of diversification for generating new analogs. Thus, chemodiversification was achieved and preliminary insights were obtained. The antiviral activity of these modified extracts against SARS-CoV-2, were correlated with these modifications. In conclusion, our study highlights the efficacy of photochemistry in expanding the chemical space of natural products, offering innovative solutions for drug discovery and development.

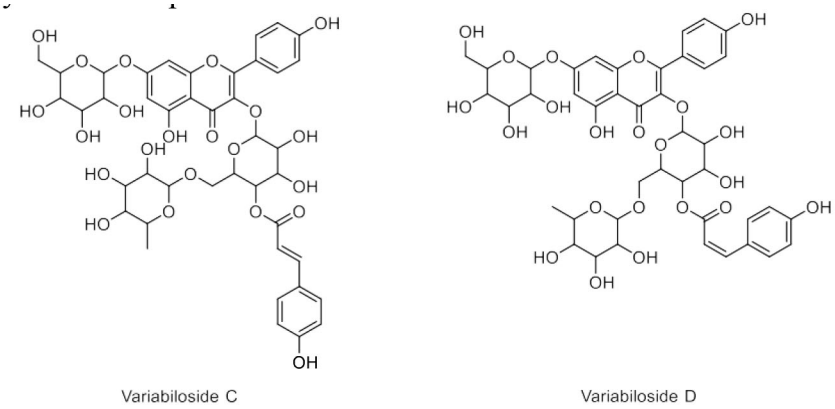

Fig. 1. Variabilosides C and D isolated from *S. variabilis*.

Keywords: *Strychnos variabilis,* SARS-CoV-2, chemodiversification, late-stage functionalisation

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S3.P142 Effects of metabolic modulators on the accumulation of specialized metabolites in *Hypogymnia physodes* (L.) Nyl.

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Lichens are symbiotic organisms consisting of a fungal component (mycobiont) and a plant component (photobiont). These organisms are a source of many biologically active substances, among which the most significant group are the so-called lichen acids. This group of compounds includes about 1,000 identified metabolites specific only to lichenized fungi. Secondary metabolites of lichens may account for up to 25% of their dry weight. They are characterized by antioxidant, antimicrobial, anti-inflammatory, photoprotective, and anticancer properties. Six common lichen species were used in this study: *Hypogymnia physodes* (L.) Nyl, *Xanthoria parietina* (L.) Th. Fr., *Evernia prunastri* (L.) Ach., *Cladonia uncialis* (L.) Weber ex F.H. Wigg., *Physcia adscendens* (Fr.) H. Olivier., and *Pseudevernia furfuracea* (L.) Opf. The lichen thallus was subjected to different metabolic modulators (methyl jasmonate, salicylic acid, nitrate oxide, selenite, zinc, chitosan lactate). After 48-hour incubation in stress conditions, the accumulation of lichen acids and allantoin was examined. The antioxidant potential exhibited by the prepared extracts was also studied. Although the results obtained did not provide sufficient evidence for a common pattern of action of metabolic modulators in all the lichen species studied, alterations in the metabolite content related to the metabolic modulator-induced lichen response were observed. The data showed significantly increased accumulation of specific compounds in some lichen species, especially after the exposure to salicylic acid, methyl jasmonate, NO, and chitosan.

Keywords: allantoin, antioxidant potential, elicitation, lichens, lichen acids, secondary metabolites

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S3.P143 Synthesis of polymethoxyflavone - zapotin (5,6,2',6'-tetramethoxyflavone) and its new structural analogues

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The synthesis of 5,6-substituted flavonoid derivatives is intricate. Synthesis methods used so far are multi-step, long-lasting, time-consuming with a low total product yield, and therefore uneconomical (Maiti et al., 2007). Polymethoxyflavones (PMF) are of particular interest due to their broad spectrum of biological activities, including anti-inflammatory and anticancer properties. According to the literature data, zapotin, is an extremely interesting example of PMF (Strawa et al., 2021) with broad biological potential, especially as an anti-tumor and chemo-preventive factor (Changhong et al., 2022). Difficulties related to the synthesis of 5,6-substituted PMF using known synthetic methods are the reason why they have been obtained mainly through isolation from plant material so far. The amount of zapotin isolated from plant raw materials is scarce, and the process of obtaining it is very troublesome and inefficient. The synthesis of zapotin and its analogues was carried out to optimize synthesis conditions. Optimization of the conditions aimed at reducing the number of synthesis steps which led to the shortening of total synthesis time. Changing the synthesis strategy from linear to more branched allowed to easily obtain zapotin **1** and its analogues **2-3** using more selective and easiest to perform transformations. The synthesis we propose does not require using completely anhydrous conditions as well as halogenated solvents, at any stage of the synthesis. Intermediate products can be easily purified by crystallization leading to the final products - compounds **1-3** - in a crystalline, stable form, with relatively good yield (46%-48%) (Fig. 1).

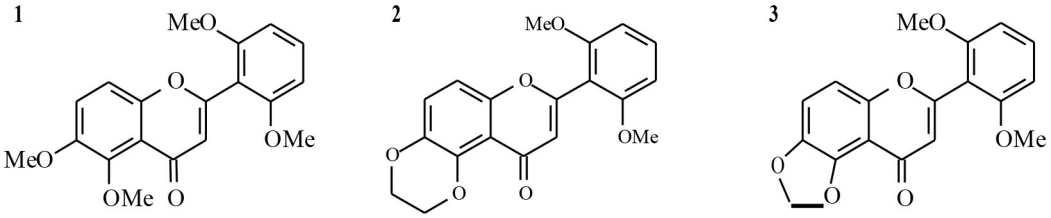

Fig. 1. Structure of zapotin **1** and its synthetized analogues **2-3**

Keywords: zapotin, polymethoxyflavones, synthesis

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S3.P144 Anti-leishmanial activity of synthesized and characterized semisynthetic derivatives of betulin

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Betulin is a naturally occurring pentacyclic triterpene that is isolated from the bark of *Betula utilis* D. Don, a member of the Betulaceae family (Verma et al., 2014). It belongs to the lupane series and is known by the systematic name 3, 28-dihydroxy-20(29) lupen or Lup-20(29)-en-3, 28-diol. Betulin has three available sites for simple chemical modification, namely the secondary hydroxyl group at position C-3, the primary hydroxyl group at position C-28 and the isopropenyl side chain at position C-19. A broad range of biological activities, including antibacterial, antiviral, anticancer, and hepatoprotective qualities, are exhibited by betulin and its derivatives (Amiri et al., 2020). The aim of the present study was to anti-leishmanial activity of synthesized and characterized semisynthetic derivatives of betulin. The synthesized derivatives were characterized using various spectroscopic techniques, including NMR, IR, and MS. Anti-leishmanial activity was done by *in vitro* promastigote assay, *ex vivo* cytotoxicity assay and amastigote-macrophages assay (Sharma et al., 2023).The synthesized betulin derivatives was attached on C3 hydroxyl group and it was characterized by spectroscopic techniques. The synthesized derivatives was tested against *L. donovani* and confirmed its antileishmanial activity against the standard drug amphotericin B by *in vitro* studies.

Keywords: *Betula utilis,* betulin, synthesis, characterization, antileishmainal

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S3.P145 Exploring novel amino acid synthesis for plant-derived active cyclopeptide acquisition

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MVA, a novel plant cyclic heptapeptide containing dimethylcyclopropyl-glycine (DMCPA) unique amino acids, exhibits remarkable anticancer activity with IC_{50} values in the nanomolar range against various cancer cell lines (Zhang, 2014). The need for the total synthesis of MVA is underscored by its rare occurrence in nature for further bioactivity studies and elucidation of its mechanism of action. In our efforts to create analogs of MVA, we substituted some common amino acids like cyclopropyl- glycine for DMCPA. However, the anticancer activities of the synthesized analogs significantly decreased, highlighting the crucial role of the unique amino acids in maintaining the anticancer potency of MVA. As DMCPA is not commercially available, the critical step in acquiring MVA is the synthesis of DMCPA to advance towards our ultimate goal of preparing MVA. The current research outlines our total synthetic approach for DMCPA.

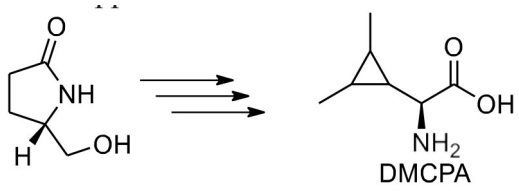

Fig. 1. Total synthesis of DMCPA

Given that DMCPA and glutamic acid share the same backbone length, the chiral centre of the resulting amino acid does not necessitate construction due to the predetermined configuration of the starting material (Fig. 1). The synthesis of DMCPA involves crucial stages like forming cyclopropane ring and converting the carboxylic acid into a methyl group. Consequently, it was determined to use it as the primary ingredient for DMCPA preparation. Through a series of subsequent reactions, including Corey-Chaykovsky and the Appel reactions, the final DMCPA can be produced.

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Keywords: cyclopeptide, plant compound, cytotoxicity, unique amino acid, synthesis

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S3.P146 Structure modification of plant-derived miliusanes compounds to explore their therapeutic potential as anticancer agents

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Geranyl meroterpenoids, a class of naturally occurring compounds produced through a hybrid biogenetic pathway, have recently captured significant attention due to their multifaceted bioactivities. Miliusanes represent a striking example discovered in plants of the *Miliusa* genus, characterized by an 18-carbon skeleton formed from the fusion of homogentisic acid and a geranyl monoterpene. In our screening program involving a wide range of plant extracts, *Miliusa sinensis* emerged as a promising anticancer plant lead (Zhang et al., 2016). Subsequent bioassay-guided fractionation of two *Miliusa* species led to the discovery of 43 novel miliusanes (Zhang et al., 2006; Xu et al., 2019; Xie et al., 2023). Some of them exhibited potent *in vitro* and *in vivo* antitumor activities, with their mechanisms of action partially attributed to their ability to modulate the p21- dependent cellular senescence pathway. The current study presents our ongoing endeavors aimed at obtaining novel miliusanes through the structural modification of the miliusol prototype. Consequently, we have successfully synthesized over 100 miliusane derivatives (Fig. 1), some of which demonstrated 5-10 times higher potency than miliusol *in vitro*. Further *in vivo* assessments revealed that two compounds featuring Michael adducts exhibited a more balanced activity profile concerning anticancer efficacy and toxicity.

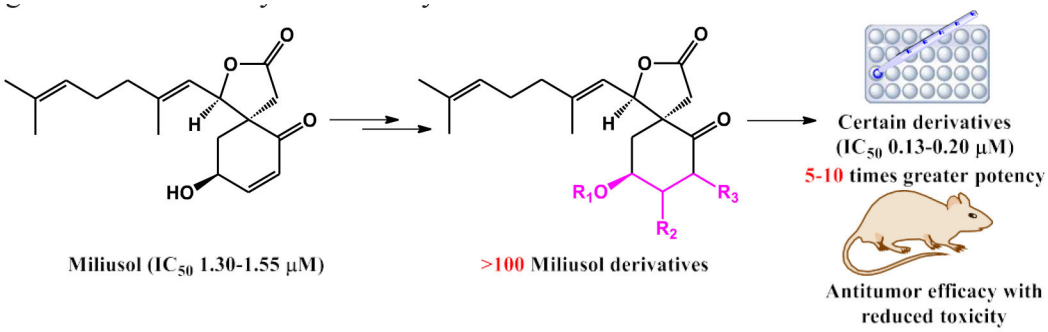

Fig. 1. Structure modification strategy based on the miliusol lead molecule.

These findings have provided us with valuable structure-activity relationship insights, which will facilitate the advancement of miliusanes as potential anticancer drug candidates.

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Keywords: *Miliusa sinensis*, meroterpenoids, miliusanes, antitumor

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S3.P147 Seed propagation, tissue culture and *in-vitro* conservation studies in *Aristolochia tagala* Cham: an endangered medicinal plant of western ghats of India

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Aristolochia tagala Cham. is a threatened medicinal plant of Aristolochiaceae family. It is mainly used to treat snake bites and other poisonous bites. Apart from this, roots are also used to treat bone fracture, malaria, and various dermatological conditions. Due to indiscriminate use of its roots and deforestation, its population in reducing in its natural habitat. Seed germination is low due to the presence of insufficient endosperm, which leads to low seed viability. Hence the conservation of this species is crucial. Hence, this study was undertaken. Fresh seeds of *A. tagala* were collected from the Field Gene Bank of RET medicinal plants. Among different treatments, the water soaking treatment responded well with the maximum in all growth parameters like rate of germination, seedling height and seedling vigor index followed by treatment with thiourea 2.0 per cent. In tissue culture, nodal segments of seedlings as an explant and cultured on MS medium supplemented with various combinations of growth regulators like BAP, kinetin and NAA. Among different combinations, minimum days taken for shoot initiation (6.06±0.04), the maximum number of shoots (3.20±0.04) and the maximum number of the leaves (3.18±0.02) were obtained in treatment combination of BAP (2.0 mg/L) and NAA (0.5 mg/L). The maximum shoot length (1.98±0.02) was obtained in BAP (2 mg/L). The satisfactory results after three months of *in vitro* conservation were obtained by using the combination of BAP (2.0 mg/l) + NAA (0.50 mg/L) in terms of high survival per cent (100), maximum number of shoots per explant (2.40±0.04) and the maximum number of leaves (2.73±0.02). Thus, MS media supplemented with BAP (2.0 mg/l) + NAA (0.50 mg/L) can be used for both *in vitro* propagation and also conservation of *Aristolochia tagala* Cham. under reduced culture condition and low light intensity (2.97 $\mu m^{-2} s^{-1}$) and low temperature (10^0C).

Keywords: propagation, conservation, endangered, seeds, treatment, germination

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S3.P148 Efficient genetic transformation of *Nigella damascena* callus

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Nigella damascena L., commonly known as love-in-a-mist, originates from the Mediterranean basin and Southwest Asia. Its seeds are abundant in essential oils, rich in β-elemene and damascenine, which demonstrate antimicrobial and molluscicidal properties. Recent investigations have also explored the pharmacological effects of damascenine, unveiling its potential as an analgesic, antipyretic, anti-inflammatory, and antiedematous agent. As conventional methods of producing these compounds can be inefficient, various biotechnological approaches are increasingly being used to improve their production efficiency. The use of medicinal plants in *in vitro* cultures provides a controlled environment for the production of therapeutic compounds, thereby improving access to natural remedies. Regulation of the production levels of target compounds can be achieved through a variety of approaches, including elicitation, manipulation of growth parameters, and modification at the molecular level. Despite the promising potential of *N. damascena*, research on this species remains limited, with only a few scientific studies available, none of which involve genetic transformation, highlighting the need for further research in this area. In our study, we developed an efficient protocol for the genetic transformation of *N. damascene* callus cells using *Agrobacterium tumefaciens (Rhizobium tumefaciens)*. We achieved a remarkably high transformation efficiency, which opens the door for further research and applications. The introduction of reporter genes has facilitated the evaluation of transformation efficiency, simplifying the process. As a result, it is now possible to insert different genes or use the clustered regularly interspaced short palindromic repeats (CRISPR) – Cas9 system for targeted mutagenesis, paving the way for genetic manipulation in *N. damascena* and deepening our understanding of its medicinal properties.

Keywords: *Nigella damascena*, genetic transformation, *in vitro*, callus, *Agrobacterium tumefaciens*

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S3.P149 Identifying antioxidant compounds within a mixture through dereplication and online DPPH strategies: A study on the CO_2 byproduct of the Makwaen Pepper

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Natural antioxidants are widely used in cosmetics, nutraceuticals, and food supplements as preservatives or to protect against reactive oxygen species (Brewer, 2011). Exploring byproducts from various industries - often considered waste - presents a substantial and promising approach for developing natural and sustainable antioxidant ingredients in a circular economy perspective.

This study aimed to develop a robust and sensitive online screening workflow to identify compounds with anti-free radical activity in chemically complex natural extracts. Compared to previously reported results (Irina et al., 2000), the response's sensitivity and resolution were significantly improved by introducing centrifugal partition chromatography as a first separation dimension before the online workflow HPLC-DPPH-UV-HRMS^2. The dereplication step was then performed by combining NMR and MS (Cordonnier et al., 2023) data to improve the annotation confidence level and finally merged with the free-radical activity information.

As a proof of concept, the developed original and efficient workflow was performed on the CO_2 byproducts of the Makwaen pepper (*Zanthoxylum myriacanthum*), known for its medicinal properties and culinary seasoning. While CO_2 extract of pepper fruits has gained commercial attention, particularly in the fragrance industry for their sulphury fruity olfactive profile (Sriwichai et al. 2019), non-volatile compounds remain insufficiently explored.

The results were visualized as a multi-informative molecular network by matching annotated compounds with a reliability level score and anti-free radical activity activities. For instance, hydroquinone, isoquercetin, hyperoside, dicaffeoylquinic acids, chlorogenic acids and lignan glucosides were annotated and identified to be responsible for the activity of CPC fractions.

Keywords: antioxidant, dereplication, molecular networking, centrifugal partition chromatography, *Zanthoxylum sp*

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S3.P150 Establishing human intestinal slice cultures as an ex vivo model of inflammatory bowel diseases for the pharmacological characterization of herbal extracts

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Inflammatory bowel diseases (IBD) are characterized by recurrent or progressive inflammatory alterations of the intestine. Besides standard therapy, complementary treatments, especially phytomedicines, are frequently used. The traditional herbal combination of myrrh, coffee charcoal and chamomile flower extract is included in the German S3-guideline for 'Colitis ulcerosa' based on clinical investigations (Kucharzik et al. 2020). Previous cell culture studies demonstrated anti- inflammatory, spasmolytic and barrier-stabilizing effects of these herbal extracts (Vissiennon et al. 2017; Weber et al. 2020).

This project aims to capture the complex human pathophysiology in an ex vivo model by establishing precision cut intestinal slices (PCIS) from colon biopsies of IBD patients. Influences of the herbal test substances (reference compound: budesonide) on inflammation were monitored. Initial results from hematoxylin-eosin staining confirm the preservation of intestinal morphology in PCIS ex vivo for 24 h (88,4% of samples) and compared to healthy tissue, IBD-typical morphological changes were observed in PCIS of IBD patients. Using immunohistochemistry, distinctions in specific immune cell populations could be detected, revealing comparable percentages of $CD68^+$ macrophages (IBD: 1,57%±0,80%; healthy: 0,96%±0,27%) but increased neutrophils (IBD: 3,96%±1,02%; healthy: 0,82%±0,44%). The release of 24 mediators (such as TNFα, IL8, IL6) into the culture media was quantified using multiplex-ELISA. Compared to untreated controls, budesonide (1µM) treatment showed less inflammation, an enhanced epithelial morphology and a significantly altered the mediator pattern.

Combining results from histology and mediator release, this model allows investigation of the effects of herbal extracts from myrrh, coffee charcoal and chamomile flower within the context of IBD's complex pathophysiology.

Keywords: inflammatory bowel diseases, ex vivo, PCIS, herbal extracts

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S3.P151 Comparative investigation of frankincense nutraceuticals: pharmacokinetic/pharmacodynamic study

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Frankincense extracts have been widely used in traditional medicine for centuries and more recently in complementary medicine. Therefore, frankincense components such as boswellic and lupeolic acids are of great therapeutic interest (Sterk et al. 2004, Syrovets et al.2005). Sixteen prescription- free commercial frankincense nutraceuticals marketed in Europe were characterized by high- performance liquid chromatography and tandem mass spectrometry (Schmiech et al. 2019). The frankincense nutraceuticals showed large differences regarding composition and total contents of boswellic and lupeolic acids. Frankincense nutraceuticals significantly inhibited the release of proinflammatory cytokines by LPS-stimulated whole human blood and isolated peripheral blood mononuclear cells. Interestingly, boswellic and lupeolic acid contents in the frankincense nutraceuticals correlated with their ability to inhibit release of proinflammatory cytokines. Among eight different boswellic and lupeolic acids tested, acetyl-β-boswellic acid exhibited the most profound effect on cytokine release.

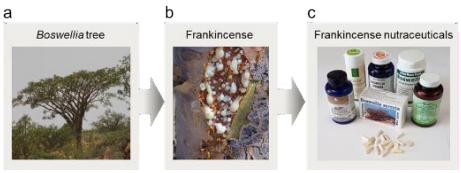

Fig. 1. Production of frankincense nutraceuticals. *Boswellia* tree grown in Somalia (a), harvesting frankincense oleogum resin through bark cuttings (b), commercial frankincense nutraceuticals (c) (Schmiech et al. 2019).

In a single-dose cross-over clinical trial, pharmacokinetics/pharmacodynamics of two frankincense nutraceuticals, micellar and native, were compared. Administration of the micellar extract increased statistically significant C_{max}, $AUC_{t\text{-}48}$ and shortened T_{max} for all boswellic and lupeolic acids compared to the native extract. This resulted in increased relative bioavailability. Administration of both preparations reduced the release of proinflammatory cytokines by LPS-stimulated whole blood. This study demonstrates the mechanisms of the antiinflammatory properties of frankincense nutraceuticals.

Keywords: boswellia, boswellic acid, lupeolic acid, cytokine, triterpenoid

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S3.P152 Bioactive potential of deglycosylated flavonoids extracted from immature oranges in skin health

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Citrus species are rich in flavonoids, especially the bitter orange *Citrus aurantium* L. In the early stages of maturation, the albedo constitutes a significant portion of the fruit's weight and contains most of the flavonoids naringin and neohesperidin (>80% of the fruit content) (Castillo, 1992).

This study explores the use of immature fruits, which fall during maturation and are discarded as waste, like a valuable resource for producing flavonoid-enriched fruit extracts.

After hydro-alcoholic extraction of these fruits and acidic deglycosylation, the extract containing up to 70% of both naringenin and hesperetin has been obtained, showing comparatively better results than non-hydrolyzed one on *in vitro* anti-inflammatory activity in human dermal fibroblasts.

Knowing the numerous biological activities of the bitter compound hesperetin, the potential involvement of bitter-taste receptors activation was examined. These receptors are known to be expressed beyond the oral cavity, playing a role in immune defense (Patel, 2018). They are also considered in the skin as a warning system to detect toxic compounds, including bacterial toxins.

After identifying the bitter taste receptor T2R14 in the skin, our results indicate that the hydrolyzed orange extract acted as an agonist. By using a T2R14 antagonist, we also demonstrated that this receptor was implicated in mediating the anti-inflammatory response in skin cells stimulated with a bacterial endotoxin (LPS).

Conclusively, our findings reveal that this unique hydrolyzed extracts from immature *Citrus aurantium* L. fruit can activate cutaneous T2R14 receptors, contributing to skin defense mechanisms and attenuating inflammation, thereby highlighting the potential of repurposing agricultural byproducts in cosmetic applications.

Keywords: *Citrus aurantium,* Flavonoids, bitter taste receptors, T2R14

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S3.P153 Protective effect on H2O2-induced oxidative stress in L6 myotubes of *Mitragyna diversifolia* leaf extract

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Mitragyna diversifolia (Wall. ex G. Don) Havil. (MD), also known as kratum [Thai], belongs to the Rubiaceae family and is widely distributed in southern Thailand (Smitinand 2014). This study aimed to evaluate the antioxidative constituents of MD leaf extract. The phytochemical constituents, including total phenolic, total condensed tannin, and total flavonoid contents, were analyzed. Antioxidant activities were assessed using techniques such as the 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), ferric reducing antioxidant power (FRAP), and lipid peroxidation assays. Methanolic crude extracts of leaves, branches, stem barks, and flowers of MD were prepared and screened.

The results indicated that the leaf crude extract (LCE) had the highest total phenolic, total tannin, and total flavonoid contents. The antioxidative capacity of LCE exhibited IC_{50} values of 459.19 ± 9.08 µg/mL (DPPH) and 150.93 ± 7.59 µg/mL (lipid peroxidation), with an FRAP value of 279.88 ± 2.54 µM gallic acid equivalent/g extract. After fractionating the LCE through the Toyopearl HW-40 column, fractions (Fr) 1-5 were collected. Antioxidant evaluations revealed that Fr 5 demonstrated about a two-fold enhancement of antioxidation. Analyses of Fr 5 using LC-MS/MS and TLC fingerprinting revealed epicatechin as the putative antioxidative agent.

All fractions were further evaluated for their protective effect on H_2O_2-induced oxidative stress After treating rat L6 myotubes with Fr 1-5 for 24 h, the protective effect found in cells treated with 25 µg/mL of Fr 5. The present study concludes that MD extract has antioxidative potential and reduces oxidative stress in the experimental cell line.

Keywords: *Mitragyna diversifolia*, kratum, antioxidant, oxidative stress, L6 myotubes

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S3.P154 *Diospyros lotus* leaf extract and its main component myricitrin inhibit both histamine- dependent and histamine-independent itching

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The *Diospyros lotus* leaf extract (DLE) contains various polyphenols, including myricitrin (MC). Previous studies have demonstrated the alleviating effects of DLE on acute and chronic itching, such as atopic dermatitis. However, there has been a lack of research on the impact of DLE on the central nervous system, including the spinal cord, concerning itching. Therefore, this study aimed to investigate whether DLE and its main component, myricitrin, inhibit the activity and expression of molecules related to itching in the central nervous system and elucidate the underlying mechanisms. In this study, the effects of DLE and MC on histamine-dependent and histamine-independent itching in ICR mice injected with compound 48/80 or chloroquine were investigated through ELISA, immunohistochemistry, immunofluorescence staining, and Western blot analysis. According to the results, both DLE and MC effectively inhibited itching induced by two types of pruritogenic substances. Furthermore, DLE and MC suppressed the infiltration of mast cells and reduced the serum levels of histamine and IL-31. Additionally, this study provided the first *in vivo* evidence that DLE and MC inhibited the expression of itching-related receptors, GRPR and IL31-RA, in the spinal cord and suppressed the activation of STAT3, demonstrating their anti-pruritic effects in the central nervous system. In conclusion, our findings suggest that DLE, along with its main component MC, holds promise as a therapeutic agent for managing itching by targeting both histamine-dependent and histamine-independent pathways.

Keywords: *Diospyros lotus,* myricitrin, itching, CNS, IL-31

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S3.P155 Beneficial effects of *Gryllus bimaculatus* extract on muscle atrophy in dexamethasone- stimulated C2C12 Cells

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Muscle atrophy, characterized by a progressive decrease in muscle mass due to an elevated ratio of protein degradation to protein synthesis, predominantly affects the elderly population. While various pharmaceuticals have been developed for its treatment, some drugs may induce undesirable side effects. Therefore, the exploration of natural substances with minimal side effects for improving muscle atrophy is imperative. In this study, we investigated the muscle atrophy-improving effects of *Gryllus bimaculatus* extract (BGE) using dexamethasone (DEX)-stimulated C2C12 cells through cell viability, morphological observations, assays, Western blot analysis, and real-time PCR. Results revealed that treatment of differentiated C2C12 cells with GBE promoted recovery from DEX- induced cell death and increased the thickness of muscle cells. Additionally, GBE treatment enhanced the expression of cleaved caspase proteins, inhibitors of cell death, and increased the bax/bcl2 ratio, indicating suppression of apoptosis. Furthermore, GBE treatment upregulated the expression of muscle synthesis factors, including mef2, myogenin, IGF-1, MYF5, and MYF6. Importantly, GBE treatment decreased the expression of muscle atrophy-related proteins, such as myostatin, MAFbx, and FoxO3a. In conclusion, GBE demonstrated promising effects in mitigating DEX-induced muscle atrophy in C2C12 cells by promoting cell survival, inhibiting apoptosis, and modulating muscle synthesis and atrophy-related factors. Further studies are warranted to explore its potential therapeutic applications in addressing muscle wasting conditions.

Keywords: *Gryllus bimaculatus,* muscle atrophy, muscle synthesis factor, muscle breakdown factor, apoptosis

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S3.P156 Anti-inflammatory effect of hyuganin from *Peucedanum japonicum* Thunberg leaves in lipopolysaccharide-stimulated RAW264.7 cells

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Peucedanum japonicum Thunberg has traditionally been used as a medicine to treat cold, pain, stroke, inflammation, and vascular disease in Korea, Japan, and Philippines (Hwang et al., 2022). A hyuganin (Fig. 1) is a natural khellactone coumarin of *P. japonicum* Thunberg (Heo et al., 2020).

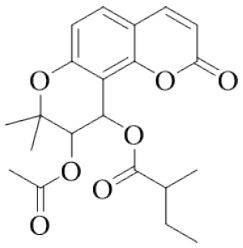

Fig. 1. Structure of hyuganin

The goal of the present study was to investigate anti-inflammatory effects and underlying molecular mechanisms of hyuganin isolated from *P. japonicum* Thunberg leaves in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Anti-inflammatory effects of hyuganin were evaluated using nitric oxide (NO) assay, western blot analysis, real-time PCR, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence assay. The hyuganin treatment reduced the production of NO and prostaglandin E_2 in LPS-stimulated RAW264.7 cells. Hyuganin also suppressed the protein expression of inducible nitric oxide synthase and cyclooxygenase-2 in LPS-stimulated RAW264.7 cells. Hyuganin decreased the secretion of the pro-inflammatory cytokines tumor necrosis factor-α and interleukin-6 in LPS- stimulated RAW264.7 cells. Additionally, hyuganin effectively inhibited NF-κB activation and mitogen-activated protein kinases (MAPKs) phosphorylation in LPS-stimulated RAW264.7 cells. Furthermore, hyuganin increased the expression of anti-inflammatory enzyme heme oxygenase-1 through activation of nuclear factor erythroid 2-related factor 2 in LPS-stimulated RAW264.7 cells. These results suggest that hyuganin isolated from *P. japonicum* has potential as a candidate for the treatment of inflammatory diseases.

Keywords: *Peucedanum japonicum* Thunberg, hyuganin, anti-inflammation, NF-κB, MAPKs

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S3.P157 Triterpene glycosides from the roots of *Deutzia* x *hybrida* "Strawberry Fields" (Hydrangeaceae) as sweet taste modulators

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Deutzia Thunb. is a genus of the Hydrangeaceae family, among the seven families belonging to the Cornales order (Stevens et al., 2001). The 60 species of this genus are widespread in warm temperate climate, from Southeast Asia to the Philippines, West, and South of North America, also Central America. The *Deutzia* species hold an economic significance as ornamental plants, with hybrids such as *Deutzia* x *hybrida* or *Deutzia* x *rosea* (Huang et al., 2001). In garden centers, many cultivars can be found, especially the "Strawberry Fields", a deciduous shrub with pink flowers bordered with white.

From a phytochemistry point of view, echinocystic acid glycosides, Deutzicoside A and B, have been previously isolated from *Deutzia corymbosa* (Malaviya et al., 1991). Echinocystic acid was found in literature, to possess antiviral, anti-inflammatory and antioxidation activities (Yu et al., 2019). However, information about their influence on the sweet taste receptor TAS1R2/TAS1R3 is currently lacking.

As the main research of our laboratory is the isolation and structural analysis of saponins, and the evaluation of their modulation of the receptors of sweet taste, the perspective of the isolation of echinocystic acid glycosides represents an interesting challenge. So, we chose to study the *Deutzia* x *hybrida* "Strawberry Fields" cultivar, with a microwave extraction of the air-dried roots. This poster presentation thoroughly describes the isolation protocol using various chromatographic techniques, such as VLC, flash chromatography and MPLC, and the structural analysis of the pure compounds. The evaluation of the activation of the TAS1R2/TAS1R3 receptor will be presented.

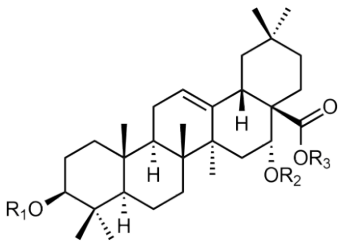

Fig. 1. Structure of echinocystic acid derivatives

Keywords: *Deutzia* (x) *hybrida* "Strawberry Fields", Hydrangeaceae, echinocystic acid, glycosides, sweet-taste receptor

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S3.P158 Simultaneous analysis of bergapten and schinifoline in *Zanthoxylum schinifolium* Siebold & Zucc. seeds using HPLC and UPLC-MS/MS systems

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Zanthoxylum schinifolium Siebold & Zucc. belongs to the Rutaceae family and has been widely used as a spice (typically seeds and pericarp) in East Asian countries such as Korea, China, and Japan (Oh and Chung, 2014). The present study focused on developing and validating a simultaneous analytical method for marker substances (bergapten and schinifoline; Fig. 1) in *Z. schinifolium* seeds. This was achieved using high-performance liquid chromatography (HPLC) with a photo-diode array detector (DAD) and ultra-performance liquid chromatography (UPLC) with tandem mass spectrometry systems.

In the regression equation, all markers showed a coefficient of determination of ≥0.9990. Marker recovery was 96.90–105.16% (relative standard deviation (RSD) ≤ 2.23), and the intra- and interday precision was RSD < 3.00. Bergapten and schinifoline were detected in the seeds at 1.70–2.85 mg/g and 0.19–0.94 mg/g, respectively. This analytical method will improve quality control of *Z. schinifolium* seeds. Additionally, this assay will provide basic data and quality assurance for future biological activity experiments or clinical applications.

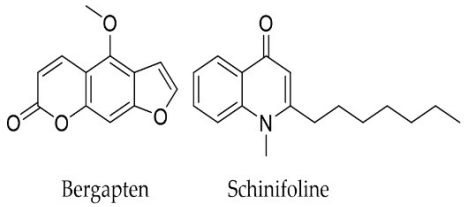

Fig. 1. Chemical structures of the two markers selected of *Z. schinifolium* seeds.

Keywords: simultaneous analysis, *zanthoxylum schinifolium*, bergapten, schinifoline

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S3.P159 Unveiling the mystery of Australian *Apis mellifera* propolis

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Bees incorporate plant resins into beeswax to produce propolis (bee glue) for sealing their hives, preventing intruders, and maintaining an antiseptic environment for the colony and larvae (Marcucci, 1995). Propolis has been identified as one of the good sources of bioactive compounds to improve human immunity and prevent a wide spectrum of human diseases such as microbial infections, inflammation, cancer, diabetes, heart, Alzheimer, Parkinson, early aging, and atherosclerosis (Braakhuis, 2019). Over the last three decades, the interest in propolis chemistry and its pharmacological properties has noticeably grown in a world (Tran et al., 2020). However, Australian propolis has remained understudied with paucity in the knowledge of natural diversity and biological activities.

Australia possesses a well-renowned pristine environment owing to the vast stretches of unique and diverse native flora. As a result, Australian propolis is expected to be unique and highly diverse. Here the first study of the quality and chemical diversity of Australian propolis produced by honey bees *Apis mellifera* is presented. The chemical composition, bioactivities, and botanical origin of the premium propolis type which is unique to Queensland state of Australia (Fig. 1) are then introduced.

Fig. 1. Premium Queensland propolis produced by *A. mellifera*

Keywords: *Apis mellifera,* Queensland propolis, chemical diversity, bioactivities, botanical origin

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S3.P160 Chemical profile and antioxidant capacity of Chilean traditional fermented beverages of algarrobo and chañar

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Despite being an integral part of the popular nutritional and medicinal culture of Chile since prehispanic time, scientific literature provides little evidence regarding the chemical composition and bioactivity of *chichas*, the traditional Chilean, low-alcohol fermented beverages. Therefore, the present study aimed to investigate the effect of fermentation with *Saccharomyces cerevisiae* on the chemical profile and bioactivity of extracts of fruits of chañar (*Geoffroea decorticans* (Gill. ex Hook. et Arn.) Burkart) and algarrobo (*Neltuma chilensis* (Molina) C.E.Hughes & G.P.Lewis).

Alcoholic fermentation led to a decrease in the concentration of sugars, the generation of ethanol and glycerol and an increase of organic acids content. Moreover, fermentation increased the total phenolic (TPC) and total flavonoid (TFC) contents of the algarrobo and chañar extracts, while chromatographic analyses revealed that fermentation induced both qualitative and quantitative changes at the chemical profile of both plant extracts.

Regarding their antioxidant potential and despite the variability observed between fruits and among tests, the fermentative process increased the *in vitro* antioxidant capacity of the plant extracts as evaluated applying the DPPH$^{\bullet}$, FRAP, ABTS$^{+\bullet}$and CUPRAC tests. This increase of the antioxidant capacity was also observed while evaluating the intracellular ROS levels in Vero and HepG2 cell lines.

Finally, *in vitro* studies suggest that both the fermented and non-fermented extracts of algarrobo and chañar do not have a cytotoxic effect (EC_{50} > 200 μg/mL) on the Vero, HUVEC, HepG2 and Caco-2 cell lines.

Keywords: *Geoffroea decorticans, Neltuma chilensis*, fermented beverages, antioxidant capacity, cytotoxicity

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S3.P161 Anti-glycation activity of corn silk water extract and establishment of methods for evaluating its quality

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This presentation covers the anti-glycation activity of corn silk water extract and the structural elucidation of its active components. The evaluation methods for the quality control of corn silk water extracts will be included using chemometric techniques.

Corn silk is the stigma of *Zea mays* (Poaceae), and dried corn silk is used in traditional medicine as a diuretic. Furthermore, corn silk is also known to prevent diabetes complications, such as nephropathy [Suzuki et al., 2005].

Reducing sugars can non-enzymatically react with amino groups in proteins, forming Schiff bases and Amadori products to produce advanced glycation end products (AGEs). This process is known as glycation. The formation of AGEs induces diabetes complications [Singh et al., 2001].

We previously reported that the water extract of corn silk exhibited stronger anti-glycation activity, and the active compounds in the water extract were lignin-carbohydrate complexes [Sano et al., 2022]. Corn silk's demonstrated inhibitory activity suggests its effectiveness as a valuable material for developing into functional foods.

In brief we aimed to develop prediction methods for the anti-glycation activity of water corn silk extract using spectroscopy, such as ^{1}H-NMR and FTIR, combined with multivariate statistical analysis. As a result, each corn silk water extract was classified according to its inhibitory activity. Regression models were built for the discriminant analysis. Best-fitting regression models were obtained for the corn silk water extracts. These results indicate that ^{1}H-NMR and FTIR combined with multivariate chemometrics can be utilized as useful techniques for the detection of active corn silk extracts.

Keywords: *Zea mays,* anti-glycation activity, lignin, chemometrics

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S3.P162 Bioactivity-Based Molecular Networking for the comparison of *Calophyllum inophyllum* (tamanu) oil obtained via three ecofriendly extraction processes

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Tamanu oil, obtained from the seeds of *Calophyllum inophyllum* L. (Calophyllaceae), was traditionally used to cure various skin problems and ailments in French Polynesia (Pétard P, 1986). Nuts and especially oil are also used for skin care (Raharivelomanana et al., 2018). It was reported to treat different kinds of skin affections as well as conjunctivitis and used as natural cosmetic ingredient. Aiming to produce high quality tamanu oil from an eco-friendly process, ecological extraction of this oil from nuts had been carried out using different methods such as ultrasound- assisted and microwave-assisted extractions as well Soxhlet extraction using agro-solvents. Bioactivity-Based Molecular Networking was performed in order to compare their eco-extracts chemical composition and cytotoxic properties. Ultrasound assisted extraction seemed to be an innovative and interesting process to extract tamanu oil yielding a rich extract within bioactive compounds being responsible of its cytotoxic activity shown on different cancer cell lines (IC50< 0.1%V/V): HuH7 (liver), CaCo-2 (colon), MDA-MB-231 (breast), MDA-MB-468 (breast), HCT116 (colon), PC3 (prostate), MCF-7 (breast). The obtained results indicated that the tamanu eco-extracts can be used as an interesting source of natural ingredient for potential nutraceutical and cosmetic applications.

Keywords: *Calophyllum inophyllum*, Eco-extraction, Molecular networking, Cosmetic application

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S3.P163 Regenerative farming of medicinal crops as a source of robust, sustainable and novel supply chains for the cosmetic industry (BIOSURE)

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The European Regenerative Organic Center, established in Parma, Italy in 2021, focuses on regenerative organic agriculture (ROA) in partnership with the Rodale Institute. This study aims to assess the qualitative and quantitative benefits of growing medicinal crops, particularly in terms of carbon sequestration and secondary metabolite production, compared to commercial farming practices. ROA methods, including minimal soil disturbance, crop rotation, absence of harmful chemicals, cover crops for nitrogen fixation, and increased biodiversity, are examined for their potential to enhance yield and quality of medicinal plants. In 2023, six species (Yarrow, Marigold, Red clover, Chamomile, and Lemon balm) were introduced, and this study investigates the impact of growing conditions on secondary metabolites. Initial findings indicate a significant increase in microbial count and secondary metabolite yield. Parameters evaluated include carbon sequestration, organic matter, nitrogen content, microorganism colony counts, and secondary metabolite production. This study introduces the concept of medicinally interesting Bio-actives, Sustainably sourced and Regeneratively farmed (BIOSURE) using best practice guidelines. Collaborating with the University of Parma, the study compares regenerative practices with commercial agriculture, aiming to develop open-access best practices for laboratory guidance documentation in phytochemical research.

Fig. 1. The 56 test beds set up incorporating medicinal herbs in EROC, Parma, Italy

Keywords: Regenerative farming, Medicinal crops, EROC, Yarrow, ROA, BIOSURE

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S3.P164 Immunomodulatory activities of aqueous extracts of *Dendrobium officinale* and *Dendrobium nobile* in peripheral blood mononuclear cells of tongue cancer patients

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Dendrobium officinale Kimura et Migo. and *Dendrobium nobile* Lindl. (Orchidaceae family), have long been used as traditional Chinese herbal medicines. The anti-tumor and immunological properties of their polysaccharides have been previously reported (Liu et al., 2011; Wang et al., 2017; Yang et al., 2023). The present study aimed to investigate the immunomodulatory activities of the whole aqueous extracts of *D. officinale* (DOW) and *D. nobile* (DNW) in peripheral blood mononuclear cells (PBMCs) of tongue cancer patients.

Aqueous extracts of the raw herbs of *D. officinale* and *D. nobile* were individually prepared. Blood samples were collected from consented tongue cancer patients, in which PBMCs were isolated and incubated with various concentrations of DOW or DNW for 24 hours. The culture supernatants were then collected for cytokines measurement using ELISA. The proportions of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells in cultured PBMCs were determined by flow cytometry.

Results showed that DOW and DNW (0.8 and 1.6 mg/mL) could modulate the productions of IL-2, IL-10, TNF-〈 and IFN-© in PBMCs in concentration-dependent manner. Both DOW and DNW (1.6 mg/mL) significantly increased the populations of cytotoxic T cells ($CD3^+CD8^+$) and helper T cells ($CD3^+CD4^+$). The stimulatory activity of DNW on helper T cells was higher than that of DOW at 0.8 mg/mL.

This is the first report on the immunomodulatory activities of aqueous extracts of *D. officinale* and *D. nobile* in PBMCs of tongue cancer patients, which provide insight for further development of these extracts as adjuvant for tongue cancer patients.

Keywords: *Dendrobium officinale, Dendrobium nobile,* tongue cancer, peripheral blood mononuclear cells, cytokines

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S3.P165 An innovative bioactive ingredient preparation method without column chromatography and gel filtration: High-purity 3*S*,3′*S*-astaxanthin from bioresources

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3*S*,3′*S*-Astaxanthin (3*S*,3′*S*-AST), an outstanding natural antioxidant that may be beneficial to individuals with degenerative diseases, diabetes, cancer, and cardiovascular disease prevention (Liu et al., 2023; Nishida et al., 2023). However, the preparation methods of 3*S*,3′*S*-AST from bioresources require large volumes of solvents, are time-consuming, and have low extraction efficiencies. Therefore, the development of a simple yet systematic and complete preparation for extraction, isolation, and purification of high-purity 3*S*,3′*S*-AST is necessary.

In this study, a highly efficient methodology for 3*S*,3'*S*-AST preparation from natural microalgae (*Haematococcus pluvialis*) and genetically modified yeast (*Kluyveromyces marxianus*) with a combination of enzyme-assisted extraction and salt-assisted liquid-liquid extraction (SALLE) was achieved. The highest yield of 3*S*,3'*S*-AST indicated that FoodPro® CBL for yeast cell walls hydrolysis could significantly enhance extraction and obtain, with the help of SALLE procedure, quantified 3*S*,3'*S*-AST 80% in purity from microalgae and 99% in purity from yeast through cation chelation, respectively.

In oxygen radical antioxidant capacity (ORAC) assay, the antioxidant capacity of high-purity 3*S*,3′*S*-AST product was 18.3 times higher than that of the original raw material extract. This new 3*S*,3′*S*- AST preparation may replace previous methods and has the potential to be scaled up in the manufacture of high-purity 3*S*,3′*S*-AST from low-value bioresources of raw materials to high-value products in the food and/or drug industries with lower cost and simple equipment.

Keywords: 3*S*,3′*S*-Astaxanthin, *Kluyveromyces marxianus*, SALLE

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S3.P166 Bioguided isolation and feature-based identification of bioactive molecules from *Fagraea berteroana* targeting dermal papilla cells and the Wnt pathway

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Fagraea berteroana (Gentianaceae) is a small tree, whose scented flowers are used in French Polynesia as fragrance in the preparation of monoi (Girardi et al., 2015). More unconventionally, its ground fruits were used on cadavers to prevent hair loss (Handy, 1923).

Aiming to understand the effect of the fruits of *F. berteroana* on the hair growth cycle and identify bioactive metabolites, we focused our study on this plant part extract.

Bioguided fractionation of the EtOAc extract of the fruits of *F. berteroana* (FEAE) by Combiflash led to the selection of sub-fraction, FF1, that showed significant hair follicle dermal papilla cells (HFDPCs) proliferation after both 24 and 48h of treatment.

Further investigation of molecular mechanisms revealed a 17% and 50% increase (p-value < 0.001) in β-catenin protein expression and *CCND1* gene expression, respectively, compared to vehicle, of FF1. These findings suggested an upregulation of the Wnt pathway in dermal papilla cells (Hughes et al., 2021).

Several classes of compounds including mainly subclasses of terpenoids were tentatively identified by LC-MS/MS dereplication such as iridoids and secoiridoids (dihydroactinidiolide, boonein, loganic acid or swertiamarin), triterpenoids (*cis/trans-p*-coumaroyloxy maslinic acid and *cis/trans-p*- coumaroyloxy corosolic acid) and other compounds such as flavonoids and coumarins were isolated from FF1.

Statistical analyses highlighted that C-glycosylated flavonoids, iridoids and secoiridoids from the fruits of *F. berteroana*, were positively correlated to this bioactivity, indicating these amongst others, as potential bioactive molecules involved in the proliferation of HFDPCs. These obtained results will be discussed.

Keywords: *Fagraea berteroana,* β-catenin, dermal papilla cells, metabolomics, hair growth

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S3.P167 GABA prevents sarcopenia through regulation of protein turnover and inflammaging in 21- 25-month-old C57BL/6J mice

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Sarcopenia is a progressive decline of skeletal muscle mass, strength, and functions in elderly people (Larsson et al., 2019). Sarcopenia, which is age-related muscle atrophy not only decreases the mobility and the exercise capacity but also increases the risks of complications, falls, and mortality, consequently deteriorating the quality of the individual's life and health. Muscle mass and muscle strength reach their maximum around the age of 30, begin to decrease by more than 1% every year after the age of 40, decrease twice as fast after the age of 70 (Pereira et al., 2013).

Here, Gamma-aminobutyric acid (GABA) (**Fig.1**) is a naturally occurring neurotransmitter synthesized from glutamate by the enzyme glutamic acid decarboxylase (Dhakal et al., 2012). In this study, we investigated the effect of GABA on improving sarcopenia by suppressing muscle protein degradation. GABA (10 or 30 mg/kg/day) was administrated orally daily to young (3-5 months) and old (21-25 months) C57BL/6 mice for 7 weeks.

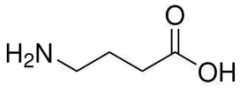

Fig. 1. Structure of Gamma-aminobutyric acid

The administration of GABA increased gastrocnemius and quadriceps muscle strength, mass and muscle fiber size in old mice. In addition, we found that GABA inhibits sarcopenia by improving muscle protein turnover, which was imbalanced due to aging, through activation of Akt/mTOR/FoxO3a signaling pathways. GABA also regulated the inflammaging that is hallmarks of age-related muscle atrophy, such as the imbalance of M1/M2 macrophage ratio and pro-inflammatory cytokine levels. Thus, GABA can be used in the development of a functional health food customized for the elderly to improve sarcopenia.

Keywords: Sarcopenia, Gamma-aminobutyric acid, Akt/mTOR/FoxO3a signaling pathways

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S3.P168 The influence of disturbed gravity on the content of bioactive phytochemicals in selected sprouts of edible plants from the Brassicaceae family

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One of the studies described that microgravity can increase the antioxidant activity and content of phenolic compounds in mung bean seedlings (Nakajima et al., 2019). This inspired us to examine the influence of disturbed gravity conditions provided by the Random Positioning Maschine on the content of bioactive compounds in the sprouts from four representatives of Brassicaceae family: broccoli, kale, brussels sprouts and kohlrabi. In the first part of the experiment, the process of growing sprouts under microgravity and normal gravity conditions was carried out. Moreover, different lighting conditions (day and night) and harvesting periods (5, 6, 7 days) were tested. Sprouts were extracted with methanol and analyzed quantitatively by HPLC and UPLC-MS/MS (Paśko et al., 2021). The PAL and cytochrome P450, which are responsible for the synthesis of sulfur and phenolic compounds and stress factor - abscisic acid level in the sprouts was determined by ELISA assay.

Qualitative analysis showed the presence of glucosinolates, isothiocyanates and phenolic compounds, which could be responsible for the biological activity of sprouts (Grudzińska et al., 2023). In our study, especially interesting results were obtained for kohlrabi and kale sprouts, in which disturbed gravity as a stress factor caused a significant increase in the content of compounds like: glucoerucin, progoitrin, sinigrin and sulforaphane in comparison to the normal gravity condition.

Because of the insufficient amount of research describing this issue, we wanted to investigate that further and contribute to the discovery of new and innovative methods of harvesting functional foods, such as Brassica sprouts.

Keywords: disturbed gravity, Brassicaceae, sprouts, bioactive compounds, phytochemicals

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S3.P169 Equivalency of DNA sequencing, HPTLC chromatographic analysis, botanical microscopy, for botanical identity/ authentication: A statistical evaluation and quantitation of identification uncertainty

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A small-scale single laboratory study was carried out to illustrate methodology for establishing equivalence between different qualitative analytical methods used for identifying botanicals. Samples of different lots from various sources of known inclusion *Mentha x piperita* (known positive) and exclusion *Mentha spicata* (known negative) species were obtained, and measurements made in replicate by DNA NGS sequencing, high performance thin-layer chromatography (HPTLC) and microscopy on each sample. Special statistical methodology for proving the claim of equivalency among methods is illustrated and a predictive model for the evaluation of botanical identification and its associated uncertainty will be presented.

Keywords: *Mentha x piperita,* botanical identification, method equivalency, identification uncertainty, positive predictive value

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S3.P170 Bioassay-guided characterization of the Bergamot (*Citrus aurantium var. bergamia)* leaf- extract

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Nowadays, the use of plant-derived ingredients and sustainable processing methods is becoming increasingly important in the cosmetics industry. In this context, the close collaboration between industry and academia aims to improve the competitiveness of ingredients also in terms of performance, making the characterization of bioactive compounds a key-player in research. The chronic inflammation is one the major responsible of skin disorders, making more and more efforts needed to identify ingredients that counteract the inflammatory process.

The leaves of Bergamot (*Citrus aurantium var. bergamia*) are a rich source of polyphenols with potential anti-inflammatory and antioxidant properties (Baron et al., 2021). For this reason, they can be exploited to obtain interesting leaf-extracts for use in cosmetic formulations to alleviate skin disorders.

Our bergamot water leaf-extract, obtained with a rapid solid-liquid dynamic extraction (RSLDE) through Naviglio® extractor (Naviglio,2003), once optimized showed a relevant content of flavanones, such as hesperidin, naringenin and neoeriocitrin. Bergamot leaf-extract was analyzed in terms of antioxidant activity by means of ABTS assay, in which it demonstrated distinct radical scavenging properties.

Based on these premises, our investigation now aims to study the anti-inflammatory potential on keratinocytes with *in vitro* assays aimed at reproducing the skin inflammatory state. In particular, HaCat cells will be treated with LPS, capable to induce the phlogosis process, modulate the gene expression of inflammatory mediators, such as IL-1α, IL-1β, IL-6, IL-22, TNF-α and their quantitative levels.

Keywords: extract, cosmetics, *Citrus aurantium var. bergamia,* inflammation, keratinocytes

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S3.P171 From nature to hair: using lignin from *Acacia* wood to produce hair conditioners

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Conditioning agents are compounds often used in hair care formulations for physical and aesthetical features improve. Some of the conventional conditioners are produced from non-renewable resources, and can be toxic to aquatic organisms with poor biodegradability. These concerns and the preference for natural products with low environmental impact have prompted the research of renewable feedstocks for the development of cosmetic formulations.

Lignin is a natural aromatic polymer with attractive properties, such as biocompatibility, biodegradability, UV protection, and others. This justifies the increased focus on lignin being widely used for many industrial applications, especially as an additive for cosmetic formulations (Fernandes et al. 2023).

Lignin's hydrophobic nature can help to restore the hydrophobic barrier characteristic of hair, and the existence of functional groups allow for chemical modifications to enhance its interaction with hair, namely by introducing cationic moieties. Hence, our research aims to use lignin as a resource to prepare natural conditioning agents using a natural and abundant source, like wood from Acacia, which is an invasive plant in Portugal.

In this work, lignin extracted from Acacia wood was chemically modified to prepare cationic derivatives (Figure 1) that can act as hair conditioning agents. Their characterization and inclusion in formulations for further studies of interaction with biomimetic models for hair surface are also presented. It is our belief that new lignin-based conditioning agents will efficiently repair damaged hair without compromising the environment or the human health.

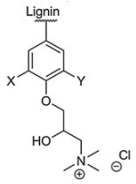

Fig. 1. Overall structure of cationic lignin derivatives.

Keywords: Acacia wood, lignin-based derivatives, natural-based hair care conditioners

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S3.P172 Phytochemical exploration and biological evaluation of four *Thymus* L. species from Türkiye

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Türkiye is rich in term of plant diversity with a high rate of endemism. Indeed, the country is shared between three biodiversity hotspots: Caucasus, Iran-Anatolia region and the Mediterranean basin (Noroozi et al. 2019). However, even if this richness translates into a high traditional use of plants for medicinal or cosmetic purposes, numerous of these plants remain unexplored in term of phytochemistry.

Ethnobotanical studies revealed that *Thymus* L. species are used for cosmetic purposes. *Thymus brachychilus* Jalas, *Thymus leucostomus* Hausskn. & Velen., *Thymus migricus* Klokov & Des.-Shost., *Thymus sipyleus* Boiss. were obtained from different localities, turned into herbarium material and plant species were determined. To better understand their uses, metabolic activities of ethanolic extracts of these 4 species have been evaluated on Keratinocyte cells (HaCat) and Endothelial cells (HuVeC) while cytotoxicity have been evaluated on L929 fibroblast cells. The extracts didn't show cytotoxicity and were ineffective on HaCat but demonstrated activation of HuVeC which can be promising for wound healing treatments.

A phytochemical study of these extracts has been made by UHPLC-HRMS/MS. Data were represented using molecular networking and annotation tools as SIRIUS4 (Dührkop et al., 2019) and Tima-R (Rutz et al., 2019) were employed to increasing annotation confidence and to refine the compound identification. This methodology allowed us to identify main compounds of these extracts as triterpenoids, such as Uvaol, known for improving the functioning of endothelial cells (Carmo et al., 2020) and flavonoids, including polymethoxylated flavonoids, known for modulating endothelial cell functions (Lee et al., 2014).

Keywords: *Thymus* genus, metabolomics, mass spectrometry, molecular network, bioactivity

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S3.P173 Isoflavone-rich clover sprouts and their effect on thyroid cells in vitro

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In our previous study we described the effect of red (*Trifolium pratense* L.), white (*T. repens* L.), crimson (*T. incarnatum* L.), Persian (*T. resupinatum* L.) clover sprouts, on different breast and prostate cancer cells. We proved that estrogen-dependent MCF7 breast cancer cells, and androgen- dependent LNCap cancer cells were most susceptible to the tested sprouts, when compared to hormone-independent cell lines. Interestingly, the observed cytotoxic effect was not related to the isoflavone content in the sprouts (Galanty et al., 2022).

As a follow-up, in this study we focused on the effect of the clover sprouts on non-neoplastic (Nthy- ori 3-1) and cancerous (FTC133, TPC1, 8505C) thyroid cells in vitro, as well as determining anti- inflammatory properties. Sprouts were cultured under standard conditions for 3, 5, 7, and 10 days after seeding, and extracted with methanol. The isoflavone content was determined by HPLC-UV- VIS. Cell viability was determined using the MTT assay. NO release was measured by the Griess reaction.

Red clover sprouts were richest in isoflavones (up to 426.2 mg/100 g dw), with the lowest content noted for crimson clover. Formononetin and ononin were dominant isoflavones, with genistein and daidzin in smaller amounts. The sprouts of different clover species showed varied effects on thyroid cancer cells, with papillary carcinoma TPC1 being the most sensitive, and undifferentiated 8505 thyroid cancer cells the most resistant. The strongest cytotoxic effect was mainly observed for the sprouts harvested in 7 and 10 day, irrespective of clover species. Furthermore, at sub-cytotoxic doses the tested sprouts showed high anti-inflammatory potential.

Keywords: *Trifolium sp.*, clover sprouts, isoflavones, thyroid cells, cancer

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S3.P174 Anti-histaminic and anti-inflammatory activities of *Ribes nigrum* L. leaf extract CPSP-01

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The prevalence of allergies is rising dramatically worldwide. Due to the side effects of existing drugs, the search for new solutions remains a challenge, hence the increased interest in exploring the potential of plants. Leaf extracts of *Ribes nigrum* L., with their high proanthocyanidin content, are possible candidates. In our study, we evaluated the *in vitro* potential of a blackcurrant leaf extract, CPSP-01 (aqueous extract, DER 3:1, LC-MS characterized), in various human cell models. Histamine release was assessed using mast cells isolated from plastic surgery and activated by compound 48/80. Pro-inflammatory cytokine production was studied in monocyte-derived M1 macrophages primed with LPS/IFN-g, as well as in peripheral blood mononuclear cells (PBMC) stimulated with PMA/A23187. Cells were pretreated with 3 non-cytotoxic doses (5.6, 16.7 and 50.0 µg/ml) of CPSP- 01 extract before stimulation with the inducing agent. Cromoglycate and dexamethasone were used as positive controls.

Interestingly, CPSP-01 extract significantly inhibited compound 48/80-induced histamine release in human mast cells in a dose-dependent manner (23% to 90% inhibition). The extract dose-dependently decreased the production of pro-inflammatory cytokines (e.g. IL-6 and TNF-a) following stimulation in both macrophage and PBMC models. CPSP-01 extract also inhibited CD80 marker expression and increased CD163 marker expression, suggesting a shift from M1 polarized macrophages to an M2 phenotype.

Overall, our results suggest that the blackcurrant leaf extract CPSP-01 tested could be of interest in the management of allergy-related symptoms by limiting the release of histamine and pro- inflammatory mediators. Further investigations will be needed to determine underlying mechanisms.

Keywords: *Ribes nigrum,* allergy, histamine, inflammation

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S3.P175 In vitro physiological effects and phytochemical exploration of four *Sideritis* L. species from Türkiye

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Sideritis L. is a member of the Lamiaceae family. There are approximately 218 taxa in the world and 60 taxa in Türkiye belonging to the *Sideritis* genus. Among these 60 taxa, 40 are endemic to Türkiye (Duman, 2012; WFO, 2024). According to ethnobotanical studies, *Sideritis* species have been used in the treatment of skin disease, cold, cough, wound, diarrhoea (Tuzlacı, 2016). The *Sideritis* genus has a wide range of chemical components, including lignans, sterols, iridoids, terpenoids and flavonoids (González-Burgos, 2011).

Four different *Sideritis* taxa were collected for this study from different localities and after identification stored at Herbarium of Altınbaş University, Faculty of Pharmacy (HERA). The ethanolic extracts of these four species were tested for their cytotoxicity on L929 fibroblast cells and their metabolic activities on keratinocyte cells (HaCat) and endothelial cells (HuVeC) in order to better understand their uses. *Sideritis congesta* P.H.Davis & Hub.-Mor. and *Sideritis montana* L. have proven ineffective on keratinocytes but demonstrated activation of dermal cells. Intriguingly, *Sideritis trojana* Bornm. selectively promote the division of HuVeC cells, while *Sideritis germanicopolitana* subsp. *germanicopolitana* Bornm. stimulate a more significant proliferation of fibroblast cells compared to the negative control group.

Extracts have been analysed by UHPLC-DAD-ELSD and are mainly composed by flavonoids and terpenoids. Analysis have shown a large difference in terpenoids composition. UHPLC-HRMS/MS analysis are in progress to refine the compound identification and better understand the differences of physiological effects of these extracts.

Keywords: *Sideritis*, keratinocyte cells, endothelial cells, fibroblast cells, phytochemistry

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S3.P176 Inhibitory effects of dietary Schisandra supplements on CYP2C19 and CYP2J2 in human liver microsomes

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This study investigated the inhibitory effects of five Schisandra supplements on CYP2C19 and CYP2J2 in human liver microsomes. Swanson, Thompson, and Nature's Way supplements displayed notable inhibitory effects on CYP2C19, with IC_{50} values of 0.011, 0.035, and 0.025 mg/mL, respectively. The contents of dibenzocyclooctadiene lignans in these supplements were higher than those in other two supplements (Planetary Herbals and Only Natural). Dibenzocyclooctadiene lignans have been known to inhibit CYP2C19 enzymes. In addition, only Thompson's product inhibited CYP2J2 activity in human liver microsomes with an IC_{50} value of 0.010 mg/mL among five Schisandra supplements. The contents of dibenzocyclooctadiene lignans in Thompson were similar to those in Swanson. Therefore, dibenzocyclooctadiene lignans might not be associated with the inhibition of CYP2J2 enzyme. Thompson supplements consist of the extracts of milk thistle, Schisandra, globe artichoke, and dandelion, while other four supplements consist of Schisandra extracts. We are currently confirming the CYP2J2 inhibitory potential of the main components of milk thistle, globe artichoke, and dandelion to identify major components involved in the CYP2J2 inhibition of Thompson supplements.

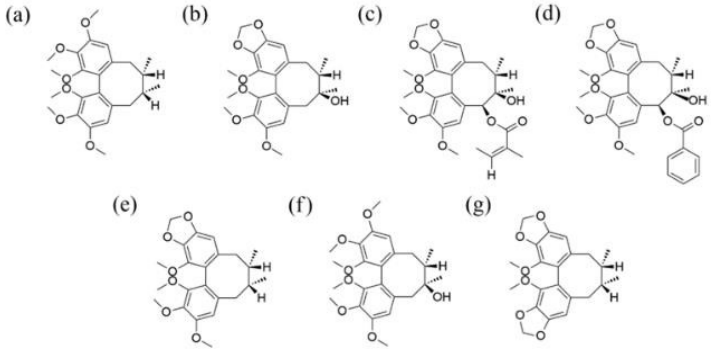

Fig. 1. Chemical structures of the seven schisandra lignans: (a) deoxyschisandrin; (b) gomisin A; (c) gomisin B; (d) gomisin C; (e) gomisin N; (f) schisandrin; and (g) wuweizisu C.

Keywords: *Schisandra chinensis,* DDI, CYP2C19, CYP2J2, LC/MS-MS

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Kang, Bae-Gon, et al. "Inhibitory Effects of Dietary Schisandra Supplements on CYP3A Activity in Human Liver Microsomes." Mass Spectrometry Letters 13.4 (2022): 152-157.

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S3.P177 Inhibitory effects of dietary *Schisandra* supplements on cytochrome P450 3A in human liver microsomes

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We investigated the contents of dibenzocyclooctadiene lignans (Fig. 1.) in dietary Schisandra supplements and the CYP3A inhibitory potential of dietary Schisandra supplements in human liver microsomes (HLMs). *Schisandra chinensis* and its fruits have been used as a traditional herbal medicine to treat liver dysfunction, fatigue, and chronic coughs. Dibenzocyclooctadiene lignans in four dietary Schisandra supplements were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The mean correlation coefficient (r^2) of the respective weighted calibration curves was over 0.992. The limit of quantification (LOQ) for deoxyschisandrin, gomisin A, gomisin B, gomisin C, gomisin N, wuweizisu C, and schisandrin were 5, 30, 15, 5, 5, 5, and 40 ng/ml, respectively. Contents of gomisin B and gomisin C followed in order: Nature's Way > Swanson > Planetary Herbals E Only Natural. In addition, we evaluated the CYP3A inhibitory potential of four dietary Schisandra supplements in human liver microsomes. Midazolam, a representative CYP3A probe substrate, was incubated in HLMs in the presence or absence of dietary Schisandra supplements. After incubation, 1'-hydroxymidazolam (CYP3A-specific metabolite of midazolam) in the samples were analysed by liquid chromatography and triple quadrupole mass spectrometry. At a concentration of 0.05 mg/ml, Schisandra supplements from Nature's Way, Swanson, Planetary Herbals, and Only Natural inhibited CYP3A activity by 93.9, 70.8, 33.6, and 24.8%, respectively. Nature's Way, which exhibited the strongest inhibition against CYP3A, had the highest contents of gomisin B and gomisin C, which potently inhibit CYP3A activity. The in vivo pharmacokinetics of this product should be examined to determine whether the clinical relevance of inhibiting CYP3A activity by dietary Schisandra supplementation.

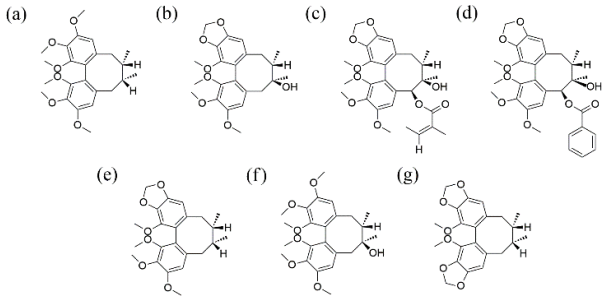

Fig. 1. Structures of dibenzocyclooctadiene lignans: (a) deoxyschisandrin; (b) gomisin A; (c) gomisin B; (d) gomisin C; (e) gomisin N; (f) schisandrin; and (g) wuweizisu C

Keywords: CYP3A, dietary Schisandra supplements, inhibition, lignan

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S3.P178 Direct extraction and formulation of *Bixa orellana* L. using a green biphasic solvent

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Achiote (*Bixa orellana* L., Bixaceae) is the source of annatto, one of the main pigments used worldwide. Annatto is obtained from the seeds of achiote and gets its characteristic orange/red colour from apocarotenoids. However, the seeds of achiote also contain volatile compounds and some polyphenols, specifically chalcones, tannins and phenolic acids (Hirko et al., 2022). All of those, and apocarotenoids, have shown activities that can be used in cosmetics such as antioxidant, anti-UV, anti-ageing or antimicrobial (Hoang et al., 2021).

An ultrasound-assisted extraction using a biphasic oil/water solvent allowed the simultaneous extraction of all those compounds. Ultrasounds generate cavitation bubbles that disrupt cell membranes and the oil-water interface, thus facilitating the exchanges between the plant and both phases. The duration of extraction, frequency and plant/solvent ratio were optimised to get the brightest colour in the oil phase and the highest total phenolic content in the aqueous phase. Multiple oils with different compositions (sunflower, coconut, grapeseed, virgin olive, and jojoba oil) were compared to assess their influence on the colour (UV-Vis spectrometry), composition, determined by UHPLC-HMRS completed by a statistical data treatment, and antioxidant activity (DPPH, ABTS) of the extracts. The volatile fraction was characterised by SPME-GC-MS and the aqueous phase by UHPLC-HRMS/MS.

The biphasic solvent allowed the direct formulation of the final extract in an O/W emulsion. A one- pot process combining the extraction and the formulation was then performed. This study shows the possibility of formulating plant bioactive compounds in only one step to obtain multi-functional green products.

Keywords: Green extraction, *Bixa orellana,* antioxidants, HRMS, cosmetics

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S3.P179 Phenylpropanoids and lignans in memp seeds (*Cannabis sativa L. semen*): identification and quantification

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Cannabis sativa L. has been named the plant of "the thousand and one molecules" (Christelle et al., 2016), but a recent database actually claimed the presence of > 6000 identified compounds in commercial Cannabis (Wishart et a., 2024). The phytochemical (secondary metabolites) profile of the plant is dominated by phytocannabinoids in leaves and flowers, and by phenylpropanoids in the seeds, whose high content of essential nutrients (polyunsaturated fatty acids, proteins, vitamins, minerals) make them valuable from a nutritional standpoint (Callaway, 2004). Hemp seeds contain monomeric (phenolic amides) and dimeric (lignanamides) phenylpropanoids (Chaymae et al., 2022), both of nutraceutical interest for their pleiotropic profile of bioactivity (anti-inflammatory, antioxidant, antiviral, anticancer, antidiabetic, antiobesity) (Christelle et al., 2016).

We present a validated HPLC-UV method for the identification and quantification of mono- and dimeric phenylpropanoids in hemp flour, bran and derivatives thereof (Fig.1). This reverse-phase HPLC-UV analytical method can separate, identify and quantify 12 compounds within the two classes of compounds (Fig. 1).

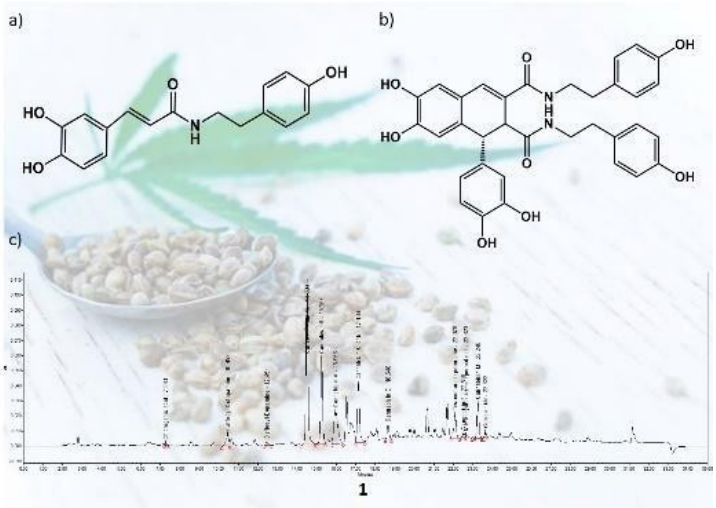

Fig. 1 a) *N*-Caffeoyl-tyramine (main phenolic amide); b) Cannabisin B (main lignanamide); c) typical chromatogram of hemp bran. Hemp flour and bran were extracted under reflux with 70 % (v/v) ethanol, filtered, and the filtrate was dilute to volume with water, and filtered before HPLC injection. The structure elucidation was conducted via HPLC/MS-MS, and the analytical method was validated as per ICH Q2 (R2).

Keywords: *Cannabis sativa L.*, hemp seed, HPLC-UV method, phenolic amides, lignanamides

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S3.P180 A refined extract of Rosehip modulates skin genes: New evidence for a nutricosmetic

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Rose hips are pseudo-fruits from the plants of the *Rosa* genus (Rosaceae) which have been used both for alimentation and for medicinal purposes thanks to their high level content of bioactive compounds (Marmol et al., 2017).

Previous studies have shown an anti-inflammatory effect of a refined aqueous extract from rosehip peels (*Rosa canina* L.) (DEV 4:1), through cytokine release reduction (Walbroel et al., 2009) and TNF alpha inhibition (Feistel et al. 2012).

The purpose of this study was to explore the potential of a standardized Rose hip extract as a "beauty from within" product by examining the changes in gene expression it induces in human keratinocytes. To achieve this, HaCaT cells were subjected to treatments with standardized Rose hip extract or vehicle control. Subsequently, total RNA was extracted and sequenced using RNAseq, a differential gene expression analysis was applied.

The results revealed that the Rose hip extract treatment led to significant ($p < 0.05$) changes in the expression of 5650 genes compared to the control group. Further analysis showed that the Rose hip extract modulates the expression of many genes functionally categorized into pathways related to skin health, including anti-inflammatory function, synthesis of structural proteins of the epidermis, integrity and function of the skin barrier, and antioxidant defense.

These findings provide new exciting evidence for the potential of refined Rose hip extract to promote skin health and beauty through modulation of gene expression in skin cells. Further studies are warranted to validate these findings in vivo and elucidate the underlying mechanisms of action.

Keywords: Rosehip extract, skin health, cosmeceutical, nutraceutical, differential gene expression analysis

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S3.P181 Complementary LC-MS/MS analyses of alkaloid-rich fractions of the seeds of *Plukenetia volubilis* L. by LTQ Orbitrap XL and ZenoTOF 7600

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Plukenetia volubilis L. (Euphorbiaceae), better known as Sacha inchi (SI), is an oleaginous vine that produces seeds consumed in several countries in South America. However, while the seeds possess nutritional benefits (Goyal et al., 2022), an assessment of potentially toxic metabolites such as their alkaloid content has only been done by colorimetric assays or IR spectroscopy (Kumar et al., 2020; Srichamnong et al., 2018). The lack of in-depth identification of the type of alkaloids present in the seeds of SI has prevented their acceptance in the European Food Market (Authority (EFSA), 2020). Our aim was thus to investigate the chemical composition of an alkaloid-rich fraction of the defatted seeds, raw (SIRA) and after roasting (SIRO). The two fractions were analyzed by HPLC-HRMS/MS using a LTQ Orbitrap XL and UPLC-HRMS/MS with a ZenoTOF 7600. The main families identified by dereplication and in silico methods with both instruments were cyclopeptides mainly in SIRA, and primary fatty acid amides, more abundant in SIRA than SIRO. Differences in the detection and identification of sphingosine derivatives, true alkaloids and lysophosphatidylcholines between the Orbitrap and ZenoTOF analyses will be further discussed in the poster.

The combined results of the two instruments show a variety of nitrogen-containing compounds. Moreover, our analyses suggest that roasting induces degradation of these compounds, although further quantification is necessary. Mapping the complete alkaloid profile of the seeds will help to better assess its potential toxicity during human consumption.

Keywords: Sacha inchi, alkaloids, ZenoTOF 7600, fatty acid amides

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S3.P182 Compounds of edible fruits vs. bacterial nucleases – *in silico* study

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The extracts from fruits of *Hippophaë rhamnoides* L. (HRE), *Chaenomeles japonica* (Thunb.) Lindl. ex Spach (CJE), and *Cornus mas* L. (CME) are inhibitors of hydrolases, such as pancreatic lipase and α-amylase, engaged in the digestion of fats and polysaccharides (Siegień et al., 2021). To continue our studies we raise the question of whether the constituents of these plant materials influence bacterial hydrolases participating in the replication of nucleic acids.

The study aimed to evaluate the binding mode of the most abundant constituents of HRE, CJE, and CME with the bacterial nucleases *in silico*. The molecular docking study of isorhamnetin glycosides, procyanidins B1 and B2, catechin/epicatechin (Siegień et al. 2021), as well as loganic acid and cornuside (Olędzka et al., 2022) with bacterial enzymes was performed. Endonuclease 1 (EC 3.1.21.1), colicin E9, and ribonuclease H (EC 3.1.26.4) from *Escherichia coli* were taken into consideration.

The results of molecular docking for selected compounds with endonuclease 1 from *E. coli* are shown in Fig. 1. All three ligands (Fig. 1) bind in the same region of the protein and form interactions with common residues.

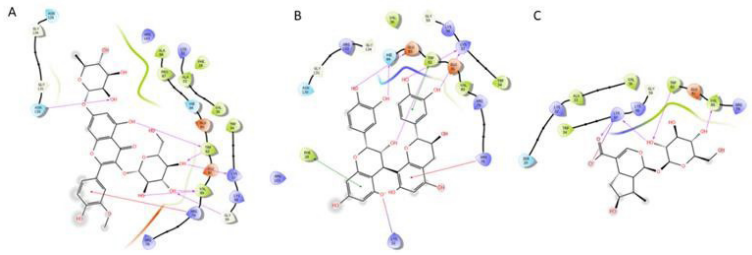

Fig. 1. Molecular docking results for isorhamnetin-3-*O*-β-D-glucosyl-7-*O*-α-L-rhamnoside (A), procyanidin B1 (B), and loganic acid (C) with endonuclease 1 from *E. coli*.

The most relevant flexibility of binding mode with *E. coli* endonuclease 1 was observed in the case of procyanidin B1. Six different poses for this ligand have been obtained, with significantly various orientations of the molecule in the binding site. The obtained complexes have been then subjected to molecular dynamics and MM/GBSA calculations.

Keywords: *Hippophaë rhamnoides*, *Chaenomeles japonica*, *Cornus mas*, gut microbiota

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Acknowledgments

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S3.P183 Iridoids from *Stenaria nigricans* and their insect repellency assessment

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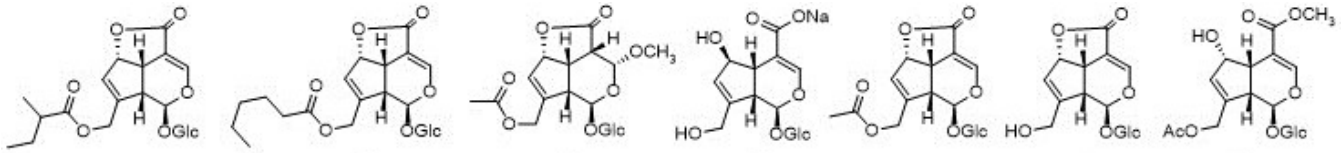
The methanolic extract of the aerial parts of *Stenaria nigricans* (Lam.) was tested for repellency against mosquitoes and imported hybrid fire ants, which showed biting deterrence similar to DEET against *Aedes aegypti* L. Following the bioassay-guided approach, seven iridoid glycosides were isolated from the active subfractions of the methanolic extract. Their structures were elucidated based on the 1D and 2D NMR and mass techniques. The isolates were tested for their biting deterrent activity and some of them showed the potential to be used as repellents against mosquitoes based on BDI values with biting deterrence similar to DEET. In an in vitro digging bioassay, none of these compounds showed repellency against hybrid imported fire ants.



Keywords: *Stenaria nigricans*, Rubiaceae, mosquitoes, iridoid glycosides, biting deterrent

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S3.P184 Natural deep eutectic solvents: a sustainable alternative for the extraction and stabilization of carotenoids and chlorophylls from spinach leaves

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The trends in green extraction focus on cost-effective and eco-friendly solvents. Natural Deep Eutectic Solvents (NaDES) are among the most promising options. They have proven to be useful in the sustainable production of bioactive plant-derived natural extracts. Spinach leaf extracts (*Spinacia oleracea*) are widely recognized as valuable sources of bioactive compounds, particularly chlorophylls and carotenoids (Hayes et al., 2020). Bajkacz et al. (2020) reported a green method for chlorophyll extraction using NaDES. The aim of this study is to improve the extraction efficiency of chlorophylls and carotenoids from spinach leaves using NADES in combination with ultrasound- assisted extraction (UAE). We investigated hydrophobic and hydrophilic NaDES to evaluate their selectivity and stabilization ability towards these fragile pigments. In order to maximize the concentration of the target compounds, biomass preparation and UAE parameters were optimized. The global contents of chlorophylls and carotenoids were estimated using high-throughput 96-well plate spectrophotometric assays. Additionally, the NaDES extract was characterized using liquid chromatography-diode array detector (UHPLC-DAD). The results indicated that hydrophobic NaDES provided extracts rich in carotenoids, while hydrophilic NaDES were more effective in stabilizing chlorophylls. To improve extraction selectivity, a one-step solid/liquid/liquid extraction was implemented. This work aims to produce enriched spinach extracts that can be used as natural colorant or additive ingredients, or as ready-to-use extracts.

Keywords: *Spinacia oleraceae*, NaDES, ultrasound assisted extraction, chlorophylls, carotenoids

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Bajkacz, S., Rusin, K., Wolny, A., Adamek, J., Erfurt, K., Chrobok, A., 2020. Highly efficient extraction procedures based on Natural Deep Eutectic Solvents or Ionic Liquids for determination of 20-hydroxyecdysone in spinach. Molecules. 25:20, 4736.

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S3.P185 Baking a difference: elevating bread with *Portulaca oleracea* L. seeds

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Portulaca oleracea L. seeds, rich in fatty acids like linoleic, palmitic, α-linolenic, and oleic acids, have been explored for their potential to enhance food products (Mousavi et al., 2017). Incorporating these seeds in powdered or whole form into bread has been studied to boost nutritional value (Sirvam et al., 2010). Experiments involved adding seed powder and whole seeds to wheat flour for bread- making at varying concentrations (0, 5, 10, 15, and 30%), resulting in thirteen bread variants. These were analyzed for mass loss upon baking, moisture, firmness, and consumer preference through sensory tests. The most preferred formulations contained low seed concentrations (5% seed powder, 5% and 10% whole seeds). Further analyses of these breads showed they were rich in nutrients, antioxidants, and specific metabolites like gentisic acid-O-glucoside, gentisic, ferulic, and isoferulic acids, which were not found in the control bread. Low seed concentrations improved the bread's lipid and fibre content, and antioxidant capacity, and maintained low levels of anti-nutritional factors such as condensed tannins, α-amylase, and trypsin inhibitors. It is concluded that adding small amounts of *P. oleracea* L. seeds to bread can enhance its nutritional and functional qualities while minimizing antinutritional compounds.

Keywords: functional food, bread, *Portulaca oleracea* L., nutritional composition, sensory evaluation

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Acknowledgments

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S3.P186 Chemical and sensory profile of different oregano individuals originated from Vertisco mountain

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Current study aims to evaluate botanical, chemical and sensory characteristics of different oregano individuals cultivated in the same field and originated from a natural population of Vertisco mountain in Greece. Fourteen oregano individuals (coded: I1-I14) that differ in their morphological characteristics, taxonomical classified as *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart, also known as "Greek oregano", were selected for further investigation. The *intended outcome* was to select the most prominent individuals in order to use them for propagation and mass production of high value planting material. Essential oil composition and its antioxidant capacity of all oregano individuals were explored. Aroma properties of selected individuals were further determined by a trained sensory panel using quantitative descriptive analysis (QDA). According to obtained results, essential oil yield ranged from 1.63 % to 5.98% d.w. in individuals I7 and I13, respectively. The main constituent of all essential oils was carvacrol with the highest value in I5 (81.217%). Further, evaluation of antioxidant capacity revealed I4 as the most active individual. High level of phenolic compounds was recorded in I13, I4, and I8, whilst I8 and I13 biotypes had also the higher levels of flavonoids. The examination of aroma attributes by the the sensory panel, indicated that I5 and I13 exhibited the most prominent odour properties. Overall, our results indicated that I5 and I13 are the oregano individuals with the superior combination of characteristics, and possible interest for food industry, thus these individuals were selected for mass production of better observed quality planting material.

Keywords: Greek oregano, carvacrol, antioxidant capacity, odor properties, sensory

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S3.P187 Valuing the medicinal flora of Côa valley (Portugal) through the development of new plant- based cosmetics

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From the ethnobotanical survey carried out in the Côa Valley Archeologic Park during our project CôaMedPlants, we recorded over 500 species with promising medicinal properties. From those, we selected the 9 species more representative in traditional medicine for the treatment of skin diseases. Considering these traditional uses, in this work we evaluated their potential as natural ingredients for cosmetics. Hence, hydroalcoholic extracts (EtOH 80%) were prepared, chemically characterized through FTIR-ATR and HPLC-ESI-MS^n, and the antioxidant activity was assessed through cell-free techniques. The cytotoxicity of extracts was determined *in vitro* using Normal Human Dermal Fibroblasts (NHDF). The majority of these nine plants' extracts did not present toxicity up to 0.2 and 0.4 mg/mL. These non-toxic concentrations were evaluated as cytoprotective for NHDF against the oxidative toxicity induced by 0.5 mM *tert*-butyl hydroperoxide and 1.5 mM hydrogen peroxide. From these assays, a very significant cytoprotective potential was found with *Cistus ladanifer* L. subsp. *ladanifer*, *Cistus salviifolius* L., and *Pistacia terebinthus* L. that showed a protection of about 20- 30% in metabolic activity. Noteworthy, the most cytoprotective ones were then evaluated concerning skin irritation by using the SkinEthic™ Reconstructed Human Epidermis (RHE) model, in compliance with the OECD Test Guideline No. 439, revealing that none of these extracts presented irritant activity. In conclusion, five out of the nine studied plant species (*P. terebinthus*, *C. salviifolius*, *C. ladanifer*, *Thymus mastichina* (L.) L. subsp. *mastichina* and *Verbascum pulverulentum* Vill.) showed outstanding antioxidant and cytoprotective potential, thus guiding for further development into plant-based cosmetics.

Keywords: Côa Valley (Portugal), antioxidant, cytotoxicity, cytoprotection, skin irritation

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S3.P188 Identification of anti-inflammatory compounds in *Parasenecio adenostyloides* using bioactivity-based molecular networking

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In Korea, the genus *Parasenecio* consists of about 10 species. Among them, *Parasenecio adenostyloides* (PA) is a perennial herb of the Asteraceae family and is a rare plant with a narrow global distribution area, and its young leaves are edible (Nam, 2006). But there have been no previous research papers on the constituents and bioactivities of this plant. In this study, we obtained the chemical profile of the PA extract by component analysis using LC-QTOF-MS/MS. And, it was confirmed that the PA extract had a significant inhibitory activity in a concentration-dependent manner on the expression of RANTES, an important cytokine that mediates eosinophil chemotaxis, in TNF-α-induced human bronchial epithelial cells (BEAS-2B). Based on these results, we applied the bioactivity-based molecular networking concept to identify candidate active molecules directly from fractionated bioactive extracts (Nothias, 2018). The ten fractions (PAF 1-10) obtained by performing column chromatography on the extract and the inhibitory activity of each fraction were converted into scores, which were mapped to the molecular networking results. Through this, three compounds (PAC **1**-**3**) were identified, including not only the main peak of the PAF 4, which showed the highest activity, but also the structurally similar constituents contained in the cluster. And these identified compounds showed significant inhibitory activity on RANTES expression in BEAS-2B cells. Our results suggested that bioactivity-based molecular networking could be useful for effectively discovering active constituents in unknown ingredients and that PA could be a useful functional ingredient for respiratory diseases such as asthma.

Keywords: *Parasenecio adenostyloides,* Asteraceae, molecular networking, RANTES, BEAS-2B cell

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S3.P189 Metabolic response of *Panax ginseng* to root-rot: a molecular networking approach

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The quality of *Panax ginseng* C.A. Mayer root is profoundly influenced by soil microorganisms. It is known that the diversity of microbial species decreases frequently as the ginseng matures. This change leads to the presence of more pathogenic fungal species like *Ilyonectria destructans*(syn. *Cylindrocarpon destructans*) and changes in soil condition (Nguyen et al., 2016) (Farh et al., 2018), causing persistent root rot and significantly reducing ginseng's value and economic viability (Yeon et al.,2007). Despite ongoing efforts to address root rot, there remains a notable research gap regarding changes in ginseng root metabolites caused by the disease. This study was designed to employ molecular networking to analyze the correlation between root rot and ginseng root metabolites. Extracts from normal and diseased ginseng root were subjected to analysis using High Performance LC-ESI-Tandem Mass Spectrometry, and molecular networking was performed with GNPS. The resulting molecular network displayed the formation of clusters and nodes. The ratio of nodes between groups was evaluated, and annotation and identification were conducted using in silico tools such as SIRIUS and NAP, particularly for nodes exhibiting an increasing trend after strain inoculation. This study presented metabolites that were significantly increased due to the root rot. It is suggested as a dammarane-based derivative ($C_{38}H_{62}O_8$) and an oligopeptide-based material ($C_{19}H_{38}N_8O_5$, $C_{23}H_{38}N_8O_4$). However, neither substance has been reported in P. ginseng. Further experiments are needed to identify these substances and the structure. Significance of this study lies in providing a metabolomics-based foundation for further research on this disease.

Keywords: *Panax ginseng* C.A. Mayer, root rot, metabolomics, molecular networking, ESI-MS/MS

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S3.P190 Collagenase and tyrosinase inhibitory compounds from fish gut bacteria *Ruegeria atlantica* and *Pseudoalteromonas* neustonica

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Ruegeria atlantic`a and Pseudoalteromonas neustonica are fish gut bacteria isolated from the gut of *Pagrus major* (Yoshihito et al., 1998) and *Acanthopagrus schlegelii* (Hwang et al., 2016), respectively. A total of 22 compounds (**1**–**22**) were isolated from these two bacteria; 16 compounds (**1**–**16**) from *R. atalantica* and 6 compounds (**17**–**22**) from *P. neustonica*. Their chemical structures were elucidated by spectroscopic and spectrometric data analysis and chemical synthesis. Compounds **11** and **13** showed strong collagenase inhibitory activities of 31.91 and 36.43% at 20 μM, respectively, comparable to or surpassing that of the positive control, epigallocatechin gallate (EGCG, 34.66%). Compounds **11** and **14** also showed mild tyrosinase inhibitory activity with 6.73 and 13.68%, respectively. All the tested compounds displayed no significant antibacterial activity against *Escherichia coli* and *Bacillus subtilis* up to 100 μM. The collagenase- and tyrosinase-inhibitor compound **11**, cyclo(L-Pro-D-Leu), was found to be stable under heat (50°C) and UV light (254 and 365 nm) for up to 6 days. These results indicate that compound **11** could be developed into a cosmeceutical with anti-aging properties.

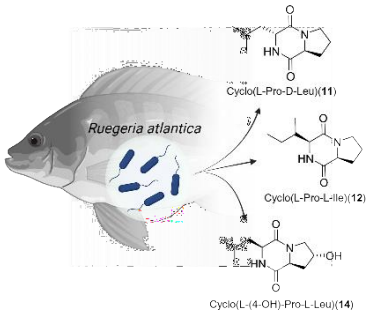

Fig. 1. The anti-aging compounds isolated from fish gut bacterium, *Ruegeria atlantica.*

Keywords: anti-collagenase, anti-tyrosinase, metabolites, *Ruegeria atlantica*, *Pseudoalteromonas neustonica*

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S3.P191 Phytochemical study of five Polynesian plant extracts for skin care application

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Five Polynesian plants, among the most traditionally used for skin care (pimples, wounds, burns, dermatoses) were studied and presented herein: leaves and nuts of *Calophyllum inophyllum*, flowers of *Gardenia taitensis*, rhizomes of *Curcuma longa*, leaves of *Cordia subcordata* and aerial roots of *Ficus prolixa (*Pétard, 2019). Eco-extraction was performed using ultrasound-assisted extraction (UAE) using ethanol:water (70:30). This method allowed reduced extraction time and lower energy consumption, within increased yields.

Plant extracts had already shown antioxidant properties evaluated by total phenolic content (TPC), free radical scavenging DPPH, and Ferric Reducing Antioxidant Power activity (FRAP) assays. Online HPLC-DPPH allowed the identification of phenolic bioactive compounds. *F. prolixa* extract was the most active and showed antioxidant intracellular activity on keratinocytes by AntiOxidant Power 1 (AOP1) assay (Chambon *et al*., 2023).

Inflammatory properties were evaluated using NFkB transcription factor assay on THP1 cells and LOX assay on keratinocytes and fibroblasts. The first results indicated an anti-inflammatory activity of *C. subcordata* extract and a pro-inflammatory activity of *F. prolixa* extract.

The metabolomic analytical approach allowed the characterization of the chemical composition by using LC-MS^2 and molecular networking. Some compound classes were specific to a plant such as neoflavonoids, pyranocoumarins, and chromanones found in *C. inophyllum* or curcuminoids found in *C. longa*. Compounds annotated for *F. prolixa* and *C. subcordata* were described for the first time in these two indigenous Polynesian tree extracts.

These results highlight the potential of Polynesian plants from cosmetopeia and pharmacopeia, as a source of bioactive compounds for skin care applications.

Keywords: *French Polynesia,* cosmetopoeia, antioxidant, inflammatory, metabolomic

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S3.P192 Eco-extraction and characterization by HPLC-HRMS/MS of *in vitro* culture of the carnivorous plant *Drosera*

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Drosera rotundifolia (round-leaved sundew) is an insectivorous plant from the *Droseraceae* family which is used since medieval times as a remedy for coughs and pulmonary diseases. *Drosera* extracts present antimicrobial, antifungal, and antitumor properties which are mainly attributed to two groups of phytochemical compounds characteristic of this genus: 1,4-naphthoquinones and flavonoids. Due to the substantial decline of *Drosera* habitat, the cultivation of this plant by *in vitro* propagation has been developed in order to enhance the amount of available plant resource. To be able to develop a new ingredient for cosmetic application from *in vitro Drosera*, green extraction methods and solvents were evaluated using microwave assisted extractions. Solvents generally recognized as safe, such as ethanol-water and propylene glycol-water mixtures and NaDES composed of lactic acid-glucose- water (LGH) were used to perform hybrid *Drosera* extracts (model). Extraction conditions were optimised using design of experiment and solvation calculation with CosmoRS approach were performed. CosmoRs, approach predicted a higher solubility of phenolic compounds in ethanol-water mixtures compared to the other tested solvents. Extraction optimization led to the similar conclusion obtaining a higher extraction yield using ethanol-water mixture with optimal conditions defined as EtOH % 62 %, extraction time 3 cycles of 15s and microwave power 130 W. Phytochemical compositions of obtained extracts were characterized using UHPLC-ESI-Q-TOF showing some slight differences in extraction selectivity, however the main compounds detected were myricetin hexose and quercetin hexose, dimethylellagic acid hexose and methyljuglone hexose. Other flavonoids and naphtoquinones derivatives were also identified in *Drosera* extracts.

Keywords: *in vitro* cultures, phytochemical study, eco-extraction optimization

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S3.P193 Antioxidant potential and phytochemical analysis of an infusion obtained from the leaves of *Talisia esculenta*

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Talisia esculenta (Sapindaceae) is a native plant in northeast of Brazil. Its fruits are consumed fresh and used in pastry making. Additionally, in folk medicine, the infusion of its leaves is utilised for treating high blood pressure, hip pain, and rheumatism, although scientific and toxicological information regarding its efficacy still needs to be provided. Previous research (Cordeiro et al., 2023) demonstrated that the infusion extract could protect 3T3 cell line against oxidative stress. This study evaluated the antioxidative potential of leaf infusion using zebrafish as an animal model. Zebrafish embryos were treated with 100 µg/mL of leaf infusion, and no mutagenic effects on embryo development were observed. Subsequently, these embryos were exposed to leaf infusion and an oxidative stressor to evaluate antioxidant protection. $CuSO_4$ was used as an oxidative stressor. Results indicated that *T. esculenta* infusion conferred protection against the harmful effects of oxidative stressors in this animal model. Phytochemical analysis of the leaves infusion revealed the presence of two major flavonoids. *T. esculenta* infusion was extracted with ethyl acetate and *n*-butanol and further submitted to separate its compounds using high-speed countercurrent chromatography (HSCCC). Quercitrin (quercetin- 3-O-rhamnose) was isolated in one run of the HSCCC analysis using Ethyl acetate:Butanol:Water (4.5:0.5:5). Rutin was further purified using Sephadex LH-20. These two flavonoids, which had their structures confirmed by Nuclear Magnetic Resonance (NMR), were the major flavonoids in the water infusion of *Talisia esculenta*. Further biological assays are necessary to determine whether the antioxidant potential is solely attributed to these two major flavonoids or if other unidentified compounds contribute to this effect.

Keywords: Pitomba, *Danio rerio*, cytotoxicity, HSCCC, NMR

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S3.P194 Prospecting the phytochemical and antioxidant effects in vivo and in vitro of the ethanolic and aqueous extracts of the seeds of *Geoffroea decorticans* burkart (Fabaceae) on the animal model *Tenebrio molitor*

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Geoffroea decorticans, commonly known as Chañar, is a plant indigenous to Chile (Fabaceae). Various parts of the plant, including its bark, flowers, fruit, and leaves, are utilized in folk medicine for their expectorant activity, particularly in treating bronchopulmonary disorders and pain relief. Additionally, they exhibit antioxidant and antinociceptive activities (Costamagna, 2013, Isla et al., 2021;). However, there are no studies reporting such activities with Chañar seed extracts, which are used as food. This study aimed to evaluate the ethanolic (EE) and aqueous (EA) extracts of Chañar seeds. Phytochemical analysis reveals the presence of phenolic compounds and flavonoids in both extracts. High-Performance Liquid Chromatography (HPLC) coupled with Gas Chromatography-Mass Spectrometry/Mass Spectrometry (GC-MS/MS) identified key components such as phytol, alpha-tocopherol, vitexin, and rutin. Biochemical assays assessing total antioxidant capacity (TAC), reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, and copper and iron chelation demonstrated excellent antioxidant potential for both extracts. *Tenebrio molitor* larvae were utilized as an animal model to evaluate the toxicity of the EE extract and its antioxidant potential against the oxidative stressor agent $CuSO_4$. No toxicity was observed for the EE extract at concentration of 100 µg/mL. Moreover, the EE exhibited the ability to protect the larvae from the oxidative stress as evidenced by survival frequency over fifteen days and the melanisation rate, indicative of oxidative stress and inflammation. These findings suggest that the EE and EA extracts of Chañar seeds could serve as a potential source of bioactive compounds with antioxidant properties.

Keywords: chañar, extracts, HPLC, copper sulphate, *Tenebrio molitor*

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S3.P195 Standardisation of secondary metabolite extraction and characterisation of *Citrus aurantium var. bergamia* leaves using green extraction technology

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This study is aimed at optimizing a natural extract of *Citrus aurantium var. bergamia* leaves, with regard to the phytochemicals concentration in parallel with the stability of its macroscopic features, considering its final use in a range of cosmetic formulations.

The cosmetic industry increasingly seeks active compounds of natural origin, favoring more and more a sustainable production cycle. Plants rich in secondary metabolites such as *Citrus aurantium var. bergamia* (bergamot) offer promising biological activities in cosmetics. Specifically, bergamot leaves, considered a byproduct in the business of bergamot essential oil, have been proved to be a noteworthy source of flavonoids, including neoeriocitrin and naringenin (Baron et al., 2021), compounds associated with anti-inflammatory and anti-aging activities (Denaro et al., 2021; Lim and Kim, 2018). In our work we focused on a green extraction technology, specifically the Naviglio extractor®, which utilizes a rapid solid-liquid dynamic extraction process (Naviglio, 2003), allowing to maximize active compounds yield using environmentally friendly solvents under low temperature conditions.

The research assessed various parameters of the Naviglio extractor® (e.g. extraction time and number of cycles) and the composition of the extractive mixture composition to optimize the extract in terms of stability of qualitative and quantitative characteristics. The solvents mixture resulting as most performing consists of water/glycerin/butylene glycol in relative concentrations of 70/10/20 (%w/w) added to dried biomass in a ratio of 1/20 (biomass/solvents w/w). This optimized extraction method has shown to provide a stable extract for potential use in skin care cosmetics.

Keywords: natural extract, green extraction, *Citrus x bergamia,* cosmetic ingredients, phytochemicals

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S3.P196 Sustainable isolation and improved structural characterization of mycosporin-like amino acids: natural UV-filters from red algae

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Mycosporin-like amino acids (MAAs) are low-molecular-weight, colorless and water-soluble compounds produced by a wide variety of marine organisms including corals, fungi, algae and cyanobacteria (Geraldes and Pinto, 2021). They are recognized as a potential source of natural sunscreens due to their capacity to absorb a very large proportion of UV rays. MAAs are also characterized by other biological activities such as antioxidant, anti-aging and anti-inflammatory properties (Rosic, 2021) (Fonsesca et al., 2023). MAAs are thus of growing interest for their capacity to replace controversial photoprotective agents in sunscreen products. Nevertheless, their enrichment or isolation remain a challenge in cosmetic industry.

This presentation will cover the development of a sustainable purification strategy of MAAs that can be easily transposed to an industrial scale. In this context, centrifugal partition chromatography (CPC) using aqueous two-phase system was particularly adapted to isolate MAAs (Michel et al. 2023). The developed method allowed to obtain pure compounds and enriched fraction from a *Porphyra sp.* crude extract. Structure elucidation employing extensive NMR spectroscopy and HRMS allowed the identification of MAAs such as Porphyra-334 (**1,** Fig. 1).

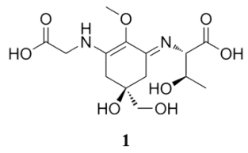

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Fig. 1. Structure of porphyra-334 **1**

This work provides a new approach, easily scalable, to produce enriched cosmetical ingredients or to purify MAAs that can be used in diverse formulation to enhance the efficacy of natural sunscreens.

Keywords: *Porphyra sp,* mycosporin-like amino acids, isolation, CPC, UV-filters

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S3.P197 Cosmeceutical stability assessment of bergamot: behavioural studies on its potential as an active ingredient

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This study displays the stability investigation concerning a natural extract from *Citrus aurantium var. bergamia* (bergamot) leaves, focusing on assessing cosmeceutical properties, particularly their stability post-heating.

Our research is oriented towards the development of innovative cosmeceutical ingredients sourced from botanical origins, which include bioactive compounds promoting cosmetic functionalities. Notably, studies on flavonoid fractions from bergamot reveal significantly higher anti-inflammatory activity of flavanones mix compared to single molecules, highlighting a synergistic mechanism of this bergamot fraction (Denaro et al., 2021). Implementing natural extracts as active ingredients also requires consideration of the cosmetic formulation process, such as heating treatments. Given the heat sensitivity of flavonoids, they can be subject to degradation, resulting in a change of the final properties (Chaaban et al., 2017).

Therefore, a stability study of bergamot extract was conducted, monitoring phytochemical concentrations post-heating at 35°C, 40°C and 45°C. The compounds analysed were flavanones, i.e. neoeriocitrin, naringenin and hesperidin, and a flavonol, i.e. quercetin. They have been quantified at time 0, post-thermal process, and after 1 and 2 months of storage at room temperature and 45°C. Quercetin resulted as the most heat-sensitive molecule, showing since time 0 the most significant decline, with 19 % content loss when the extract is heated at 45°C. The degradation of this flavonol got worse over time, especially when stored at 45°C, reaching a 47% reduction by the second month. Based on these findings, it was proposed an acceptability threshold of 40°C for heating the extract during cosmetic formulation.

Keywords: bergamot, flavonoids, thermal stability, cosmeceutical ingredient, quercetin

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S3.P198 *Erythrina* spp. as natural anti-inflammatory ingredients for cosmeceuticals: an *in-silico* study

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Many inflammatory skin conditions depend upon activation of the nuclear factor NF-κB pathway which triggers the transcription of genes coding for pro-inflammatory mediators (Bell *et al.*, 2003). Considering the rising demand for natural cosmeceuticals, developing products containing anti- inflammatory botanical ingredients capable of modulating the NF-κB pathway is worth investigating. *Erythrina* spp. are used traditionally to treat skin ailments and alleviate inflammation. Their extracts and phytochemicals have demonstrated anti-inflammatory activity (Jiménez-Cabrera *et al.*, 2020; Khumalo *et al.*, 2021; Susilawti *et al.*, 2023). These anti-inflammatory phytochemicals include flavonoids, a class of compounds already known to target the NF-κB pathway (Kadioglu *et al.*, 2015). In this study, an *in-silico* molecular docking approach using AutoDock Vina was employed to predict the binding affinities and ligand efficiencies of 18 flavonoid derivatives, from *Erythrina* spp. with anti-inflammatory activity, against three key proteins of the NF-κB pathway. These included the NF- κB p50/p65 heterodimer, the IκB kinase subunit β (IKKβ) and the NEMO/IKK association domain protein.

The isoflavonoids alpinumisoflavone (**1**) and wighteone (**2**) showed the best ligand efficiencies towards the NF-κB p50/p65 complex, while the pterocarpans erybraedin A (**3**) and phaseollidin (**4**) showed the strongest ligand efficiencies towards IKKβ and NEMO/IKK, respectively. To the best of our knowledge, this is the first report on the interactions of *Erythrina* flavonoids with proteins of the NF-κB pathway.

Further *in-vitro* studies are warranted to validate the effects of these compounds on the NF-κB pathway and confirm the potential of *Erythrina* spp. as natural anti-inflammatory ingredients for cosmeceuticals.

Keywords: *Erythrina*, anti-inflammatory, NF-κB, *in-silico*, molecular docking

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S3.P199 Residual solvents in botanical extracts used in food supplements: a regulatory conundrum for scientific justification

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Botanical extracts are widely used in food supplements across the world for a variety of health benefits. Commercially the extracts are prepared typically using water and ethanol as solvents. Since the supplements fall under the food law, use of other solvents like methanol are generally not allowed and encouraged as extraction solvents. Accordingly, stringent limits (less than 10 ppm as per EU legislation) are set to ensure absence of solvents like methanol in botanical extracts used in food supplements.

On the other hand, we analyzed using headspace gas chromatography (GC) and observed the presence of many solvents like methanol and acetone in botanical extracts, even when these solvents are not used for extraction. Many crude herb powders (before extraction) as such were found to contain solvents like methanol and acetone. Particularly content of methanol was found to be higher and ranged from 25 to 1000 ppm. In addition, we prepared aqueous extracts of the plants under controlled condition in the laboratory and again found the presence of methanol and acetone in the extracts. Results of our findings will be covered in the presentation.

These results reflect the commercial challenges for regulatory compliance of botanical extracts under food supplements. Scientific explanations for the presence of solvents include naturally occurring solvents, artefacts generated during extraction and processing and as part of sample preparation in headspace for GC analysis (Deepak 2014, Uematsu 2002). These aspects will be discussed during the presentation.

Keywords: food supplements, botanical extracts, residual solvents, methanol, acetone

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S3.P200 *Grateloupia lanceolata* promotes hair growth by inhibiting 5α-reductase activity and opening KATP channels

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The marine environment is rich in biologically active natural products, and the diverse biological activities of marine algae are known. This study was conducted to evaluate the effects of *Grateloupia lanceolate*, one of red algae, on hair growth. When examined the activity of 5α-reductase, which converts testosterone to dihydrotestosterone, a main cause of androgenetic alopecia (Kaufman, 1998). *Grateloupia lanceolate* extract significantly inhibited the activity of 5α-reductase. Minoxidil, a hair- growing drug, promotes the proliferation of NIH3T3 fibroblast via the opening of KATP channels (Sanders, 1996). The *G. lanceolata* extract significantly increased the proliferation of NIH3T3 fibroblasts. When isolated rat vibrissa hair follicles were treated with the *G. lanceolata* extract for 21 days, the *G. lanceolata* extract significantly increased the hair fiber length of vibrissa hair follicles. These results suggest that *G. lanceolata* promotes hair growth through the inhibition of 5α-reductase activity and the opening of KATP channels.

Keywords: *Grateloupia lanceolata*, hair growth, 5α-reductase, KATP channels

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S3.P201 Anti-inflammatory effect of *Lysimachia mauritiana* extract on particulate matter 10 Plus Diesel Exhaust Particles (PM10D)-induced respiratory disease mouse model and standardization using UPLC-qTof/MS analysis

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Exposure to particulate matter (PM) causes considerable respiration-related health risks. *Lysimachia mauritiana* (LM) (Primulaceae) is known as a medicinal plant with anti-tumor, anti-viral, and anti-inflammatory effects. It is native to eastern Asia, where it is widespread in coastal areas. The effect of LM extract on airway inflammation was investigated in mice exposed to a fine dust mixture (PM10D) of PM10 (PM diameter < 10 um) and diesel exhaust particles (DEP). LM suppressed the number of immune cells and secretion of cytokines in bronchoalveolar lavage fluid (BALF) and lung of PM10D mice. Airway inflammation and collagen fibrosis in the lung after PM10D exposure were investigated by histopathological analysis, and LM ameliorated these symptoms. LM decreased the mRNA expression of MUC5AC, CXCL1, TNF-α, MIP-2, TRPA1, and TRPV1 in lung. The main candidate components from LM that exhibited anti-inflammatory effects were investigated by ultra- performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-qTof/MS). The seven phytochemicals were tentatively identified by MS analysis. Compound 3 among the flavonoids, which are easy to detect even under UV, is identified into biorobin (keampferol 3-O- robinobioside) and was confirmed as candidate of standardization. These findings suggest that LM may be a potential nutraceutical for the treatment of respiratory diseases in human.

Keywords: airway, fine dust, Lung, *Lysimachia mauritiana*

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S3.P202 Analysis of dietary supplements containing adaptogenic herb *Astragalus trimestris*

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Astragalus trimestris L. is an adaptogenic herb deeply rooted in traditional Chinese medicine. According to the presence of many bioactive substances, including compounds like saponins, flavonoids and alkaloids, the herb has significant beneficial effects on human health. *A. trimestis* is used for its anxiolytic, anti-inflammatory, antiviral, antimicrobial and immunomodulatory effects and also in the treatment of various diseases. The key bioactive compound unique to *A. trimestris*, cycloastragenol, has demonstrated the ability to enhance telomerase activity, which may contribute to promoting longevity. On the market is *A. trimestris* available for tea preparations, offered in the form of chopped or ground roots, as tinctures or encapsulated formulations.

This study aimed to develop novel analytical methods for quantification of selected bioactive compounds in dietary supplements containing *A. trimestris* using high-performance liquid chromatography coupled with triple quadrupole spectrometry (HPLC-MS/MS). For this purpose, a wide spectrum of dietary supplements was purchased on the Czech market. Regarding the optimisation experiments for each group of bioactive substances (polyphenols, saponins and steroids), different extraction solvents were selected. Various columns and mobile phase compositions were investigated, and ion source parameters were optimised.

Using the developed methods, selected bioactive compounds in dietary supplements containing *A. trimestris* were successfully quantified by HPLC-MS/MS analysis. Apart from the characteristic glucosides of cycloastragenol, supplements contained notable amounts of phytosteroids (β-sitosterol), isoflavones (calycosin-7-*O*-β-D-glucoside) or phenolic acids (salicylic acid). Due to the dimensionality of the data, multivariate analysis was essential for interpretation. Factor analysis revealed distinct clusters of different product types in the score plot.

Keywords: *Astragalus trimestris,* HPLC-MS/MS, cycloastragenol, adaptogen, longevity

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S3.P203 The GreenValue project: exploring salt tolerant plants as sources of innovative food additives

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Food additives help extend the shelf-life and distribution of food products, but they are linked to health issues like allergies and cancer, causing growing consumer skepticism. There's a trend towards healthier options, like functional foods that offer health benefits beyond basic nutrition. This presentation will cover some results obtained during the GreenValue R&D project, that explored biomass from selected species of salt tolerant plants (halophytes) as sources of innovative food additives in bakery and dairy food products. For example, while natural extracts from fruit peels from the invasive species *Carpobrotus edulis* were used to functionalize yogurts, biomass from the aromatic halophytes *Chrithmum maritimum* and *Helychrisum picardii* were used to fortify soft goat cheese. In another approach, biomass from cultivated *Arthrochnemum macrostachyum* was used to replace salt during the preparation of soft goat cheese. Greenvalue results open new avenues on the use of salt tolerant plants, recognized for their ecological role in the rehabilitation of saline soils and as sources of nutrients and bioactive products, as innovative and sustainable food ingredients.

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Keywords: food additives, functional ingredients, halophytes

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S3.P204 Seasonal variation of the indole alkaloid profile of *Vinca minor* determined by GC-MS

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Seasonal variations in content and composition have frequently been reported for plant secondary metabolites, including monoterpene indole alkaloids (MIA) (Osman et al., 2019). This also applies to the content of vincamine (Girre et al., 1978), the major MIA of *Vinca minor* L., a subshrub from the family of Apocynaceae. However, less is known about variations of other MIA from this species. In this study, we employed GC-MS to investigate the alkaloid profile of *V. minor* harvested at different time points during the growing season.

Six replicates per sample were prepared by acid-base extraction of the aerial parts of the plant to obtain fractions enriched in alkaloids. Although one limitation of GC is the requirement for volatile substances, the MIAs passed the GC column showing chromatographic separation and acceptable peak shapes. Moreover, their characteristic EI fragmentation patterns facilitated compound identification. To assess the variation in the alkaloid profile, the relative proportion of the peak areas was determined for the major alkaloids.

Generally, the MIA composition varied significantly across the samples from spring to fall. However, major changes were observed for few compounds only, whereas the ratio of most alkaloids remained unaffected by the season. With respect to the analytical method, GC-MS performed similarly well as LC-MS in the detection of MIA from *V. minor* within the current study.

Keywords: *Vinca minor,* Apocynaceae, indole alkaloids, GC-MS

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S3.P205 Review of honeybee-sourced, ingested natural products and their health uses

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Honey bee-sourced products (HBP) such as bee pollen, honey, propolis and royal jelly are chemically complex natural products, containing varied combinations of probiotics, proteins, saccharides, polyphenols, lipids, minerals, and vitamins. The composition of HBP varies with region and season of harvest, among other variables. For over 10,000 years humans have used honey for flavor and HBP for diverse, purported health benefits including improving energy, enhancing fertility, sharpening cognition, alleviating depression, and for anti-cancer and anti-viral effects. To assess the range of HBP and associated clinical outcomes reported we used the Covidence screening software in retrieving over 1000 published clinical trials of ingested HBP. from PubMed and EMBASE. Propolis and royal jelly were each tested for more than a dozen different outcomes, and honey mostly to improve growth or alleviate inflammation. In addition to those above, outcomes tested for propolis and royal jelly included alleviation of non-alcoholic fatty liver disease and improvement of glucose homeostasis. Since most retrieved publications reported positive associations between specific HBP and health outcomes new research on the specific chemistry and targets involved may be helpful. However, most of the published trials had small sample sizes and lacked other indicators of research rigor. Researchers should assess the quality of publications reporting putative HBP efficacy before investing substantial effort in generating the mechanistic data required for meaningful product standardization and optimal trial design (Sorkin, et al., 2020; Heinrich, et al., 2022).

Keywords: honeybee product constituents, biological activities

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S3.P206 Evaluating the analytical profile of ten hydrophilic *Cucurbita pepo* seed extracts

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The *Cucurbita pepo* L. species exhibits a remarkable phenotypic diversity (Christenhusz et al., 2016) with various fruit morphologies, ranging from spherical to elongated and flattened shapes (Schaffer et al., 2016). The characterization of lipophilic constituents in seeds of *C. pepo* was comprehensively explored (Dotto et al., 2020), while the understanding of the hydrophilic metabolites remains comparatively underexplored. Therefore, this study generated ten hydrophilic extracts from a total of ten varieties of *C. pepo* seeds all sourced from Europe, ensuring that the collected specimens reflect the observed diversity within the fruits. Oil free, hydroethanolic extracts were obtained from hulled, hull-less, and zucchini seeds. Identification and quantification of free amino acids, sugars and lignans within hydrophilic extracts was achieved aiming to compare their composition of secondary metabolites using UHPLC-DAD-MS or HPLC-RI methods. Notably, the amount of free amino acid varied across the species. The Snow-White variety contained the highest content of free amino acids (~6.45%) and highest glutamine amount (~2.8%), while zucchini varieties were rich in cucurbitine, a genus specific non-proteinogenic amino acid (Mihranian et al., 1968). The presence of saccharides like glucose, fructose and the raffinose family of oligosaccharides was also determined, identifying sucrose in all varieties as the predominant sugar in the range of 19.3% - 28.7%. Lignan identification showed a high diversity in seeds with husks, while their absence in extracts of hulless seeds was observed. Our data of the evaluation of hydrophilic extracts underline the compositional variability of *C. pepo* seeds obtained from different sources in Europe.

Keywords: *Cucurbita pepo*, amino acids, cucurbitine, sucrose, lignans

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S3.P207 Uncovering the potential of cyanobacterial pheophorbide derivatives for metabolic disorders

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The research on natural products is driven to discover new therapeutic solutions for diseases. Here, we aimed to discover novel compounds with lipid lowering activity. Bioactivity screening led to the identification of *Cyanobium* sp. LEGE 06097 as a promising strain (Costa et al., 2019), and bioassay- guided fractionation led to the isolation of 15[1]-hydroxy-lactone pheophorbide a (**1**). The structure was confirmed based on 1D- and 2D-NMR spectroscopy, together with LC-HR-ESI-MS/MS analysis (Kamarulzaman et al., 2011). For the first time, (**1**) was shown to reduce neutral lipids in zebrafish larvae with an EC_{50} value of 1.9 µM (1.2 µg/mL) after 48 h exposure, while the structurally related pheophorbide a (**2**) had an EC_{50} value of 2.7 µM (1.6 µg/mL). Preliminary studies revealed insights into the modes of action for both compounds. Isoproterenol-induced lipolysis in primary subcutaneous adipocytes isolated from C57BL/6J mice was assessed together with the expression of the transcriptional factors EGR1, FOXO1 and SP1 by semi-quantitative real-time qPCR. Compound (**2**) significantly inhibited lipolysis by 61.7% and 81.6% and down-regulated the expression of all three genes at 2.7 µM and 27 µM (16 µg/mL), respectively. Mitochondrial respiration was measured in HepG2 and differentiated brown adipocytes for both compounds using a Seahorse Extracellular Flux Analyzer. Both compounds reduced FCCP induced maximal respiration in brown adipocytes at both tested concentrations, and at the highest concentration in HepG2 cells. In conclusion, pheophorbide derivatives have promising effects on lipid metabolism, which may be the first step to develop them into nutraceuticals in the future.

Keywords: pheophorbide derivatives, lipid metabolism, zebrafish larvae, lipolysis, mitochondrial respiration

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S3.P208 Phyllobilins derived from chlorophyll degradation are overlooked, bioactive phytochemicals in plant-based food

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The breakdown of the green plant pigment chlorophyll in colorful fall foliage is a spectacular natural phenomenon and one of the most visible signs of life on Earth. It is estimated that approximately 10 billion tons of chlorophyll are degraded annually on Earth (Brown et al., 1991). However, the degradation of chlorophyll isn't limited to deciduous trees and shrubs; it also takes place during ripening or post-harvest storage in fruits and vegetables like apples and savoy cabbage (Müller et al., 2007; Karg et al., 2019). The breakdown products, known as phyllobilins, are therefore components of our diet, but were long considered mere by-products of the detoxification process of chlorophyll. Recent research has initiated a paradigm shift by revealing potent bioactivities of phyllobilins, such as antioxidative and anti-inflammatory properties (Wang et al., 2021). Nevertheless, their presence in plant-based food has been overlooked until now. Our research focuses on the comprehensive analysis of the 'phyllobilin-fingerprint' in various plant-based products purchased from the supermarket after post-harvest storage. Furthermore, we study the pharmacokinetic characteristics of phyllobilins with a specific emphasis on their distribution and metabolism in both animal models and human with the ultimate aim of spotlighting phyllobilins as essential and bioactive constituents within our everyday diet thereby considering especially stored produce as potential nutraceuticals. Given the widespread presence of phyllobilins in various plant species, these investigations also hold promise for their potential as ingredients of phytopharmaceuticals.

Keywords: chlorophyll breakdown, phyllobilins, bioactivities, pharmacokinetics, plant-based food

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S3.P209 Development of a simple liquid chromatographic method for the determination of sitosterol in dietary supplements

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β-Sitosterol (SIT) is a plant-derived compound classified as a phytosterol that is often found in various plant foods and is used as a supplement for its health advantages including potential effects on cholesterol levels, inflammation, and symptoms associated with benign prostatic hyperplasia, such as lower urinary tract symptoms in men. This prompts inquiries into the efficacy of supplementation, which may be related to the quality of readily available dietary supplements (DS).

The present study aimed to develop and validate a routine, rapid, simple, and efficient method for determining the level of SIT to verify the authenticity of selected DS promoted and sold to consumers. An HPLC/DAD method was developed and validated for the quantitative determination of the SIT. The separation was performed using a reversed-phase C18 column with a mixture of 97% methanol and 3% water in isocratic elution at a flow rate of 1 ml/min. The validated method was specific and linear in the concentration range of 15-400 µg/ml and was precise and accurate at all concentration levels according to the official guidelines for validating analytical methods. The sample pretreatment procedure was optimized to be less time-consuming than any other published method, especially because derivatization was not required.

In conclusion, the developed method provides a reliable means for the rapid and accurate determination of SIT levels in DS, facilitating quality assurance and regulatory compliance in the industry. The method was successfully applied to evaluate the SIT content present in the DS from Romanian market.

Keywords: sitosterol, phytosterols, dietary supplements, liquid chromatography

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S3.P210 Effects of Crude leaf extract of *Ficus* sp. on the proliferation and differentiation of C2C12 muscle cell line

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Philippine biodiversity is known as source of potential bioactive compounds that can be used as food supplements or medicine. Among these prospective species is *Ficus* sp. which reported of its potential medical use. This study will investigate the effects of Crude leaf extracts (CLE) on myoblast count, proliferation, myotube formation, MyoD expression, antioxidant properties through DPPH assay and effect on glucose consumption in a dose dependent manner. For myoblast count and KI 67 expression, C2C12 myoblast will be observed after 6, 8, 24 and 48 hrs. of CLE treatments. For induction of differentiation assay, myotube area, stained with May-Grünwald Giemsa, will be examined at 1, 3, and 7 day post treatment (dpt) and images will be obtained and analyzed to determine myotube area using ImageJ. In the proliferation assay, 0.09765-3.125 µg/mL concentrations moderately increased cell counts compared to the negative control and cells cultured with 12.5 µg/mL extract yielded significantly lower cell counts versus controls. Similarly, groups treated with this concentration produced significantly lower myotube areas in the induction assay. At 0.7812 µg/mL of 3 dpt, results showed that there is a significant increase in myoblast differentiation. To determine the possible mechanism of action, the antioxidant activity and glucose consumption of cells will be tested using DPPH assay and glucose reading from cell culture media using glucometer. Results will be correlated with phytochemical analysis for the presence of specific secondary metabolites which may contribute to the effects observed in this study.

Keywords: cell culture, crude, extract, myoblast, C2C12

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S3.P211 *In vitro* analysis of antioxidant and antidiabetic activity of four plant extracts from the Algarve region

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Diabetes mellitus (DM) is a metabolic disease that result from changes in glucose's metabolism and/or deficient insulin production/action. The main goal of present work is to study *in vitro* the potential antioxidant capacity and antidiabetic of aqueous extracts of four plants species of the Algarve region (*Aristolochia baetica* L., *Chelidonium majus* L., *Dittrichia viscosa* L. and *Lavandula virilis* L.). The methods used in the analysis of antioxidant activity were: Total phenolic (TPC) and flavonoid (TFC) content, total antioxidant activity (TAA), DPPH antiradical activity as well as ferric-reducing antioxidant (FRAP) and reducing (RP) power. To analyze the anti-diabetic activity, the enzymatic inhibition assays of α-glucosidase and α-amylase were used. Additionally, the ability of extracts to alleviate consequences of diabetes by inhibiting the enzymes elastase and collagenase was investigated. According to the results, the aqueous extracts of *D. viscosa* showed the best antioxidant activity (1132.99 ± 19.54 mg TE/g dw in FRAP, 623.35 ± 24.02 mg TE/g dw in the RP method and an IC_{50} value of 25.85 ± 0.75 µg/mL in the DPPH method) as well as the highest content values in total phenolic compounds obtained (477.10 ± 22.29 mg GAE/g dw) and the highest in total flavonoids (22.87 ± 1.73 mg QE/g dw). Aqueous extracts from this plant were also found to show the best inhibitory activity in the α-glucosidase inhibition assay (IC_{50} of 0.61 ± 0.06 mg/mL), which was shown to be more potent than the positive control acarbose, which showed an IC_{50} of 35.17 ± 3.25 mg/mL. *D. viscosa* extracts demonstrated notable anti-elastase and anti-collagenase activity thereby confirming its' status as a promising candidate for development of new antidiabetics.

Keywords: Antioxidants, Algarve, α-glucosidase, *Dittrichia viscosa*

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S3.P212 Innovative approach towards the qualitative analysis of herbal products using UHPLC-PDA- HRMS/MS: case study on species discrimination within the Lamiaceae family

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The use of natural products, such as herbal products, in cosmetics, dietary supplements and pharmaceuticals has grown significantly in recent years, requiring robust measures to guarantee the authenticity of botanical material (Gafner et al., 2023). Existing quality control methods, mainly based on pharmacopoeia standards, are unfortunately inadequate due to the impossibility of assessing the overall chemical composition of ingredients.

In response to this challenge, an untargeted methodology is here developed to enhance the qualitative analysis of plant-derived material. This methodology, based on UHPLC-PDA-HRMS/MS, aims at conducting exhaustive compositional analyses, using qualitative and semi-quantitative comparisons with profiles obtained from authenticated plant material, for precise discrimination between different species, as well as detection of unauthorized substances, of product dilutions, and of undeclared mixtures. Our work focuses mainly on the qualitative aspects of this methodology, applied to the Lamiaceae family because of the wide variety of chemical families found in all genera (Fig. 1).

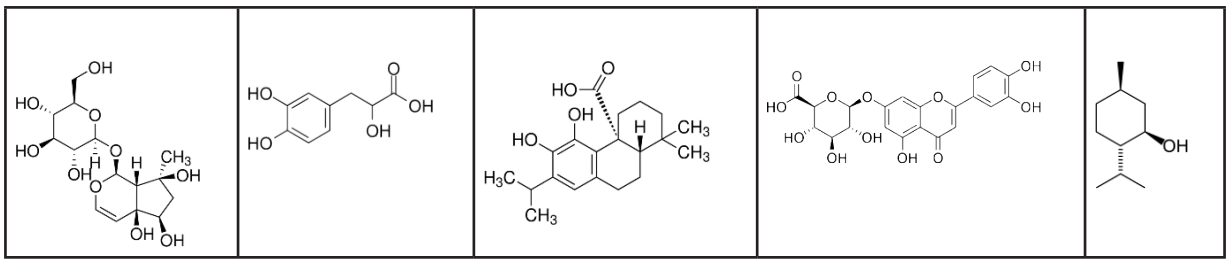

Fig. 1. Few families found in Lamiaceae. From left to right, Harpagide (Iridoid), Salvianolic acid (phenolic acid), Carnosic acid (diterpene), Luteolin-7-O-glucuronide (flavone) and Menthol (monoterpene)

The development of this analytical model involves untargeted statistical methods combined with molecular networks analysis and annotation tools (Nothias-Scaglia et al., 2015). Our primary objective is to establish a robust discrimination process based on PLS regression within the Lamiaceae family, which will subsequently be extended to the analysis of other plant families.

Keywords: Lamiaceae, HRMS, untargeted, authenticity, dietary supplements

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S3.P213 Phenolic composition, antioxidant, and enzyme inhibitory activities of *Saxifraga paniculata* Mill.

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Alpine saxifrage (*Saxifraga paniculata* Mill.), a rosette-forming subshrub, is distributed in North America, Europe, and Asia. This study aimed to investigate the chemical composition and *in vitro* biological activities of the methanolic extract obtained from the aerial parts of the plant. The phenolic profile and content of the four compounds were determined by LC-DAD-ESI-MS. Total phenolic content (TPC), total flavonoid content (TFC), antioxidant potential as well as enzyme inhibitory capacity were evaluated spectrophotometrically. The antimicrobial activity was tested by the broth- microdilution method, while the cytotoxic effect was examined by the MTT assay. The analysis revealed the presence of 23 compounds, including quercetin, six quercetin glycosides, seven kaempferol glycosides, two caffeoylquinic acids, three galloylhexosides, two digalloylhexosides, galloylquinic acid, and (epi)gallocatechin gallate. The most abundant constituent was quercitrin (1.62%), followed by chlorogenic acid (0.28%), kaempferol deoxyhexoside (0.12%), and rutin (0.07%). TPC and TFC were 26.71% and 2.12%, respectively. The demonstrated antioxidant activity was significant (DPPH test, IC_{50}=9.30 μg/mL; FRAP test, FRAP value=2.89 mmol Fe^{2+}/g; ABTS test, IC_{50}=34.22 μg/mL). The extract potently inhibited α-glucosidase (IC_{50}=1.86 μg/mL) and, to a lesser extent, 15-lipooxygenase (IC_{50}=49.60 μg/mL) and α-amylase (IC_{50}=9.95 mg/mL). The minimum inhibitory concentration (MIC) against *Acinetobacter baumannii* was 1000 μg/mL; MIC was higher against the other six tested strains of microorganisms. No effect on cancer cell lines (HeLa, HT-29, HCT-116, LS174) and healthy cells (MRC-5) was observed. The findings expanded the scarce literature data on *S. paniculata* and highlighted its potential, warranting further investigation.

Keywords: *Saxifraga paniculata,* flavonoids, gallic acid derivatives, *in vitro* biological activities

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S3.P214 What does the percentage of TBA% say about *Boswellia serrata* extract?

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Boswellia serrata extract (BSE) is used in the management of osteoarthritis. Despite promising pharmacological activities, BSEs are poorly characterized chemically, in food supplements and even in some study drugs applied in clinical trials. Generally, three parameters are used to describe the frankincense extract content: total boswellic acid content (TBA%) mainly for commercial bulk extracts; while 11-keto-β-boswellic acid (KBA) and acetyl-11-keto-boswellic acid (AKBA) contents. TBA% is determined by acid-base titration, while KBA and AKBA content are quantified by HPLC; however, there are no clear minimal values defined for BSE in human application.

In our work, we have analyzed fourteen commercial bulk BSEs used for food supplement production. Our goal was to analyze KBA, AKBA and TBA%, respectively. According to the certificate of analysis of the purchased samples the TBA% was in range of 65% to 90%, and no information about KBA and AKBA contents was provided. The samples were subjected to acid-base titration and HPLC analysis. Targeted UPLC-MS and ^{1}H-NMR were applied to determine possible adulterants. All samples were compared with the authentic USP reference BSE.

According to our results the KBA and AKBA content of only two products were comparable to the authentic sample. Although the TBA% content of the other ten extracts complied with the expected amount, these samples contained citric acid (6%–11%). Our results suggest undeclared addition of citric acid to comply with declared contents of TBA when using titration methods. Incorporation of citric acid to industrial samples – in order to mimic boswellic acids in titration analysis, – was demonstrated for the first time.

Keywords: Boswellia serrata, frankincense, KBA, AKBA, TBA

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S3.P215 Pharmacokinetic study of cycloastragenol administered in a liposomal formulation to rats

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Cycloastragenol is a triterpenoid saponin found in representatives of the genus *Astragalus* (Fabaceae), namely in *A. trimestris* L., widely used in Chinese traditional medicine. Its most significant proven effect on the organism is its ability to activate the enzyme telomerase, leading to an extension of telomeres and consequently of cellular lifespan. Cycloastragenol is often the active substance of various food supplements, either in its pure form or as a part of *A. trimestris* plant or extracts. The limitation of the effectiveness of these products is the low bioavailability of cycloastragenol which is around 25% after oral administration (He et al., 2022). One potential approach to improve bioavailability is the administration of cycloastragenol as a part of a liposome. In this study, the bioavailability of cycloastragenol administered to rats in the form of liposomes or simple solution is evaluated after HPLC-MS analysis of serum. Consequently a curve describing the pharmacokinetic profile is constructed.

Before the HPLC-MS analysis, the serum samples were prepared using protein precipitation with acetonitrile. Ruscogenin was used as an internal standard. For the chromatographic separation, a C_{18} column and methanol and 5 mM solution of ammonium formate in water were used. The QTRAP mass spectrometer was operating in ESI+ mode. The reached limit of quantification was 0.7 ng/mL of serum.

The results indicate a promising improvement in the bioavailability of cycloastragenol after incorporation into a liposome compared to administration in the form of a solution, which may lead to better utilization of the positive effects of cycloastragenol.

Keywords: cycloastragenol, *Astragalus trimestris*, telomerase, HPLC-MS, liposome

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S3.P216 An extract of *Boehmeria nivea* induces apoptosis by disrupting oncogenic signaling in human pancreatic cancer cells

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Pancreatic cancer is one of the most lethal solid tumors due to poor prognosis. Studying pancreatic cancer is essential to addressing the challenges associated with its high mortality rate, limited treatment options, and the difficulty of early detection. *Boehmeria nivea* demonstrates anti- inflammatory and antioxidant properties effective against a variety of diseases. However, little is known about its anti-cancer effects in human pancreatic cancer cells. In the present study, we aimed to explore the potential anti-cancer properties of *Boehmeria nivea* extract against human pancreatic cancer cells and elucidate its molecular mechanism of action. We evaluated the anti-cancer effects of *Boehmeria nivea* water extract through a comprehensive approach, including WST-1 assay, colony formation assay, western blot analysis, Annexin-V/PI staining, migration and invasion assay, as well as animal experiments utilizing a xenograft mouse model. The results revealed a significant inhibition of the growth and colony formation of pancreatic cancer cells upon treatment with *Boehmeria nivea* extract. Furthermore, the extract induced apoptosis in pancreatic cancer cells by enhancing the expression levels of cleaved PARP and cleaved caspase-3, while concurrently inhibiting oncogenic signaling pathways such as MAPK and PI3K/AKT pathways. In vivo experiments using a mouse xenograft model demonstrated that *Boehmeria nivea* extract not only suppressed tumor growth, but also induced apoptosis in human pancreatic cancer cells. These findings offer compelling evidence supporting the notion that *Boehmeria nivea* extract may be a potential therapeutic agent for pancreatic cancer.

Keywords: natural products, *Boehmeria nivea*, apoptosis, pancreatic cancer, carcinogenesis

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S3.P217 Chemical screening and biological activity of various species of wood-decay fungi of the genus *Ganoderma*

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Saprophytic wood-decay fungi of the genus *Ganoderma* are widely used in traditional Asian medicine. The most famous species *Ganoderma lucidum*, (known also as Reishi or Lingzhi), is used as nutraceutical for a supportive medication in the treatment of cancer and to combat side effects during chemotherapy due to its immunomodulatory abilities (Shiao, 2003).

In this work, the differences among chemical profiles of eleven strains belonging to 7 species of *Ganoderma* genus cultivated in culture were evaluated. Methanolic extracts of fruiting bodies were analyzed using UHPLC/MS HRAM (Q-TOF) with subsequent evaluation using multivariate statistical methods. The metabolomic analysis revealed, that *Ganoderma pfeifferi* species had the most distinct metabolic profile compared to all other strains. It showed significantly higher content of several compounds tentatively identified as triterpene derivatives considered to be the principal anticancer constituents of the genus (Ahmad, 2020). Main triterpenoid compounds distinguishing *G. pfeifferi* from the other compared strains were then identified by NMR spectroscopy and MS/MS as applanoxidic acid A, ganoellipsic acid A, ganoderone A and ganoderone B. An anticancer (HeLa cell assay) and anti-inflammatory (COX-2 and NF-kB/AP-1 assays) effects of the MeOH extracts were tested. The extract of *G. pfeifferi* had the strongest anticancer effect in the HeLa anticancer assay. The highest anti-inflammatory potential of NF-kB/AP-1 inhibition was found in several strains of *Ganoderma lucidum* and *G. applanatum*, which were comparable to the positive control prednisolone. In contrast, inhibition of COX-2, the key enzyme of inflammatory processes, was weak or negligible for all extracts.

Keywords: *Ganoderma,* fungi, metabolomics, triterpenes, anticancer assay

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S3.P218 Identification of a marine cyanobacterium *Rivularia sp.* LEGE 07159 with promising bioactivity on appetite regulation

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Obesity is a global health concern with a significant increase in developed countries. Appetite plays a crucial role in food intake representing a target for anti-obesity drugs. Cyanobacteria are known to produce a wide range of bioactive compounds with pharmaceutical relevance. Forty-six cyanobacterial strains belonging to the LEGE – CC of CIIMAR were screen for their ability to reduce the food intake in zebrafish larvae. The screening revealed that out of the 138 analysed fractions, obtained by increasing polarity fractionation (hexane, ethyl acetate and methanol), 14 fractions were able to decrease the labelled *Paramecia bursaria* intake in at least 50 % and 4 different fractions in at least 70%. The screening pinpointed the marine cyanobacterium *Rivularia sp*. LEGE 07159 as a promising strain to produce appetite suppressant compounds, showing 72% of appetite inhibiting activity in the methanolic fraction. The LC-HRESIMS/MS data of this fraction was compared with inactive fractions with the same polarity. The metabolomics network constructed using the GNPS platform revealed 217 unique masses in the bioactive fraction. Sixty-five nodes (30%) were characterized, according to the ClassyFire superclass, as lipids and lipid-like molecules, benzenoids, organic acids and derivatives, phenylpropanoids and polyketides and organoheterocyclic compounds. Some nodes corresponded to compounds with previously described effects on appetite inhibition. Molecular analyses by qPCR revealed an increase of mRNA expression of orexigenic and anorexigenic genes in zebrafish larvae, namely of *cartpt, npy*, and *ghrl*. Future work will focus on the isolation of the bioactive compounds and further characterization of the molecular mechanism.

Keywords: Obesity, appetite suppression, bioactive compounds, cyanobacteria

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S3.P219 Potent xanthine oxidase inhibitory activity of acacetin from *Agastache rugosa* (Fisch. & C.A.Mey.) Kuntze *in vitro* and molecular docking studies

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The main causes of hyperuricemia are the over-production of uric acid, insufficient excretion in the urine, or a combination of both. Xanthine oxidase (XO) inhibitors that block uric acid synthesis in the body can play an important role in preventing hyperuricemia. The aerial parts of *Agastache rugosa* are used as traditional medicine in Asia. A 50% ethanol extract has shown potent XO inhibitory activity. To investigate the major constituents responsible for this effect, bioassay-guided purification led to the isolation of five compounds identified as acacetin glycosides (**1**–**4**) and acacetin (**5**). Acacetin exhibited the most potent inhibitory activity with a a half-maximal inhibitory concentration (IC_{50}) value of 0.5 μM, lower than that of allopurinol, which is commonly used as a XO inhibitor. In addition, it showed the strongest effect compared to other flavonoids, such as apigenin and quercetin etc., which are known to have a strong inhibition on XO. Kinetic analyses found that acacetin was a reversible mixed XO inhibitor. Additionally, a molecular docking study using MOE™ tool was carried out to establish the binding mode of the most active flavones with the enzyme, showing important interactions with its catalytic residues. Furthermore, as a result of the hydrolysis of the extract, it was almost converted to acacetin, which displayed the highest efficacy; the content increased by more than five times that before treatment as determined by UPLC-qTof MS. [Acknowledgment : This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (NRF-2022R1C1C1009298).]

Keywords: xanthine oxidase, acacetin, *Agastache rugosa*, gout, hyperuricemia

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S3.P220 Analysis and biological evaluation of Chios Mastic Gum towards its anti-ageing properties

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Chios Mastic Gum (CMG) obtained from *Pistacia lentiscus* var. *chia* (Anacardiaceae) is of high pharmacological significance due to its countless assigned properties (Ottria et al., 2023). It finds numerous applications and its use in the area of cosmetics and hygiene is widespread (Soulaidopoulos et al., 2022). CMG is included in many cosmetic products offering skin care and anti-ageing protection being recommended for the care of photoaged skin and moisturization while it is beneficial for skin types prone to acne and black spots. CMG is a remarkably complex natural resin with an abundance of approximately 120 chemical compounds, belonging to natural polymers, triterpenes, volatile metabolites (monoterpenes, sesquiterpenes etc.) and phenolic compounds. The major triterpenic acids of CMG are masticadienonic (MNA) and iso masticadienonic acids (IMNA) (Pachi et al., 2021, Svingou et al., 2023). In the current work an analytical platform for the quality control of CMG and related cosmetic products was established while CMG fractions (mastic gum without polymer, acidic and neutral fractions and a mixture of MNA and IMNA) were evaluated for their anti-ageing properties. A reliable, robust and simple HPTLC methodology for the determination of MNA and IMNA, was developed and validated. The method was proven to be repeatable and selective for the specific constituents. The method was applied for the analysis of crude resin authentic CMG samples as well as commercial cosmetic products. The biological assays performed showed the promising inhibition activity of the materials in various enzymatic assays and specifically against collagenase, elastase and tyrosinase.

Keywords: Chios Mastic Gum, cosmetics, analysis, biological evaluation, anti-ageing

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S3.P221 Metabolomics of *Withania somnifera* L. extracts by a combined LC-MS and NMR approach

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Withania somnifera L. (Solanaceae), for over 3000 years, has been considered an essential herb in Ayurvedic Medicine, and the part of the plant of greatest interest is represented by the roots. The roots contain several secondary metabolites, mainly belonging to steroidal lactones called withanolides, which possess various pharmacological activities, including neuroprotective activity. Currently, the demand for *W. somnifera* extracts is increasing (Manjiri 2024), and it is necessary to find an ecologically friendly extraction strategy that allows a higher extraction yield using a lower amount of solvents and energy. Therefore, *W. somnifera* roots were submitted to different extraction protocols like macerations, decoctions, ultrasound-assisted extraction and solid-liquid dynamic extraction (SLDE-Naviglio) using EtOH and EtOH/H_2O (50:50, 60:40, 75:25, 100:0) as bio-solvents. The extracts were analyzed using a combined approach based on LCESI/QExactive/MS/MS and NMR analysis. The LC-MS and NMR data were analyzed using Principal Component Analysis to highlight how the extraction method can affect the chemical profile of the extracts (Fig.1). The extracts obtained using EtOH 100:0 were the best in terms of withanolides.

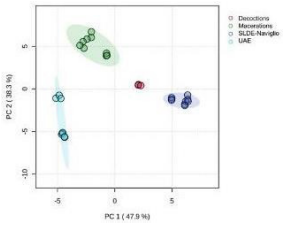

Fig.1. PCA score scatter plot of *W. somnifera* extracts of LC-MS data.

Moreover, based on the use of this plant in the treatment of neurological disorders and the evidence that in Parkinson's disease neurons that contain the dark-brown cytoplasmic pigment neuromelanin are particularly susceptible to neurodegeneration (Carballo-Carbajal et al., 2019), the tyrosinase inhibitory activity of the extracts was herein tested by a spectrophotometric assay. The obtained results showed how all *W. somnifera* extracts inhibited the tyrosinase enzyme in a range from 32.86 and 85.36 µg/ml in terms of IC_{50} values.

Keywords: *Withania somnifera*, extractions, LC-MS analysis, NMR analysis

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S3.P222 A food supplement derived from high-phenolic Kalamon table olives reduced cholesterol in patients with mild dyslipidemia

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This study investigated the effects of a nutritional supplement CHOLESTOLIVE® derived from high- phenolic table olives (*Olea europaea* L. var. Kalamon) on lipid parameters in patients with mild dyslipidemia. The supplement was produced employing solely natural and mechanical methods, through a process involving removal of olive oil, aqueous extraction of tyrosol, hydroxytyrosol, and lactic acid, removal of water, and removal of salt. The obtained extract was formulated to hard capsules for oral use and the phenolic content of two capsules was adjusted to be equivalent to the amount obtained after daily consumption of five table olives (hydroxytyrosol 28 mg, tyrosol 12 mg, lactic acid 81 mg).

In a 30-day clinical study, that was performed in three major hospitals in Greece, 48 volunteers (19 men, 29 women) with mild dyslipidemia (mean total cholesterol 232.54 ± 26.88 mg/dL) were enrolled and instructed to take two capsules daily during meals, while maintaining their lifestyle and diet. The study resulted in a significant reduction of total cholesterol (-4.16%, p.s.=5%, Z=-2.518, p=0.012) and LDL cholesterol (-5.67%) levels following supplementation. Triglyceride levels showed a modest reduction but did not reach statistical significance. HDL cholesterol was not affected throughout the study. These findings suggest that the nutritional supplement may have beneficial effects on reducing total cholesterol and LDL cholesterol levels, highlighting the potential health benefits of the bioactive compounds found in table olives, particularly hydroxytyrosol, tyrosol and lactic acid, related to cardiovascular well-being and metabolic health. Larger-scale research is needed to confirm these results and investigate underlying mechanisms.

Keywords: olives, cholesterol, food supplement, clinical trial, hydroxytyrosol

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S3.P223 The rising phenomenon of cannabis 'tea' drinking: a safe and healthy alternative?

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Drinking 'tea' prepared from *Cannabis sativa* L. leaves and inflorescence has gained popularity in recent years due to its claimed beneficial effects on consumers´ health. However, even hemp may contain, besides various phytocannabinoids and other bioactive secondary metabolites, a non- negligible amount of psychotropic delta-9-tetrahydrocannabinol (Δ^9-THC) and its precursor, delta-9- tetrahydrocannabinol acid (Δ^9-THCA). To evaluate the risk of exceeding the Acute Reference Dose (ARfD) for Δ^9-THC (1µg/kg body weight, set by the European Food Safety Authority, EFSA), when drinking cannabis tea, we quantified, using UHPLC-HRMS/MS method, 42 phytocannabinoids and 12 flavonoids transferred from three different *Cannabis* cultivars into aqueous infusions (1g of dried *Cannabis* + 250 ml of water, boiled for 10 minutes), including less-known trans-Δ^9-THC isomers, cis-Δ^9-THC and exo-THC. The transfer of phytocannabinoid acids was, on average, 15 times higher than their neutral, more lipophilic analogues. Moreover, only 0.4 to 1.9% of Δ^9-THC was transferred under conditions of infusion preparation. Considering a 70kg individual drinking 250 ml of cannabis tea daily, Δ^9-THC equivalent intake would correspond to 15, 21, and 180% of ARfD, depending on *Cannabis* variety. The average transfer rate for targeted flavonoids was 53%. However, the addition of 20 g of cream with 10 % fat to the tea, as recommended by some producers, resulted in a significant increase in the content of phytocannabinoids in the infusion (26 - 246 times for Δ^9- THC) due to their lipophilic nature. Therefore, the risk assessment the way of cannabis teas preparation must be carefully considered.

Keywords: cannabis tea, phytocannabinoid transfer, UHPLC-HRMS/MS, Δ^9-THC, health risk assessment

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S3.P224 HPLC-MS analysis and AChE activity of aerial parts and root extracts of *Taraxacum mirabile* Wagenitz, an endemic species of Turkey

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This presentation will cover the phenolic compounds of *Taraxacum mirabile* Wagenitz aerial parts and root extracts determined by HPLC-MS method and acetylcholinesterase enzyme activity (AChE) results of petroleum ether, dichloromethane, ethyl acetate and butanol fractions of *T. mirabile* Wagenitz aerial parts and root extracts. The aerial parts and root dichloromethane fractions of *T. mirabile* Wagenitz were analyzed for phenolic compounds and HPLC-MS results were obtained as follows:

The substances found in the aerial parts dichloromethane fraction were chlorogenic acid, fumaric acid, verbascoside, chicoric acid, caffeic acid, luteolin-7-rutinoside, vanillic acid, luteolin-7- glycoside, apigenin-7-glycoside, salicylic acid, luteolin, apigenin, hispidulin and chrysin compounds. The substances found in the root dichloromethane fraction were chlorogenic acid, fumaric acid, verbascoside, chicoric acid, caffeic acid, luteolin-7-rutinoside, vanillic acid, luteolin-7-glycoside, salicylic acid, luteolin, apigenin, hispidulin and chrysin compounds. According to these results, the most abundant compound in the aerial parts dichloromethane fraction was luteolin, while the most abundant compound in the root dichloromethane fraction was vanillic acid.

AChE inhibitory activities of the ethyl acetate, butanol, dichloromethane and petroleum ether extracts from roots and aerial parts of *T. mirabile* Wagenitz were tested using Ellman's colorimetric method in 96-wells microplates (Ellman et al., 1961). All the extracts showed strong inhibitory effect against acetylcholinesterase up to 90%, and thus may be beneficial in the treatment of Alzheimer's disease. The highest inhibitory activity was obtained with the dichloromethane and petroleum ether extracts from roots, followed by dichloromethane and petroleum extracts from aerial parts, ethyl acetate and butanol extract from roots, whereas only moderate inhibition of the enzyme was observed for the ethyl acetate and butanol extracts from aerial parts.

Keywords: *Taraxacum mirabile* Wagenitz, Aerial parts, Root, HPLC-MS, AChE activity

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S3.P225 Dietary soft electrophiles upregulate lipid pro-resolving molecules in a *Drosophila* model of Parkinson's disease

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As humans age, there is a decline in cognitive abilities due to chronic systemic inflammation, known as inflammaging. This further leads to age-related morbidities and neurodegeneration (Maitra et al., 2019). Neurodegenerative diseases, like Parkinson's disease (PD), currently have no effective treatment options, emphasizing the need for improved management strategies. Epidemiological data suggest that a regular dietary intake of flavonoids and omega-3 fatty acids enhances cognitive capacity in both animal models and humans and delays the onset of age-related neurological diseases (Seigler et al., 2021). We propose that dietary phytochemicals like flavonoids may mimic the physiological effects of omega-3-derived pro-resolving and anti-inflammatory molecules. Therefore, they might be involved in the process of the resolution of neuroinflammation. Additionally, we propose that the anti-inflammatory and neuroprotective activity of these dietary molecules is directly related to their soft electrophilic properties. Through a variety of analytical approaches, we have identified these compounds *in vivo* in the context of a living organism (*Drosophila melanogaster*), focusing on their anti-inflammatory properties (Maitra et al., 2021). Our preliminary results suggest that dietary soft electrophiles, such as gardenin A, thymoquinone, and vitamin E vitamers, enhance the production of lipid pro-resolving molecules in a paraquat-induced *Drosophila* model of PD. We have recently shown that oral administration of a polymethoxyflavonoid, gardenin A, modifies the lipid profile in a mammalian model of PD (Hack et al., 2023). My future studies will include an insight into the mechanisms by which these phytochemicals might bring about pro-resolution effects in this *Drosophila* PD model by inhibiting cyclooxygenase (COX) activity. Thus, lipophilic soft electrophiles may have potential therapeutic applications in addressing neuroinflammation-related conditions.

Keywords: neurodegenerative, inflammation, cognitive, phytochemicals, pro-resolution

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S3.P226 Comprehensive analysis of commercial kratom products: alkaloids, polyphenols, heavy metals and microbial contaminations

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Kratom (*Mitragyna speciosa*) leaves have been traditionally used in Asia and Indochina for pain relief, fatigue reduction, or mood lifting. These effects are primarily attributed to its indole and oxindole bioactive alkaloids, among which mitragynine is the major one, accompanied by over 40 others, which interact with opioid, serotonin, and dopamine receptors (Brown et al., 2017). Kratom has recently become popular in Western society as well. Here, however, it is often sold in the form of finely ground leaves or concentrated extracts and drinks (Swogger et al., 2022). In many countries, these products are outside legislative regulations, and their production is therefore not subject to any quality and safety requirements (Prozialeck et al., 2019).

The aim of this study was to develop analytical methods for quantification of five kratom alkaloids (mitragynine, 7-hydroxymitragynine, speciogynine, speciociliatine, and paynanthein) and 33 polyphenols in kratom products, utilizing a Vanquish UHPLC system and Orbitrap Exploris 120 mass spectrometer. We successfully developed analytical methods for the quantification of alkaloids and polyphenols in kratom samples. We employed these methods to analyze a number of commercial samples sourced from the Czech market, subsequently conducting assessments for heavy metal content and microbial contamination.

Our comprehensive analysis and critical evaluation of the results significantly enhances the understanding of the diverse pharmacological effects exhibited by various types of kratom. Particularly, our investigation contributes substantially to the assessment of safety parameters associated with the commercial products available for consumption.

Keywords: Kratom alkaloids, *Mitragyna speciosa,* HPLC-MS, mitragynine, polyphenols

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S3.P227 Diarylheptanoids from *Corylus avellana* cultivar "Tonda di Giffoni": antioxidant effects through the activation of the Nrf2 pathway as assessed via molecular docking and biological evaluation

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Hazelnut (*Corylus avellana* L., Betulaceae) represents one of the most consumed foods, with a high content of healthy lipids. Food and Drug Administration (FDA) has recognized hazelnut as a "heart- healthy" food due to its nutritional and nutraceutical properties related to a large range of bioactive and health-promoting compounds (Bottone, 2019).

Kernels and byproducts of the Protected Geographical Indication (PGI) hazelnut cultivar "Tonda di Giffoni" contain diarylthereptanoids derivatives, natural compounds able to prevent oxidative damage of human plasma lipids (Bottone, 2019).

Based on the effects of diarylheptanoids on oxidative stress, the antioxidant effects of giffonins E (**1**), H (**2**), J (**3**), and carpinontriol B (**4**), isolated from "green" extract (EtOH: H_2O, 50:50) of green leafy covers and kernels, were assessed using molecular docking experiments and *in vitro* tests. The isolated compounds were tested for their ability to disrupt the interaction between Kelch-like ECH- associated protein 1 (Keap1) and nuclear erythroid 2-related factor 2 (Nrf2), triggering the activation of the Nrf2-mediated pathway and consequently promoting the transcription of antioxidant enzymes (Bello, 2017). The results suggested an antioxidant effect of giffonin H and carpinontriol B. Moreover, compounds **1**-**4** were tested for the antioxidant and cytoprotective properties in human neuroblastoma SH-SY5Y and human embryonic kidney cell lines HEK/tau. showing a significant protection against H_2O_2-induced cytotoxic damage in both cell models. Finally, tested compounds were able to reduce cellular oxidative stress by decreasing ROS production and levels of lipid peroxidation, increasing cell viability, and attenuating cell apoptosis (caspase-3 activity).

Keywords: diarylheptanoids, *C. avellana* "Nocciola di Giffoni" PGI, green leafy covers, molecular docking, antioxidant activity

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S3.P228 Valorization of Almond Okara as a valuable source of polyphenols and proteins

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Almond milk is one of the most popular plant-based beverages. However, its production generates a solid residue byproduct called "okara", which is underutilized despite its potential bioactive constituents. Our objective is to deepen our knowledge of almond okara, composition with a focus on the levels of polyphenols, fibers, proteins, lipids, and vitamin E.

Solid-liquid extractions were conducted, varying several parameters including extraction time, solvent concentration, solid/solvent ratio, and extraction temperature. The results showed that the highest extraction yield was obtained after 90 minutes of extraction, which could be explained by considering that the extended exposure allowed the solvent to infiltrate into the matrix, thereby facilitating the solubility process.

Moreover, the results highlighted that the polyphenol yield in almond okara was positively associated with solvent concentration and temperature (Fig.1). Specifically, we found that using 50% ethanol at 60°C resulted in the highest levels of polyphenols, tannins, flavonoids, and antioxidant activity. This can be explained by the fact that hydro-ethanolic solvent increases the permeability of plant tissues enabling better mass transfer by diffusion. Moreover, high temperatures decrease surface tension and solvent viscosity and in return enhance the degree of diffusion and solubility of analytes.

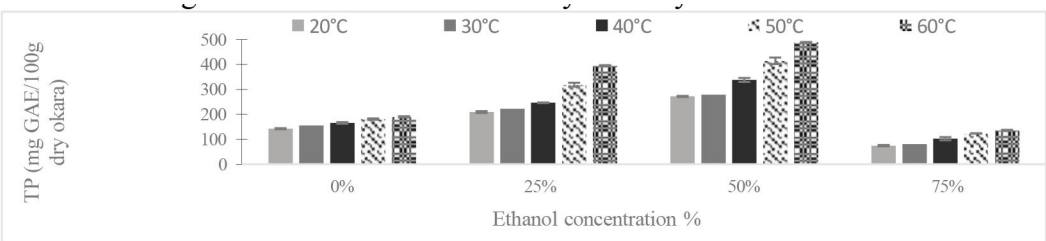

Fig.1: Total polyphenol (TP) content, in mg gallic acid equivalent/ 100 g dry okara, obtained in different ethanol concentrations and extraction temperatures.

In conclusion, our results demonstrated that through its composition, almond okara has health- promoting potential. This makes it a very promising byproduct to facilitate the formulation of innovative industrial approaches, including cosmetics and nutraceuticals.

Keywords: *Almond okara,* solid-liquid extraction, phenolic compounds, antioxidant activity

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S3.P229 Application of annular centrifugal extraction (ACE) in honey - isolation and identification of honey bioactive compounds

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Honey's intricate chemical composition and viscous nature pose challenges in handling. In recent literature, different extraction techniques have been extensively discussed (Hanim Hassan et al., 2022). Nevertheless, these conventional methods present many limitations, i.e., low yields, so there is a need for an efficient, repeatable, and reproducible extraction method. Thus, the primary aim of this study was the development of an extraction protocol using annular centrifugal extraction (ACE) that would allow the recovery of an enriched in non-sugar compounds extract while simultaneously eliminating the sugar content of honey. This approach involves single-stage extractors based on the continuous counter-current liquid-liquid extraction principle with applications mostly in the industrial field (Hamamah et al., 2022). As for food commodities, ACE has only been applied by our group in the recovery of biophenols and lignans from olive and sesame oil, respectively, and is its first application in honey (Michailidis et al., 2019). Following extraction and characterization of the obtained extracts with liquid chromatography - high-resolution tandem mass spectrometry (LC- HRMS/MS), the second aim of this study was the isolation and identification of possible bioactive compounds. After fractionation with fast centrifugal partition chromatography (FCPC) and purification with other chromatographic techniques, more than 20 compounds were isolated and identified from Greek and French honeys. Among these compounds belonging to simple phenols, megastigmanes, and fatty acids, 1-(4-methoxyphenyl)-ethane-1,2-diol, indicated in our previous study as a biomarker for heather honey, was isolated and identified for the first time in honey.

Keywords: Honey, extraction, ACE, isolation, identification

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S3.P230 Ornamental varieties of *Achillea millefolium* L. as a source of skin-lightening ingredients for natural cosmetics

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Achillea millefolium L. is one of the best known medicinal plants with long history of usage in both traditional and modern medicine. (Strzępek-Gomułka et al., 2021a) Achillea Millefolium Oil, Extract, Flower Extract, Flower Water, Flower/Leaf/Stem Extract and Flower/Leaf/Stem Juice are also listed in the Cosmetic Ingredients Database CosIng as anti-seborrheic, cleansing, refreshing, skin conditioning, shooting, antioxidant and fragrance ingredients of cosmetics. (CosIng) In addition to the common *A. millefolium* with white flowers, there are many ornamental varieties with colorful flowers. In this study the phytochemical content, antioxidant and tyrosinase-inhibitory properties of *A. millefolium* hydroethanolic extracts obtained from the flowers of ornamental cultivars Skysail White (W), Yellow (Y), Bright Pink (P) and Fire (W) were compared. The content of phytochemicals between varieties was significantly different – W extract showed the highest polyphenolic and flavonoid levels (160.16 and 62.12 µg/mL, respectively), whereas Y cultivar contained the least polyphenols and flavonoids (86.77 and 35.05 µg/mL, respectively). The W and Y extracts showed also the highest and lowest antioxidant activity, respectively. Interestingly, Y extract was the most potent inhibitor of tyrosinase (41% inhibition at 50 µg/mL). The observed activity may result from the specific content of phytocompounds previously described as tyrosinase inhibitors. (Strzępek- Gomułka et al., 2021b) The extracts from *A. millefolium* varieties were also analysed using EpiDerm Phototoxicity Test according to OECD TG 498 guidelines. None of the tested extracts were phototoxic following UVA exposure. The Y and P extracts slightly increased the viability of UVA- treated EpiDerm tissues, suggesting their photoprotective potential.

Keywords: *Achillea millefolium,* cosmetic, tyrosinase, LCMS, phototoxicity

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S3.P231 Impact of the drying process and incorporation of natural preservatives on the viability of lactic acid bacteria in yogurts

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Dairy products are among the most widely consumed foods globally, with yogurts particularly demanded for their flavour and nutritional benefits, including essential macronutrients, vitamins, minerals, and probiotics. To mitigate food waste, methods to prolong shelf life are emphasized, such as the incorporation of food preservatives or the creation of powdered foods (Ueda *et al.*, 2023). Therefore, the aim of this study was to assess the survival of total lactic acid bacteria in yogurts after the incorporation of natural extracts (rosemary, basil, and sage), as well as to evaluate their viability after the freeze-drying process. Yogurts were prepared in the laboratory, with lyophilized plant extracts (30 mg/kg) added. Total lactic acid bacteria were monitored for 14 days, alongside the assessment of lactic acid and lactose level using HPLC-DAD and HPLC-RI, respectively. Subsequently, the viability of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was evaluated in freeze-dried yogurts without the addition of the vegetable extracts (Kok and Hutkins, 2018; Terzaghi and Sandine, 1975). The results indicated a slight increase in lactic acid bacteria counts, as well as an increase in lactic acid and a reduction in the lactose content over the 14-day period, confirming the yogurt fermentation process. During the lyophilization, a slight decrease in the viability of both bacteria was observed, but it remained within the Codex Standards limits (minimum of 10^7 CFU/g). Therefore, the incorporation of plant extracts and the freeze-drying process did not significantly change the viability of lactic acid bacteria in yogurts, remaining a probiotic food.

Keywords: yogurts, lactic acid bacteria, powdered foods, freeze-drying, natural products

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S3.P232 NMR metabolomics and chemometrics of *Malus domestica* (Suckow) Borkh. varieties from Molise region of Italy

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Natural products identified in plants are an important resource for both food and drug discovery field. This is because they offer not only primary metabolites that satisfy our food requirement but also a large library of secondary metabolites which can show different bioactivities and can find a use in different areas such as nutraceuticals, cosmeceuticals and for the drug discovery.

This project is based on the valorization of the Molise region of Italy, exploiting several autochthonous varieties belonging to *Malus domestica* (Suckow) Borkh. (Fig. 1): the metabolic profile, with primary and secondary metabolites, can vary in the same species qualitatively and quantitatively if it grows in different environmental conditions, including the type of soil (Sampaio et al., 2016). Apples are rich of nutritional properties and represent an important source of polyphenols which are responsible for their antioxidant and anti-inflammatory properties and possess a role in the prevention of degenerative diseases (Geană et al., 2021).

The phytoconstituents of the varieties of *Malus domestica* from Molise region of Italy will be identified through 1D and 2D-NMR analysis of the extracts obtained by a specific extraction protocol. The qualitative analysis will be followed by a quantitative approach of the most important metabolites identified and by a chemometric analysis with the PCA and PLS-DA techniques in order to observe differences among the varieties. Moreover, the total phenolic, flavonoids, and condensed tannins content will be assessed, and in vitro antioxidant activity will be evaluated by using DPPH scavenging activity, the ABTS scavenging assay, and ferric-reducing antioxidant power (FRAP).

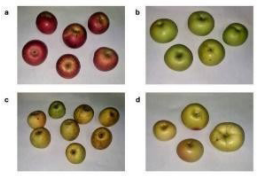

Fig. 1. Selected *Malus domestica* varieties of Molise region in Italy (**a**: Annurca; **b**: Gelata; **c**: Limoncella; **d**: Zitella)

Keywords: *Malus domestica,* NMR, metabolomics, chemometrics, metabolic profile

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S3.P233 IExploring *Genista tridentata* leaves extract: novel insights for functional food in diabetes management

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Genista tridentata (L.) Willk., a Leguminosae (Fabaceae) species native to the Iberian Peninsula, Morocco, Algeria, and Tunisia, boasts versatility in both culinary and traditional medicine (Laranjeira et al., 2023). Recognized for its properties, this plant is used in folk medicine and by populations for the management of several pathologies like, diabetes, hypertension, respiratory and digestive disorders (Coelho et al., 2011; Balanč et al., 2016). This study aimed to evaluate the in vivo antinociceptive effects of *G. tridentata* leaves (GtL) methanolic extracts in rats with experimental Type 2 Diabetes mellitus (T2DM). Using the Streptozotocin-Nicotinamide model (Masiello et al., 1998), male Wistar rats were induced with T2DM, divided into Sham (n = 14) and Diab (n = 38) groups. Diabetic rats were further divided into three experimental groups: (i) Diab treated with PBS (n = 14), (ii) GtL_100 treated with 100 mg/kg (n = 11), and (iii) GtL_300 treated with 300 mg/kg of GtL extract (n = 13), administered daily via gavage, for four weeks. Sham animals received PBS (n = 14). Weekly nociceptive assessments included mechanical and thermal hyperalgesia and allodynia. Post-euthanasia, internal organs underwent histopathological examination. T2DM induced thermal allodynia and thermal and mechanical hyperalgesia, which was decreased after *G. tridentata* treatments. Concomitantly, *G. tridentata* treatment ameliorated the histopathological changes in T2DM animals' internal organs. These findings suggest *G. tridentata* leaves may offer beneficial effects in addressing T2DM-related complications, potentially due to its antioxidant and anti- inflammatory properties, supporting its potential in developing nutraceutical products.

Keywords: *Genista tridentata, Pterospartum tridentatum,* Type 2 Diabetes, Medicinal Plants, nociception, Functional foods

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S3.P234 A formulation containing palmitoylethanolamide and phenolic compounds counteracts diabesity and related hepatic damage in mice: in vivo and in vitro evidence

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Gluco- and lipotoxicity play a key role in the development of diabesity, namely diabetes-related-to- obesity (Michaelidou et al., 2023). The N-acylethanolamine palmitoylethanolamide (PEA) and some phenolic compounds, such as rutin and hydroxytyrosol (HT), have shown different metabolic, anti- inflammatory and antioxidant effects (Annunziata et al., 2020, Pirozzi et al., 2016, Muvhulawa et al., 2022). Here, we evaluated the beneficial activity of a formulation containing PEA co-micronized with rutin and associated with HT, named NORM3, in counteracting insulin resistance (IR) and hepatic metabolic alterations in high-fat diet (HFD)-fed mice.

Male C57Bl/6J mice received standard chow diet or HFD for 19 weeks; a HFD group administered NORM3 (PEA 10 mg/kg/die-Rutin 2 mg/kg/die, HT 0,5 mg/kg/die *per os*) from week 12 up to week 19.

First, NORM3 limited body weight of obese mice, after 4 weeks of treatment, and fat mass. HFD+NORM3 animals showed an improved insulin sensitivity, as shown by oral glucose and insulin tolerance tests and the reduced HOMA-IR index. In liver, morphological analysis showed that NORM3 reduced macro- and microvacuolar steatosis caused by HFD. Contextually, NORM3 treatment restored hepatic insulin signaling, activating AKT/PI3K pathway, and reduced the mRNAs of crucial enzymes of gluconeogenesis. Moreover, NORM3 counteracted hepatic lipid dysmetabolism caused by HFD, and associated inflammation and oxidative stress. In HepG2 cells, we proved the synergistic effect of the single components of NORM3 in reducing ROS production and inflammation and increasing antioxidant defense.

Our findings identify NORM3 as a potential nutritional approach in counteracting IR and related hepatic dysmetabolism due to diabesity.

Keywords: N-acylethanolamines, polyphenols, obesity, diabetes, glucose metabolism, oxidative stress, MAFLD

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S3.P235 The *Vitis vinifera* cvs. callus *in vitro* culture extracts as a potential cosmetic antioxidant

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The innovative active ingredients of natural origin, especially the new antioxidants, are constantly in demand in the production of cosmetics (Oresajo et al., 2012).

Grapevine (*Vitis vinifera* L.) is one of the major fruit crop over the world. Several studies on *Vitis vinifera* extracts showed their strong antioxidant potential for which phenolic compounds are most likely responsible (Sharafan et al., 2023).

The aim of the study was to evaluate the antioxidant potential of *in vitro* callus extracts obtained from different grapevine cultivars (3 red cultivars: Marechal Foch, Regent, Rondo and 3 white cultivars: Chardonnay, Hibernal, Seyval Blanc). The cultures were maintained over 30-day growth cycles on two media: Murashige and Skoog (MS) and Schenk and Hildebrandt (SH) with various concentration of plant growth regulators: 6-benzylaminopurine (BA) and indole-3-butyric acid (IBA) or 1- naphthaleneacetic acid (NAA). The extracts were prepared using ultrasound mediated extraction, in 96% ethanol. The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and FRAP (ferric reducing antioxidant power) assays were used to determine the antioxidant potential of extracts.

The results of ABTS assays showed that all the extracts possessed relatively high antioxidant activity. The ABTS inhibition reached 51.38%. In the case of the FRAP assay, the ferric ion reducing activity reached up to 10.4%. The best results in ABTS and FRAP assays were obtained for cv. Hibernal maintained on medium 'W2' (MS, 1.5 ml/l BA and 0.2 ml/l NAA).

The obtained results indicate that *V. vinifera* cv. callus *in vitro* cultures could be proposed as valuable alternative of natural cosmetic ingredient that is custom-produced in a controlled environment.

Keywords: *Vitis vinifera,* vine grape*, in vitro* cultures, plant callus culture, antioxidant capacity

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S3.P236 Phytochemical analysis of *Echinacea* preparations from the pharmacies and herbal markets in Türkiye

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Echinacea species are Asteraceae family plants containing mainly chlorogenic acid, cichoric acid, caffeic acid, echinacoside. Aerial parts, roots, and especially flowering tops of *Echinacea* sp. were used for treatment of recurrent respiratory tract infections and wounds, ethnobotanically. This study aims to evaluate the phytochemical quality (according to European Pharmacopoeia) of 5 different *Echinacea* containing commercial products (ECCP) available on pharmacies/herbal markets in Türkiye. Detailed information about ECCP planned to be analyzed is given in Table 1.

Table 1. Detailed information about 5 different *Echinacea* preparations used in this study

Echinacea preparations	Dosage form	Ingredients/Dosage (if available)
Sample-1	Capsule	265 mg *E. purpurea* (aerial parts) powder and 65 mg *Echinacea* root extract
Sample-2	Capsule	100 mg *E. purpurea,* zinc, vitamin C, β-glucan, *Rosa canina L.* / 2 capsules (daily reccomended dosage)
Sample-3	Capsule	150 mg *E. purpurea,* β-glukan, vitamin C, zinc
Sample-4	Liquid	300 mg *Echinacea* extract, β-glukan, propolis, vitamin C, zinc
Sample-5	Powder	*Echinacea spp.* (roots) powder

Sample 1, 2, 3 and 4 was directly macerated with ethanol for 3 days. Sample-5 was purchased as dried root parts of the plant. Then, it was grinded and macerated with ethanol for 3 days. After maceration, extracts were filtered through Whatman No:1 filter paper and concentrated by using rotary-evaporator.

Standard compounds (cichoric acid, chlorogenic acid, caffeic acid, and echinacoside) and ECCP extracts were dissolved in ethanol (10 mg/mL) and filtered via 0.22µm membrane filters prior to HPLC analysis. An Agilent 1100 HPLC-DAD system was used to conduct HPLC studies. C18 column (100 x 4.6mm, 10µm) and A: water:formic acid (100:0.1, v/v), B: acetonitrile were used as stationary and mobile phases, respectively. The gradient elution in HPLC was applied as 10-78% B (0–18 min), 90-10% B (18-21 min). Flow rate was 1.2 mL/min and injection volume was 5 µL with a temperature of 26°C. The detection was performed at 330 nm. The reference standards were prepared and analyzed at the concentrations of 10, 20, 30, 40 and 50 µg/mL. The quantitative analysis of ECCP extracts was calculated by using the calibration curve of reference standards. Results of the quantitative analysis of ECCP extracts are given in Table 2. Chlorogenic acid was the only standard detected in Sample-1 as 0.1% (w/w). Sample-2 and Sample-4 were lack of any standard compounds. Cichoric acid, chlorogenic acid and caffeic acid were detected in Sample-3 at the concentration of 0.4%, 0.6% and 0.2% (w/w), respectively. Sample-5 revealed that it contains cichoric acid, chlorogenic acid, echinacoside and caffeic acid at the concentration of 0.1%, 0.6%, 0.2% and 0.3% (w/w), respectively. All results were evaluated according to European Pharmacopoeia. To the best of our knowledge, in this study, pharmacy and herbal market's *Echinacea* supplements were tested together for their phytochemical content for the first time.

Table 2. Phytochemical contents of the samples evaluated by HPLC method (w/w)

	Cichoric acid	Chlorogenic acid	Echinacoside	Caffeic acid
Sample-1	Not detected	Not detected	Not detected	0.1%
Sample-2	Not detected	Not detected	Not detected	Not detected
Sample-3	0.4%	0.6%	Not detected	0.2%
Sample-4	Not detected	Not detected	Not detected	Not detected
Sample-5	0.1%	0.6%	0.2%	0.3%

Keywords: *Echinacea*, herbal supplement, HPLC, pharmacy

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S3.P237 Untargeted metabolomics analyses to identify a new sweet compound released during post- fermentation maceration of wine

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The sensory quality of a wine is mainly based on its aroma and its flavor. In particular, gustatory balance of red wines relies on sourness, bitterness and sweetness. Even if certain compounds were already identified as contributing to sweetness, certain taste modifications remain largely unexplained (Cretin et al., 2019, Fayad et al., 2021). Some empirical observations combined with sensory analyses have shown that an increase of wine sweetness occurs during post-fermentation maceration. This step is a key stage of red winemaking during which the juice is left in contact with the marc, that contains the solid parts of the grape (seeds, skins and sometimes stems). The present work aimed to identify a new taste-active compound that contributes to this gain of sweetness.

Recent developments have highlighted the interested of untargeted metabolomic analysis for natural product research (Hubert et al., 2017, Wolfender et al., 2019). Using similar tools, an original approach using UHPLC-HRMS/MS has been developed to discover new sweet molecules released during post-fermentation maceration. Among different markers highlighted, one compound was selected and considered for a targeted purification by various separative techniques (SPE, CPC and HPLC-preparative). It was unambiguously identified by NMR as N6-succinyladenosine and was reported for the first time in wine. It was quantitated at an average concentration of 3.16 mg/L in 85 red wines. Furthermore, sensory analysis revealed its pronounced sweetness. In addition to discovering a new sweet compound in wine, this study offers new tools for studying taste-active compounds in natural matrices.

Keywords: Untargeted metabolomics analysis, UHPLC-HRMS/MS, wine, sweetness, taste

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S3.P238 Do you know what's really hiding in popular herbal supplements? – evaluation of products containing *Cistus* leaves and extracts

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Cistus plants, also known as rockroses, are Mediterranean-native shrubs belonging to the Cistaceae family. Their flowers, leaves, and aerial parts have been used in traditional medicine to treat many conditions, including the common cold, gastric ulcers, wounds, and many inflammatory diseases (Barrajón-Catalán et al., 2016). Nowadays, products containing those species are becoming increasingly popular due to their somehow proven efficacy in reducing the average duration and severity of upper respiratory tract infections (Kalus et al., 2009; Guzelmeric et al., 2023). Manufacturers supply and market a wide range of *Cistus* dietary supplements, including infusion teas, capsules, and tablets. In order to evaluate their quality, we tested sixteen of those products that are available on the Polish market, using HPTLC methods to compare their fingerprint profiles to authentic standards. Additionally, we used HPLC-DAD-MS/MS method to in deep analyze their qualitative profile. Five out of sixteen supplements were found to be adulterated. The majority of the counterfeit preparations were in the form of tablet and capsules. Instead of Cistus extracts, products probably contained buckwheat, green tea, or oak extracts. In the other products, we identified typical *Cistus* phytochemicals, including characteristic myricetin derivatives, tiliroside, punicalagins, and dimeric prodelphinidin. The investigation showed that, despite what the producers claim, several items on the market do not contain *Cistus*. This study also pointed out the significance of controlling the raw herbal material used in the manufactures.

Keywords: *Cistus*, Cistaceae, supplements, HPTLC, LC-MS

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S3.P239 A smartphone-based methodology for analyte quantification and antioxidant activity determination directly in emulsions

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This work investigated the width of applicability of a methodology for the quantification of analytes directly into emulsions with oil internal phase and aqueous external phase (O/W). The methodology relied on a specific reaction (enzyme-catalysed, or not) between the analyte of interest and a suitable chromogen towards a coloured product. Colour intensity was captured by a mobile phone camera and analysed by the ImageJ software (Schneider et al., 2012) into its R, G and B components (Fig. 1). A linear relationship was observed between at least one of the R, G or B components or their average, and analyte concentration in O/W emulsions for the investigated analytes (glucose (Tsotsou and Tsara, 2023), urea (Tsotsou, 2024), and total iron). Satisfactory coefficients of correlation (R^2) (≥0.984), and relative error values (≤ ±15.9 %), as well as wide linear dynamic ranges were determined. Interferences were also reported.

Fig. 1. Setup for the proposed extraction-free quantification methodology

The robustness of the methodology was demonstrated in terms of camera specifications, emulsion properties (pH, viscosity, conductivity, transparency), ambient temperature and light conditions. Preliminary results suggested its applicability to antioxidant capacity measurement by the FRAP assay, directly into an O/W emulsion containing Unisooth EG-28® (R^2 of calibration curve: 0.989). Unisooth EG-28® named according to INCI system as water, propyl gallate, gallyl glucoside, epigallocatechin gallatyl glucoside, is an antioxidant cosmetic active ingredient complex that is composed of gallic acid glucoside, of epigallocatechin gallate glucoside, and propyl gallate. This methodology is proposed as an extraction-free, green procedure, suitable even for minimally equipped settings, to serve quality control needs of emulsions. It may also prove useful for quantifying antioxidant capacity of natural extracts into emulsified matrices.

Keywords: emulsion, smartphone-based analysis, digital image colourimetry, urea/glucose/phenolics/total iron analysis, antioxidant, cosmetic ingredients

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S3.P240 Phytochemical analysis of *Laurus nobilis* L. leaves essential oil: rationalization of antioxidant and anti-inflammatory activities by combined *in vitro* and *in silico* approach

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This research focuses on natural product chemistry, explicitly targeting the structural characterization of secondary metabolites derived from plant matrices. These secondary metabolites have been assessed as prospective "new drugs" due to their intriguing pharmacological properties demonstrated in both *in vitro* and *in vivo* studies (Newman and Cragg, 2020). These properties include anti- inflammatory, antioxidant, antitumor, antiviral, and antibacterial activities.

The evergreen Mediterranean shrub *Laurus nobilis* L., commonly known as bay laurel, has a long history of traditional use in cuisines and folk medicine due to its health-promoting properties. These properties are now being scientifically elucidated through various biological activities.

Samples of *L. nobilis* were collected from three different regions in central-southern Italy. The leaves were subjected to hydro-distillation, and essential oils (EOs) were analyzed using gas chromatography (GC/FID) for quantification of volatile compounds and gas chromatography/mass spectroscopy (GC/MS) for identification. The multivariate analysis of the main components establishes the correlations of the different chemical compounds with the corresponding antioxidant and anti-inflammatory activities.

In vitro, antioxidant activity was then evaluated by different methods (DPPH, ABTS, FRAP assay) and anti-inflammatory activity through inhibition of selected enzymes involved in eicosanoid biosynthesis through the three metabolic routes: cyclooxygenase (COX), 5-lipoxygenase (5-LO), cytochrome P450 (CYP450) pathways (Sun et al., 2021). In more detail, here, the simultaneous targeting of different, i.e., COXs and Soluble Epoxide Hydrolase (sEH) of the EOs was evaluated. Furthermore, the *in silico* studies were applied to rationalize the biological activities in inflammatory events observed for the main secondary metabolites of the different varieties of *L. nobilis* EOs at the molecular level.

Keywords: *Laurus nobilis* L., phytochemical analysis, essential oil, metabolomics, natural product

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S3.P241 A study on the biological activities of loquat leaf extract using various extraction methods

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Loquat (*Eriobotrya japonica*) is a member of the Rosaceae family and the Pomoideas subfamily, cultivated in East Asia. The aim of this study was to examine the physicochemical properties of extracts obtained through different extraction methods of Eriobotrya japonica leaf extract. We used 70% ethanol extraction (EE), infrared-assisted extraction (IAE), and ultrasound-assisted extraction (UAE). To determine the antioxidant and anti-aging effects of Eriobotrya japonica leaf extract, we investigated various biological activities induced by different extracts. Extraction using IAE resulted in the highest chlorogenic acid content compared to other methods, as analyzed by high-performance liquid chromatography. It was observed that the IAE-associated extract exhibited higher antioxidant activities than the others, as determined by DPPH and ABTS assays. Significant inhibitory effects on elastase and collagenase activities were found using the IAE method, which might be attributed to its anti-wrinkle effects. These results suggest that selecting the extraction method is crucial in obtaining loquat leaf extract with specific bioactive properties, which can be useful in functional materials.

Keywords: loquat, *Eriobotrya japonica*, antioxidant, anti-wrinkle, cosmetic

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S3.P242 Study on modulation of inflammation expression by *Brassica oleracea* L. leaves extract

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Brassica oleracea L., commonly known as cabbage, belongs to the plant family Brassicaceae. It holds significant nutritional and medicinal importance due to its rich biochemical and phytochemical composition. In this study, the antioxidant and anti-inflammatory activities were investigated to confirm the potential of *Brassica oleracea* L leaves extract. as a natural cosmetic material. The total polyphenol content of hot water extract (KRD) and 70% ethanol extract (KRE) was measured, resulting in 124 mg TAE/100 g and 144 mg TAE/100 g, respectively. To affect the antioxidant activity of various extracts, we confirmed their ability to donate electron and scavenge ABTS radical. It was observed that the activity of KRD and KRE increased in a dose-dependent manner. The inhibitory effects on inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were assessed through western blot and reverse transcription-PCR. Treatment with KRD and KRE confirmed the concentration-dependent suppression of iNOS and COX-2 protein and mRNA expression. These findings suggest that kailan could serve as a promising natural cosmetic material.

Keywords: *Brassica oleracea,* antioxidant, anti-inflammation, nitric oxide, cosmetic

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S3.P243 Glucosinolates in *Brassica incana* subsp. *raimondoi* (Brassicaceae) leaf extract: composition, content, and anti-inflammatory activity

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Brassica incana subsp. *raimondoi* (Brassicaceae) is a taxon endemic to Sicily (Italy), restricted to the specific geographic area of Castelmola (Messina). It grows at an altitude of about 500 m a.s.l. on abrupt limestone cliffs overlooking the Ionian Sea and is typified by various distinctive traits. *B. raimondoi* belongs to the *B. oleracea* cytodeme, a group of species characterized by the same diploid genome (2n=18) and which attracts great attention for the genetic improvement of the horticultural varieties of *B. oleracea* in terms of resistance to biotic and abiotic stresses and phytochemical compounds such as the glucosinolates (Malfa et al., 2020). Initially described at a specific level, it was not long ago assigned to the rank of subspecies (Malfa et al., 2020; 2022). As part of our ongoing research on Sicilian Brassicaceae taxa, in this study the methanolic leaf extract of *B. raimondoi,* analyzed by UPLC-MS/MS, evidenced the presence of one aromatic, four indolic, and eleven aliphatic glucosinolates. Among these, gluconapin, glucoraphanin, glucoraphasatin, and sinigrin represented more than 50% of the total glucosinolate content.

Fig. 1. *Brassica incana* subsp. *raimondoi*

Since we found that the phytochemicals in the methanolic extract had no cytotoxic effects on HDF cells, we tested the extract's anti-inflammatory effects on this cell line by inducing inflammation with IL-1β exposure. Quantitative analysis of ROS and nitrite/nitrate levels, which increase as hallmarks of inflammation in cells, showed that the extract can counteract the harmful effects of the pro- inflammatory cytokine in a dose-dependent manner starting from a concentration of 50 μg/mL.

Keywords: *Brassica*, glucosinolates, chemotaxonomy, oxidative stress, inflammation

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S3.P244 *Ex vivo* and clinical evaluation of efficacy and mode of action of an *Anogeissus leiocarpus* and retinol-containing eye cream

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The Estee Lauder Companies

In an ex vivo study, the phytochemistry and bioactivity of *Anogeissus leicarpus* bark, alone and when combined with retinol were investigated. Additionally, the clinical impact of a novel anti-aging eye cream with these ingredients was assessed. High-Performance Thin Layer Chromatography (HPTLC) Phytochemical scouting analysis of an ethanolic extract of *A. leiocarpus* bark revealed the presence of 10-12 unidentified flavonoids, several unidentified saponins and two compounds tentatively identified as Caffeic and Ellagic acid. Previously excised human skin (ex vivo) showed a marked increase in extracellular matrix components (Collagen, Elastin and Fibrillin) when individually treated with *A. leiocarpus* extract and Retinol. When the two ingredients were combined in a treatment, extracellular matrix components showed a significant improvement when compared to the effect of either ingredient alone. Separately, the effects of a novel anti-aging eye cream containing proprietary levels of retinol and *A. leiocarpus* were assessed via clinical trial involving 29 female subjects (aged 35 to 64, average age 57) with mild-to-moderate age-related eye conditions. After 12 weeks of usage, the cream significantly reduced wrinkles, fine lines, puffiness, dark circles, as assessed by expert grading. The study underscores the effectiveness of the cream in addressing age- related eye concerns, which can be linked to the action of its key ingredients.

Keywords: *Anogeissus leiocarpus*, phytochemical scouting analysis, retinol, clinical test, anti-aging

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S3.P245 Targeted Isolation of natural products that modify sweet taste perception through metabolomics, molecular networking and sensory evaluation of *Symplocos* spp. (Symplocaceae)

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As health consciousness rises, sugar reduction becomes a trend. Taste is paramount and achieving the right balance between health and taste is a challenge. Modifying the taste profile of high intensity sweeteners to mimic that of sugar is one solution and taste modulators have been identified from natural sources. Leaves of *Symplocos* spp. have sweet taste and were partly attributed to the presence of dihydrochalcone glucosides (Tanaka et al., 1980; Kinghorn et al. 2010). These dihydrochalcones were approved by Flavor & Extracts Manufacturers Association (FEMA) for use as flavoring substances (Cohen et al., 2022; Smith et al., 2011).

In our research, we found that *Symplocos* spp. contain additional compounds reported to have sweetness perception modifying function. Six extracts from *Symplocos* species were evaluated by a sensory panel, and one species showed more sweetness intensity. To identify molecules responsible for the variation of sensory quality, we performed molecular network and metabolomics analysis. With molecular network analysis, we investigated three classes of compounds associated with sweet modulating properties: dihydrochalcones, megastigmanes and flavonoids. Through differential analysis we identified a fourth class of compounds present at higher levels in the extract with a higher rating of sweetness. These compounds were selected for isolation to verify their taste function.

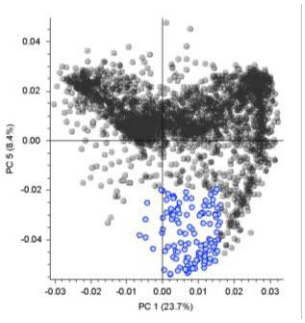

Fig. 1. PCA loadings plot of LCMS ions in negative mode. Dots in blue represent compounds unique to our target species.

Keywords: *Symplocos,* Symplocaceae, sweet, dihydrochalcones, megastigmanes

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S3.P247 Investigation of the Greek microbial diversity for the discovery of novel antiaging products

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Greek ecosystems present a rich yet under-explored area for investigating Actinobacteria, well- known for their production of bioactive compounds. To this purpose, the "AntiAging" project sought to uncover novel, promising natural compounds with anti-aging properties, which could be incorporated into cosmeceutical/ nutraceutical formulations. In total, 1000 isolates originating from the Greek natural environment and belonging to the ATHUBA collection (Athens University Bacterial & Archaea Culture Collection)—some originating from unique environments (caverns, volcanoes, thermal springs, etc.)—were investigated. A unique library of ~2000 extracts (EtOAc and MeOH/H_2O) was generated, and the tyrosinase and elastase inhibition activity was evaluated via enzymatic assays. The top 3 microbial strains, belonging to *Amycolatopsis sp.* and *Streptomyces sp.* families, combining a high production of secondary metabolites and bioactivity on both cell-free and cell-based assays, were grown using four different cultivation media and subjected to enzymatic assays. LCMS based metabolomics was then applied to detect significant differences among strains and fermentation conditions. One selected strain was then subjected to a scale up process (12L) and its EtOAc extract was fractionated. A customized bio-guided workflow was then applied, combining a high-throughput dereplication and molecular networking method (UPLC-HRMS). Purification of the targeted components led to the isolation of several compounds and structure elucidation was achieved using 1D and 2D NMR combined to HRESIMS. The latter results and the plethora of identified molecules show a hidden yet powerful potential of harnessing Greek microbial wealth in the context of the science of anti-aging.

Keywords: *Actinobacteria*, extract library, LCMS, metabolomics, NMR

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S3.P248 EthnoHERBS: Conservation of european biodiversity through exploitation of traditional herbal knowledge for the development of innovative products

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EthnoHERBS project aims to record and evaluate information on Southeast European traditional knowledge, explore in a high-throughput manner the biodiversity of Balkan Peninsula flora and elaborate cutting-edge technologies in Natural Products chemistry to discover and develop innovative cosmeceutical products against skin disorders. Based on ethnobotanical/ethnopharmacological studies and ancient-medieval manuscripts, 1289 plant species have been identified for their traditional use against skin ailments (Tsioutsiou et al., 2022). The evaluation of the aforementioned data resulted in the selection of 240 plant species, that were collected according to good botanical practices and laws. Moreover, the selected species investigated through in silico procedure which was used in combination with biodiversity data to select the most promising herbs for further phytochemical analysis. Furthermore, the selected plant material was extracted in lab-scale with Ultrasound Assisted Extraction using dichloromethane, methanol and methanol:water 50:50, successively and the generated extracts (*ca*. 720) were forwarded for the investigation of their phytochemical profile was performed using chromatometric (TFC, TPC) and analytical HPTLC, HPLC-DAD, and NMR methodologies and cell- free bioassays for evaluation of their biological properties (antioxidant, enzymes inhibition). Based on the chemical profiles and preliminary biological results *ca.* 100 extracts have been selected for further investigation for the isolation of bioactive compounds. The phytochemical characterization of *Momordica charantia, Cistus creticus* subsp. *eriocephalus* (Viv.) Greuter et Burdet and *Juglans regia* extracts, coupled with their biological evaluation on skin enzymes and on skin cell lines, prospects for the discovery of bioactive natural compounds for the treatment of skin diseases.

Keywords: ethnoherbs, skin diseases, enzyme inhibitors

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S3.P249 *Artocarpus thailandicus* heartwood extract extends lifespan and promotes healthy aging behaviour in *Caenorhabditis elegans*

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Artocarpus thailandicus (AT) and its primary bioactive compound (oxyresveratrol) were believed to possess a high antioxidant capacity. However, the beneficial effect and mechanism of AT and oxyresveratrol on healthy aging phenotype and oxidative stress *in vivo* studies have never been investigated. We aimed to study the effects and mechanisms of AT and oxyresveratrol on healthy aging phenotype and oxidative stress resistance. The total phenolic and flavonoid contents were determined. Then, the ROS scavenging activity of these extracts was tested by DPPH and ABTS assays. To study healthy aging phenotype, survival rate under oxidative stress, lifespan assay, egg laying, and pharyngeal pumping rate were performed. The molecular mechanism affected-healthy aging phenotype was investigated by measuring the expression of HSP16.2, *sod-3, gst-4*, and nuclear translocation of DAF-16 and SKN-1 has been studied. Additionally, the oxyresveratrol level was quantified by HPLC, and NMR was employed to identify the phytochemicals in AT. Its ethanol crude extract (ATE) possesses a strong scavenging activity against DPPH and ABTS free radicals. ATE and oxyresveratrol improved the pharyngeal pumping rate including attenuating ROS accumulation of juglone-induced oxidative stress. Moreover, HSP16.2, an oxidative stress resistance biomarker, was decreased. In addition, DAF-16 and SKN-1 nuclear translocation was obvious, stimulating *sod- 3* and *gst-4* gene expression. Moreover, the major phytochemical constituent of ATE can exhibit powerful antioxidant activity. In conclusion, our study exhibited a novel mechanism of ATE and oxyresveratrol against oxidative stress. Both ATE and oxyresveratrol have the potential to serve as dietary supplements to promote healthy aging.

Keywords: *Artocarpus thailandicus*, oxyresveratrol, *C. elegans*, oxidative stress

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S3.P250 Utilization of ethnobotanical data to discover potent inhibitors of skin related enzymes through the application of in silico based methodology

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EthnoHERBS aims to evaluate information on Southeastern European traditional knowledge for the treatment of skin diseases and elaborate cutting-edge technologies in Natural Products chemistry. After the systematic reviewing of published ethnobotanical surveys in Balkan countries, 1289 taxa were recorded for their skin beneficial effects. The recorded information was assessed based on current scientific data concerning the biological properties of the identified plants resulting in the collection of 240 species of Balkan flora and the preparation of 720 extracts. Complementary to enzyme inhibition bioactivity evaluation, in silico studies were introduced in the assortment process of the most promising ones.

Protein and ligand structure preparation were performed by means of the NovaMechanics Asclepios KNIME (Fig.1) pipeline (Papadopoulou et al., 2021). The metabolites of species were searched in Reaxys and their structures were converted to SMILES strings. The ligand structure preparation involved addition of missing hydrogen atoms, conversion of the 2D structure to 3D by performing energy minimization to obtain a low energy conformation. After removal of duplicates and exclusion of ligands <175 Daltons, the ligands were reduced from *ca.* 210,000 to 20,000. The latter were docked within the experimental binding cavity of selected enzymes such as elastase, tyrosinase, collagenase, xanthine oxidase, cyclooxygenase, and lipoxygenase. The receptors were prepared, protonated and hydrogen-treated with PDBFixer node and docking calculations were performed with the Vina-GPU software (Easteman et al., 2017). Among the most promising species that were identified were *Momordica charantia*, *Juglans regia* and *Cistus creticus* subsp. *eriocephalus*.

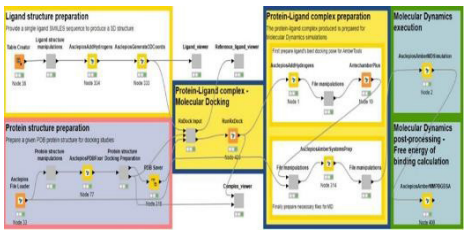

Fig. 1. Enalos Asclepios KNIME pipeline for enzyme inhibitors discovery automation

Keywords: in silico, Balka flora, enzyme inhibitors, skin disorders

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S3.P251 Chemical characterization of a *Schisandra sphenanthera* fruit extract, an adaptogen from a sustainable panda-friendly conservation program

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Schisandra sphenanthera Rehd. et Wils (Schisandraceae), commonly known as Southern Schisandra, *nan wu wei zi*, is used interchangeably with the adaptogen, *S. chinensis*, in traditional Chinese medicine (TCM). It is endemic to southern China, where it is used in indigenous medicines of ethnic minorities of Sichuan, including Qiang, Tibetan, and Yi, where it grows in forests of Minshan and Qinling mountains. Its habitat overlaps with the habitat of the Giant Panda (Brinckmann, 2018). This ingredient meets FairWild, Giant Panda Friendly Products, and Organic Wild-crop Harvesting Practice Standards.

Schisandra species contain unique dibenzocyclooctadiene lignans (DBCL) and nortriterpenoids with a wide range of biological activities. As part of our interest in finding sustainable, high functioning ingredients, we chemically characterized a *S. sphenanthera* extract obtained from a hot water (<90°C) high pressure reiterative process using UPLC-MS. Using LC-MS to obtain a chemical profile of this important botanical, we tentatively identified several schindilactone nortriterpenoids from *S. sphenanthera* fruit for the first time. This extract was shown to activate nrf2, a major transcription factor that regulates the cellular defense through the expression of genes involved in oxidative stress response and drug detoxification, measured by NQO1 activity in normal human epidermal keratinocytes.

Keywords: *Schisandra sphenanthera,* Schisandraceae, lignans, sustainable sourcing

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S3.P252 LC-MS/MS analysis of polyphenolic and triterpene components and antioxidant activity of cold-pressed rose oils

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Cold-pressed oils are preferred over refined edible oils and are gaining growing consumer interest since they are characterized by higher levels of bioactive compounds (Rabiej-Kozioł et al., 2023). Among vegetable oils, rose oil is one of the most expensive and most highly valued, especially for its cosmetic use (Ayati et al., 2018).

The aim of the study was to compare chemical composition and antioxidant activity of rose oils available on the Polish market. Special attention was paid to the liquid chromatography-mass spectrometry (LC–MS/MS) analysis of polyphenolic and triterpene components as potential markers for oil authentication. Another goal was to determine to what extent polar and non-polar components are responsible for oil's antioxidant activity. Antioxidant assays (the $ABTS^{\bullet+}$ radical scavenging, H- ORAC, and L-ORAC) were adjusted to enable fast analysis of oil samples on microplates.

It was demonstrated that the polyphenolic profile, colour and carotenoid content (1.22-79.53 mg/mL) of oils from different producers differ significantly. The dissimilarities in the content of quercetin, protocatechuic, syringic, salicylic and caffeoylquinic acids were particularly noticeable. Interestingly, analysis of triterpene content did not reveal significant qualitative differences. The TEAC values of the oils varied from 349.06 to 907.18 μg Trolox/mL, and most of this activity was found to be caused by the non-polar components of the oily fraction (515.01 - 849.17 μg Trolox/mL).

The observed differences suggest large discrepancies in rose oil production (e.g. botanical origin, quality and preparation of plant material) and the need to establish quality standards and methods for verifying oil's authenticity.

Keywords: cold-pressed oil, LC-MS, polyphenols, hydrophilic oxygen radical absorbance capacity, lipophilic oxygen radical absorbance capacity

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S3.P253 Chemical insights into a bioactive polyphenolic extract from *Camellia japonica* L. flower

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Camellia is a genus of flowering plants, which belongs to Theaceae family. Although some *Camellia* spp. are broadly exploited for their essential oils and tea for their leaves, a few species are grown as garden ornamentals (Garcia-Jares et al., 2017). Among these latter, *C. japonica* L. varieties are distinguishable based on their flower anatomical features (Pereira et al., 2022), whereas those with flowers double full in shape and red in colour are the most fascinating also in a nutraceutical and/or cosmeceutical perspective.

In this context, *C. japonica* L. flowers, collected from the botanical garden of the University of Campania (Caserta, Italy), underwent ultrasound assisted maceration in formic acid acidified ethanol, and extract obtained was chromatographed on Amberlite XAD-4 resin to achieve an anthocyanin rich fraction (CjA).

CjA fraction was first chemically investigated using spectroscopic (ATR FT-IR, and UV-Vis) techniques, and then profiled by means of Ultra-High-Performance Liquid Chromatography-High- Resolution Mass Spectrometry (UHPLC-HRMS). The fraction, which consisted of anthocyanins (mostly acylated), procyanidins and flavonols, exhibited a marked antiradical activity. In fact, it massively scavenged DPPH radical and ABTS radical cation, with calculated ID_{50} values equal to

14.2 and 2.1 μg/mL, respectively. Furthermore, when cytotoxicity was assessed by employing MTT assay on human cell lines, CjA fraction appeared to be cytotoxic, also at the lowest tested doses towards tested cancer cells, while cytotoxic effects were not observed on HaCaT keratinocytes. These preliminary data suggest further investigation needs to be carried out, for better exploiting these high- value flowers.

Keywords: *Camellia japonica*, polyphenolic extract, cytotoxicity, antioxidant activity

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S3.P254 Complementary LC-MS/MS analyses of alkaloid-rich fractions of the seeds of *Plukenetia volubilis* L. by LTQ Orbitrap XL and ZenoTOF 7600

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Plukenetia volubilis L. (Euphorbiaceae), better known as Sacha inchi (SI), is an oleaginous vine that produces seeds consumed in several countries in South America. However, while the seeds possess nutritional benefits (Goyal et al., 2022), an assessment of potentially toxic metabolites such as their alkaloid content has only been done by colorimetric assays or IR spectroscopy (Kumar et al., 2020; Srichamnong et al., 2018). The lack of in-depth identification of the type of alkaloids present in the seeds of SI has prevented their acceptance in the European Food Market (Authority (EFSA), 2020). Our aim was thus to investigate the chemical composition of an alkaloid-rich fraction of the defatted seeds, raw (SIRA) and after roasting (SIRO). The two fractions were analyzed by HPLC-HRMS/MS using a LTQ Orbitrap XL and UPLC-HRMS/MS with a ZenoTOF 7600. The main families identified by dereplication and in silico methods with both instruments were cyclopeptides mainly in SIRA, and primary fatty acid amides, more abundant in SIRA than SIRO. Differences in the detection and identification of sphingosine derivatives, true alkaloids and lysophosphatidylcholines between the Orbitrap and ZenoTOF analyses will be further discussed in the poster.

The combined results of the two instruments show a variety of nitrogen-containing compounds. Moreover, our analyses suggest that roasting induces degradation of these compounds, although further quantification is necessary. Mapping the complete alkaloid profile of the seeds will help to better assess its potential toxicity during human consumption.

Keywords: Sacha inchi, alkaloids, ZenoTOF 7600, fatty acid amides

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S3.P255 Effect of different extraction methods on determination of *Drosera capensis* active ingredients

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Drosera capensis cape sundew is a carnivorous plant native to South Africa (Jobson and Conn, 2012). Drosera capensis is also known contained the 1,4-naphthoquinones and 7-methyljuglone chemicals are used for antimicrobial and antifungal purposes and prevent tumor formation (Ziaratnia et al., 2009). In this study, Drosera capensis was propagated by tissue culture method extracted by ultrasound, soxhlet and microwave extraction methods and its 1,4-naphthoquinones content determined by HPLC method. It was concluded that different extraction methods had a significant impact on the determination of the active ingredient content in D. capensis.

Keywords: cape sundew1,4-naphthoquinones, ultrasound, Soxhlet, microwave

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S3.P256 Valorization of *Olea europaea* L. cv. Caiazzana prunning waste: a step towards a sustainable approach for for resource functional recovery

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Olive leaves by the pruning and harvesting of olive trees (*Olea europaea* L.) represent one of the olive oil industry by-products, whose valorization needs to be pursued also in an innovative food & nutraceutical scenario. In this context, also taking into account cultivar biodiversity, leaves from *Olea europaea* L. cv. Caiazzana, an autochthonous cultivar of Southern Italy, collected after pruning in an orchard near Caiazzo (Caserta, Italy), and freeze-dried, underwent ultrasound-assisted extraction (UAE) (Pacifico et al., 2022) using ethanol. The alcoholic extract was further fractionated to achieve a polyphenol fraction, which was quali-quantitatively profiled by UHPLC-ESI-Q*q*TOF-MS/MS techniques, and screened for its antiradical capability, Fe(III) reducing power, and antibacterial activity. The cytotoxicity was also carried out towards normal-like and cancer cells. Nevertheless, the exhausted leaves were explored for recovering pure cellulose, characterized chemically by ATR FT- IR tools, and morphologically by scanning electron microscopy (SEM). Thus, cellulose was properly derivatized and cellulose-based films entrapping olive leaf polyphenol fraction were fabricated. FTIR spectroscopy and SEM suggested the occurrence of hydrogen-bonding among the film components. The films maintained the antioxidant efficacy of the bioactive extract and advantageously release the polyphenols when allowed them to adhere to a depletable product, such as meat (storage time considered 10 days), preserving it from oxidation, and functionally enriching it in bioactive compounds. Thus, the recovery of olive leaves opens up to their favourable use for the creation of active films capable of enriching foods in our diet with functional constituents.

Keywords: *Olea europaea* L. leaves, polyphenols, cellulose, nutraceutical & food sectors

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S3.P257 Plants from Lublin region as potential sources of ingredients with cosmetic value

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Nowadays, ingredients used in cosmetics are expected to perform multiple functions and protect the skin against harmful effects of environmental factors. Due to ongoing environmental changes, there is a great demand for cosmetic ingredients that have both antioxidant and skin brightening properties. This project aimed to develop analytical conditions that would allow the identification of extracts with antioxidant and tyrosinase-inhibitory potential among the medicinal plants occurring in the Lublin region. From the tested ethanol, water and 50% ethanol extracts of 10 plant species four were found to be the most active, namely *Matricaria chamomilla, Sambucus nigra, Urtica dioica* and *Melissa officinalis*. Their percentage inhibition of the DPPH radical was calculated as 38,561%, 35,791%, 16,711% and 50,536% respectively. The tyrosinase inhibition was calculated as 81,16% for the water: ethanol extract (50:50 v/v) , 20,29% for the ethanol extract, 28,14% for the ethanol extract and 16,91% for the water extract respectively, whereas the total phenolic content was determined using the Folin Ciocalteu reagent. Gallic Acid Equivalent (GAE) was calculated as 13,35 for the water: ethanol extract (50:50 v/v), 16,69 for the ethanol extract 13,98 for the ethanol extract, 16,68 for the water extract respectively.

The HPLC-ESI-QTOF-MS/MS fingerprinting was performed for the analyzed extracts. Melissae water extract was found to be the most rich. Among the major components caffeic, chlorogenic, caftaric, rosmarinic, syringic, genistic, p-coumaric and ferulic acid, hydroxy jasmonic acid glucoside, rutine, apigenin, were identified.

Also, the most active extract was subjected to an on-line assay using an HPLC-MS instrument coupled with a bioreactor to determine single constituents with anti-tyrosinase properties in the extract.

Keywords: *Melissa officinalis*, antioxidant, anti-tyrosinase, cosmetics, HPLC-ESI-QTOF-MS/MS

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S3.P258 Anti-neuroinflammatory effects of *Elsholtzia blanda* benth on lipopolysaccharide-stimulated BV-2 microglial cells

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Elsholtzia blanda (Benth.) Benth. (EBB) is a medicinal plant in Nepal with traditional use. Several studies have shown that EBB has bioactive properties, including antimicrobial (Nguyen et al., 2022; Ishwori et al., 2015) and anti-inflammatory effects (Duong et al., 2023). In this study, the methanol extract of EBB was tested to evaluate its anti-inflammatory effect against the lipopolysaccharide (LPS)-induced microglial macrophage cells (BV2). In LPS-activated BV2 microglial cells, EBB significantly reduced the induced proinflammatory mediators, *e.g.* TNF-α and PEG2, as well as nitric oxide (NO) accumulation. EBB also attenuated LPS-induced ROS in BV2 cells. In addition, both protein and mRNA expression levels of iNOS and COX-2, which were upregulated by LPS, were significantly decreased by EBB extract. Also, EBB extract effectively reduced the transcriptional expression and further suppressed the increased production of inflammatory cytokines by LPS stimulation. The results obtained in this study showed that EBB extract effectively suppressed the inflammatory response induced by LPS in BV-2 cells. We suggest that EBB extract holds promise as a preventive agent against diseases triggered by microglial inflammatory responses.

Keywords: *Elsholtzia blanda* (Benth.) Benth., microglial macrophage (BV2), neuroinflammation, lipopolysaccharide, anti-inflammatory effect

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S3.P259 Metabolite profiles and biological activities of *Capsicum chinense* cell cultures

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Research and industry pursue natural alternatives to synthetic antioxidants and antiaging agents for safer use in food and cosmetics. Chili fruits harbor capsaicinoids, polyphenols, carotenoids and fatty acids, offering anti-inflammatory, antioxidant, and antitumoral effects (*Alonso-Villegas* et al., 2023). Consistent agricultural production of these constituents is challenging due to yield variability, highlighting sustainable sourcing importance for cosmetic and pharmaceutical industries. Alternatively, plant cell culture offers a renewable, eco-friendly production system.

In the frame of the EU-InnCoCells project, we established *Capsicum chinense* var. Pimento cell suspension cultures in dark and photoperiodic growth conditions. Subsequently, hydrophilic and hydrophobic compounds were extracted by hydroethanol and ethyl acetate, repectively. Extracts were then subjected to compehensive metabolite profiling. Chemical analyses revealed that the hydrophilic extracts contain capsaicinoids, sesquiterpenes, phenolic acids, flavonoids and phenolamides, whilst the hydrophobic extracts included carotenoids and fatty acids. The most aboundant components were phenolamides. Phenolamides have been reported to possess skin depigmentative and antioxidative activities (Roumani et al., 2020).

Finally, antioxidant, antimicrobial and cytotoxicity of cell culture hydrophilic extracts were investigated. Extracts exhibited potent antioxidant properties. Hydrophilic extracts effectively inhibited the growth of Gram-positive *Staphylococcus aureus* and *S. aureus* MRSA strains.

The hydrophilic extracts of all cell cultures were not cytotoxic at concentrations of 2.5 and 1.25 mg/mL, respectively, against the BALB/c 3T3 and HaCaT cell lines. Furthermore, cell extracts did not exhibit phototoxicity in the BALB/c 3T3 cell line at a concentration below 2.5 mg/mL.

Keywords: Chili, Plant cell culture, Antimicrobial and antioxidant activity, Cytotoxicity, Cosmetics

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S3.P260 Liposome encapsulation of *Lavandula austroapennina* N.G. Passal., Tundis & Upson leaf and stem extracts for cosmeceutical innovation

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Plant-derived specialized metabolites are highly researched for their efficacy against disease and for their use in cosmetics and dietary supplements (Elshafie et al., 2023). However, their poor water solubility and stability pose a challenge, impacting their efficacy and bioavailability (Bhalani et al., 2022). Liposomes are extensively applied as lipid-based carriers for topical drug delivery, improving the penetration of bioactive compounds through skin layers (Chaves et al., 2023). In this context, extracts from the endemic species *Lavandula austroapennina* N.G.Passal. Tundis & Upson, were explored for their cosmeceutical potential.

L. austroapennina plants from Mt. Cervati were fractionated into its different organs and extracted using ultrasound-assisted maceration in *n*-hexane and methanol. Phytochemical analysis through UHPLC-HR-MS/MS identified fatty acids and olean- and ursane-type triterpenes in lipophilic extracts, and hydroxycinnamoyl and 8-hydroxyphenylpropanoid acid derivatives in alcoholic ones. Previous studies highlighted the superior bioactivity of stem lipophilic and leaf alcoholic extracts (Chianese et al. 2023; Gravina et al. 2023), selected for encapsulation in liposome-based nanocarriers. Accordingly, extracts-containing liposomes, preliminarily characterized by ATR-FTIR spectroscopy, underwent cytotoxic and healing capability assessment towards keratinocytes cell models. The extracts-enriched liposomes showed a healing capacity higher than the parental extracts they were derived from. A joint approach employing UHPLC-HR-MS/MS and *in vitro* skin permeation studies revealed that encapsulation in liposomes improved bioactive delivery through/into a specific skin layer. These findings suggest the effectiveness of liposome-based nanoparticles in improving the bioavailability and biological activity of specialized metabolites, thereby reinforcing the cosmeceutical potential of *L. austroapennina* extracts.

Keywords: cosmeceuticals, encapsulation, percutaneous absorption, UHPLC-Q*q*TOF-HR-MS, ATR- FTIR

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S3.P261 Effect of combining proteolytic enzymes papain and chymotrypsin with Alexandrite laser hair removal on the degree of facial hirsutism

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Papain (**1**) is a proteolytic enzyme derived from the latex of *Carica papaya*, which is used for depilatory preparations (Mellou et al., 2020). Chymotrypsin (**2**) is a pancreatic serine protease (Fu et al., 2021). Iontophoresis of **1** and **2** to skin of experimental animals has shown long-lasting depilatory effects (Protopapa et al., 1999). Photo-epilation is the treatment of choice for hirsutism. However, it does not offer complete and persistent results (Mikiel et al., 2020). Therefore, we opted to combine photo-epilation with application of these enzymes, as a more natural approach, to enhance treatment efficacy.

In this randomized controlled clinical study, 59 adult Caucasian women with facial hirsutism were divided into two groups: Group I, treated with combination of laser Alexandrite 755nm and iontophoresis of aqueous solutions **1** (0.48%) and **2** (0.29%) and Group II, treated with laser Alexandrite 755nm alone. The degree of facial hirsutism (0-4) was assessed by a dermatologist and the participants, based on the modified Ferriman-Gallwey scoring system. After 10 sessions, a comparison between the two groups of the degree of hirsutism was performed.

Participants of Group I had a statistically significantly lower overall degree of hirsutism compared to Group II, according to both the dermatologist's ($p=0.014$) and the participants' assessment ($p=0.009$). Iontophoresis of **1** and **2** provides a more natural adjuvant treatment, with a potential to enhance laser hair removal efficacy in hirsute patients.

Keywords: papain, chymotrypsin, photo-epilation, facial hirsutism, efficacy

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S3.P262 Simultaneous analysis of bergapten and schinifoline in *Zanthoxylum schinifolium* Siebold & Zucc. seeds using HPLC and UPLC-MS/MS systems

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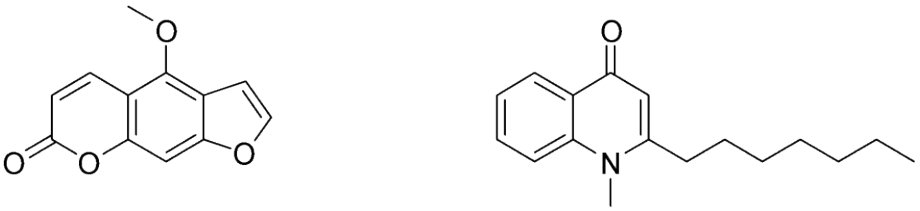
Zanthoxylum schinifolium Siebold & Zucc. belongs to the Rutaceae family and has been widely used as a spice (typically seeds and pericarp) in East Asian countries such as Korea, China, and Japan (Oh and Chung, 2014). The present study focused on developing and validating a simultaneous analytical method for marker substances (bergapten and schinifoline; Fig. 1) in *Z. schinifolium* seeds. This was achieved using high-performance liquid chromatography (HPLC) with a photo-diode array detector (DAD) and ultra-performance liquid chromatography (UPLC) with tandem mass spectrometry systems.

In the regression equation, all markers showed a coefficient of determination of ≥0.9990. Marker recovery was 96.90–105.16% (relative standard deviation (RSD) ≤ 2.23), and the intra- and interday precision was RSD < 3.00. Bergapten and schinifoline were detected in the seeds at 1.70–2.85 mg/g and 0.19–0.94 mg/g, respectively. This analytical method will improve quality control of *Z. schinifolium* seeds. Additionally, this assay will provide basic data and quality assurance for future biological activity experiments or clinical applications.



Bergapten Schinifoline

Fig. 1. Chemical structures of the two markers selected of *Z. schinifolium* seeds.

Keywords: simultaneous analysis, *Zanthoxylum schinifolium*, bergapten, schinifoline

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S3.P263 Health-promoting effects of the hydroalcoholic extract from the *Allium sativum* L. ecotype "Sulmona red garlic" in the colon

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In the present study, we investigated the chemical composition and biological properties of the hydroalcoholic extract from the aerial bulbils of the *Allium sativum* L. ecotype "Sulmona red garlic", a traditional ecotype of middle Italy cultivated in the Abruzzo region. The study aimed at exploring the potential application of a plant material, namely aerial bulbils, that is considered a waste material in the "Sulmona red garlic" supply chain. The aerial bulbils extract was tested in different biological models, including an ecotoxicological model constituted by the crustacean *Daphnia magna* and cell two different cultures (C2C12 and HCT116 cell lines), with the aim to unravel the limits of its biocompatibility and protective effects. Additionally, a deep phytochemical analysis was conducted for determining the content of specialized metabolites. NMR analyses demonstrated the content of alliiin and allicin, in the aerial bulbils where different phenolic compounds were identified and quantified, among which the prominet were catechin, chlorogenic acid, and gallic acid. The extract was biocompatible in *the Daphnia magna* model (up to a concentration of 10 mg/mL) and in the non- tumoral C2C12 cell line, in the concentration range of 10-1000 µg/mL. By contrast, a concentration- dependent cyotoxic effect was observed in the human colon cancer HCT116 cell line. This effect was related, albeit partially, to the inhibition of the transient receptor potential (TRP) M8 (TRPM8), whose upregulation has been implicated in inflammation and carcinogenesis. Additionally, the extract inhibited the gene expression of TNF-α (tumor necrosis factor), HIF1-α (hypoxia-inducible factor), and VEGFA (vascular endothelial growth factor), thus further demonstrating promising health- promoting effects, especially against inflammatory bowel diseases.

Keywords: garlic, organosulphur compounds, phenolic compounds, cytotoxicity, colon, TRPM8

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S3.P264 Anti-inflammatory effect of *Lysimachia mauritiana* extract on particulate matter 10 plus diesel exhaust particles (PM10D)-induced respiratory disease mouse model and standardization using UPLC-qTof/MS analysis

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Exposure to particulate matter (PM) causes considerable respiration-related health risks. The effect of *Lysimachia mauritiana* (LM) extract on airway inflammation was investigated in mice exposed to a fine dust mixture (PM10D) of PM10 (PM diameter < 10 um) and diesel exhaust particles (DEP). LM suppressed the number of immune cells and secretion of cytokines in bronchoalveolar lavage fluid (BALF) and lung of PM10D mice. Airway inflammation and collagen fibrosis in the lung after PM10D exposure were investigated by histopathological analysis, and LM ameliorated these symptoms. LM decreased the mRNA expression of MUC5AC, CXCL1, TNF-α, MIP-2, TRPA1, and TRPV1 in lung. The main candidate components from LM that exhibited anti-inflammatory effects were investigated by ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-qTof/MS). The seven phytochemicals were tentatively identified by MS analysis. Compound 3 among the flavonoids, which are easy to detect even under UV, is identified into biorobin (keampferol 3-O-robinobioside) and was confirmed as candidate of standardization. These findings suggest that LM may be a potential therapeutic agent for the treatment of respiratory diseases in human.

Keywords: airway inflammation, find dust mixture, *Lysimachia mauritiana,* lung

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S3.P265 Optimization, estimation, and improvement of lutein content from marigold flowers using *Lactobacillus* species by solid-state fermentation

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Lutein is primarily sourced from marigold petals which are used in the cosmetic, nutraceutical, and pharmaceutical industries (Anna et al., 2020). This contributes to overall eye health and brain diseases (Mitra et al., 2021; Yagi et al., 2021). In this study, we investigated the improvement in lutein content in marigold flowers (*Calendula officinalis* Linn) via solid-state fermentation using three different *Lactobacillus* species such as *L. rhamnosus, L. casei*, and *L. plantarum*. Microorganisms were isolated from the National Dairy Research Institute (India) and fermented for 10 days in an incubator with fresh and dried marigold petals. Fermented and non-fermented petals were extracted with hexane and ethanol, and the presence of lutein was confirmed by HPLC at 446 nm. Using hexane as a solvent, the experiment revealed variations in the lutein content of different types of petals. Fresh petals (1 kg) fermented with *L. plantarum* contained 4.0% lutein, whereas dried petals (1 kg) fermented with *L. rhamnosus* contained 0.99% lutein. Non-fermented fresh petals contained 1.1% lutein, whereas non-fermented dried petals contained 0.4% lutein. Furthermore, the use of ethanol also yielded satisfactory results. When fresh marigold flowers (1 kg) were fermented by *L. plantarum*, they contained 0.43% lutein and dried petals (1 kg) contained 0.52% lutein when fermented by *L. rhamnosus*. Based on these findings, *L. plantarum* enhanced lutein content by 3-fold in hexane. In addition, ethanol is a useful green replacement for conventional hazardous solvents, and *Lactobacillus* species are preferred to enhance the extraction of lutein from marigold petals in research laboratories and industries.

Keywords: Lutein, *Lactobacillus* species, marigold, solid-state fermentation

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S3.P266 Metabolite Profiling and Molecular Network Shows Kinkeloids as promoting of collagen synthesis from *Combretum micranthum*

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This presentation will investigate the molecular composition and a new cosmetically relevant biological activity of *Combretum micranthum* extract and enriched fractions to begin to establish a structure-activity relationship. *Combretum micranthum*, a plant native to Africa, has a well-documented traditional use in the treatment of various ailments such as fever, diabetes and malaria (Yoro, Tine et al., 2024). Its pharmaceutical benefits include nephroprotective, anti-inflammatory, antioxidant and antimicrobial properties have been demonstrated (Olajide, Olumayokun et al., 2003). In addition, its potential for cosmetic applications is being explored due to its depigmenting, anti- inflammatory and UV damage repairing properties (胡舒婷 et al., 2020). First, an extract of *Combretum micranthum* was prepared and selected for its overall biological response, then fractionated to obtain simplified molecular fractions. One fraction was particularly enriched in kinkeloids, a family of compounds specific to this species. All fractions and the crude extract were then tested on biological targets to evaluate and compare their cosmetic activities. Molecular networks were constructed from the UHPLC-MS/HRMS data to better characterize the extract and fractions and to highlight structure-activity relationships. This study highlights the metabolic profiling of a butylene glycol extract of *Combretum micranthum*, showing its main chemical families and revealing that the kinkeloids identified by HRMS and NMR promote an increase in collagen I synthesis, an interesting cosmetic activity not previously described for these compounds or for any *Combretum micranthum* extract.

Keywords: preparative chromatography fractionation, UHPLC-HRMS/MS, molecular network, NMR, collagen I synthesis

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S3.P267 Determination of the anti-inflammatory activity of fruits of some varieties of *Rubus idaeus* and *Rubus occidentalis* (Rosaceae)

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Red raspberry fruits (*Rubus idaeus* L., Rosaceae) are a remedy known in folk medicine for treating colds. In Poland, the species is cultivated in many varieties (Szymanowska et al., 2018). However, another raspberry species originating from North America - black raspberry (*Rubus occidentalis* L.) occurs in Poland in only two varieties, including the 'Litacz' variety (Kaume et al., 2012). Raspberries are a source of anthocyanins - cyanidin glycosides and ellagitannins with proven antioxidant and anti- inflammatory effects (Gomathi et al., 2024). The aim of the study was to evaluate the *in vivo* anti- inflammatory effect of fruits from selected varieties of red raspberry and black raspberry. The analysis of the biologically active compounds in the tested fruits was carried out using the HPLC–DAD– ESI/MS. In the pharmacological studies, a plethysmometric method was used to measure carrageenan-induced rat paw edema compared to indomethacin (reference compound). *R. occidentalis* 'Litacz' fruit extract at a dose of 500 mg/kg (p.o.) revealed the strongest anti-inflammatory effect (inhibition of rat paw swelling was 54.88% at 1 hour after administration of carrageenan, compared to indomethacin - 39.56%) ($p<0.05$). The anti-inflammatory effect was dose- dependent except for the dose of 1000 mg/kg (23.68%). However, at this dose, at 5 hour of the experiment there was a statistically significant increase in the inhibition of edema (55%)($p<0.05$). The richest source of cyanidin glycosides, especially its triglycosides, turned out to be the fruits of the *R. occidentalis* 'Litacz'. Their slow hydrolysis to free aglycone - cyanidin, probably determines the long-lasting anti-inflammatory effect.

Keywords: raspberry fruits, cultivar varieties, anti-inflammatory activity

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S3.P268 Unveiling Thailand's ethnopharmacological heritage to find new natural sweeteners

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Thailand's biodiverse landscape and traditional practices in parts of southwest Asia offer a unique opportunity to explore the rich array of medicinal plants and their cultural significance. In this context, three species were sourced from local thai markets to investigate their potential for yielding novel glycosides, such as sweet-tasting saponins.

First, the Monk fruit *Siraitia grosvenorii (Swingle) C.Jeffrey ex A.M.Lu & Zhi Y.Zhang* (Cucurbitaceae) (MPNS Portal, 2024) is a natural sweetener used for over 300 years to treat pharyngitis and pharyngeal pain (Gong et al., 2019). *Albizia myriophylla Benth.* (Fabaceae), is also prevalent in Thai and Vietnamese traditional medicine. Its stems are utilized as a substitute for licorice due to their sweet taste and it is highly valued for treating diseases of oral cavity like dental caries and aphthous ulcers (Joycharat et al., 2016). *Sapindus rarak DC.* (Sapindaceae) pericarp powder and extracts are widely used for their sweet taste and in hair and skin care, due to their rich concentration in saponins and thus natural foaming abilities. The pericarps also find applications in laundry detergents and in Ayurveda and folklore medicine for treating asthma, skin diseases and psychiatric disorders (Kora, 2023).

This poster presentation provides a comprehensive overview of the isolation protocol used to obtain crude saponin mixtures from each plant, beginning with microwave-assisted extraction and incorporating various chromatographic techniques including VLC, flash chromatography, and MPLC. Furthermore, it assesses their capacity to activate the sweet taste receptor TAS1R2/TAS1R3, offering insights into their potential effects.

Keywords: Glycosides, *Siraitia grosvenorii, Sapindus rarak, Albizia myriophylla*, TAS1R2/TAS1R3 receptor

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S3.P269 Recovery effect of phloroglucinol 1-O-β-D-glucopyranoside from the brown alga *Agarum clathratum* subsp. *yakishiriense* on neomycin-induced otic hair cell damage in zebrafish

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Marine plants are popular and abundant food ingredients mainly in Asian countries, and also known to have many beneficial biological activities. *Agarum clathratum* subsp. *yakishiriense* Yamada ex

G.H. Boo & P. C. Silva is a perennial brown alga in the family Costariaceae and is widely distributed in the coastal area of the East Sea in Korea (Boo et al., 2012). Previous studies on *A. clathratum* have reported neuroprotective effect (Kim et al., 2014) and anticancer effect on epithelial ovarian cancer (Kim et al., 2023).

As part of our continuing search to find potent anti-ototoxic compound, we investigated the recovery effect of hearing loss for EtOH and water extracts of *A. clathratum* subsp. *yakishiriense* on a zebrafish model. The EtOH extract of *A. clathratum* subsp. *yakishiriense* which showed ameliorative effect in a zebrafish ototoxicity model, was suspended in water and then partitioned consecutively with organic solvents to give CH_2Cl_2, EtOAc, *n*-BuOH, and H_2O fractions. Among these fractions, the H_2O fraction revealed the strongest protection against the otic hair cell damage in zebrafish models. The water fraction was subjected to column chromatographic separation. Phloroglucinol 1-O-β-D- glucopyranoside along with five compounds were isolated from the water fraction of *A. clathratum* subsp. *yakishiriense.* The isolated compounds were investigated the ameliorative effect on neomycin- induced ototoxicity in the zebrafish. Phloroglucinol 1-O-β-D-glucopyranoside showed significant recovery on otic hair cell damage at concentration of 1 μg/mL. In addition, its mechanism of action was studied. In conclusion, phloroglucinol 1-O-β-D-glucopyranoside revealed potent recovery effect for the hearing loss.

Keywords: *Agarum clathratum* subsp. *yakishiriense,* Costariaceae, phloroglucinol 1-O-β-D-glucopyranoside, neomycin-induced ototoxicity, zebrafish

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S3.P270 Giuseppe Raddi and the plants: Taxonomy, Uses and Conservation in the 21st century

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Giuseppe Raddi was an Italian naturalist who surveyed the flora of Rio de Janeiro in 1817, producing a rich collection of around 4000 plant specimens. The difficulties in studying historical collections were greatly attenuated by the digitization of biological collections and libraries, but the volume of materials produced by Raddi combined with particularities of his life history poses a greater challenge in the study of his work.

The present work aims a brief assessment of the relevance and contribution of Raddi in plant taxonomy, and, given the impossibility of tabulating for analysis all the specimens collected by him, we will use the names he described to bring his work to the 21st century reality, in relation to the uses made of the species reported by him and their confirmation by science, and their state of conservation, almost 200 years after his death.

We used the main online botanical databases to search for names containing "Raddi", resulting in 368 names which were checked as to their priority and legitimacy, generating a list of 273 names that correspond to the universe of taxa dealt by Raddi, 236 of them occurring in Brazil.

Evidence of Raddi's brilliance is the fact that he navigated with equal dexterity every group of land plants known to science, and that his name has been celebrated in 40 names of taxa, during his lifetime and after. His dedication to non-flowering plants, however, might explain the lower number of medicinal plants described in his works. From the 236 Brazilian taxa surveyed, only 20 had their conservation status evaluated, evidencing the size of Brazil´s natural heritage and our responsibility towards future generations.

Keywords: biodiversity, conservation, historical botany, Giuseppe Raddi, medicinal plants

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