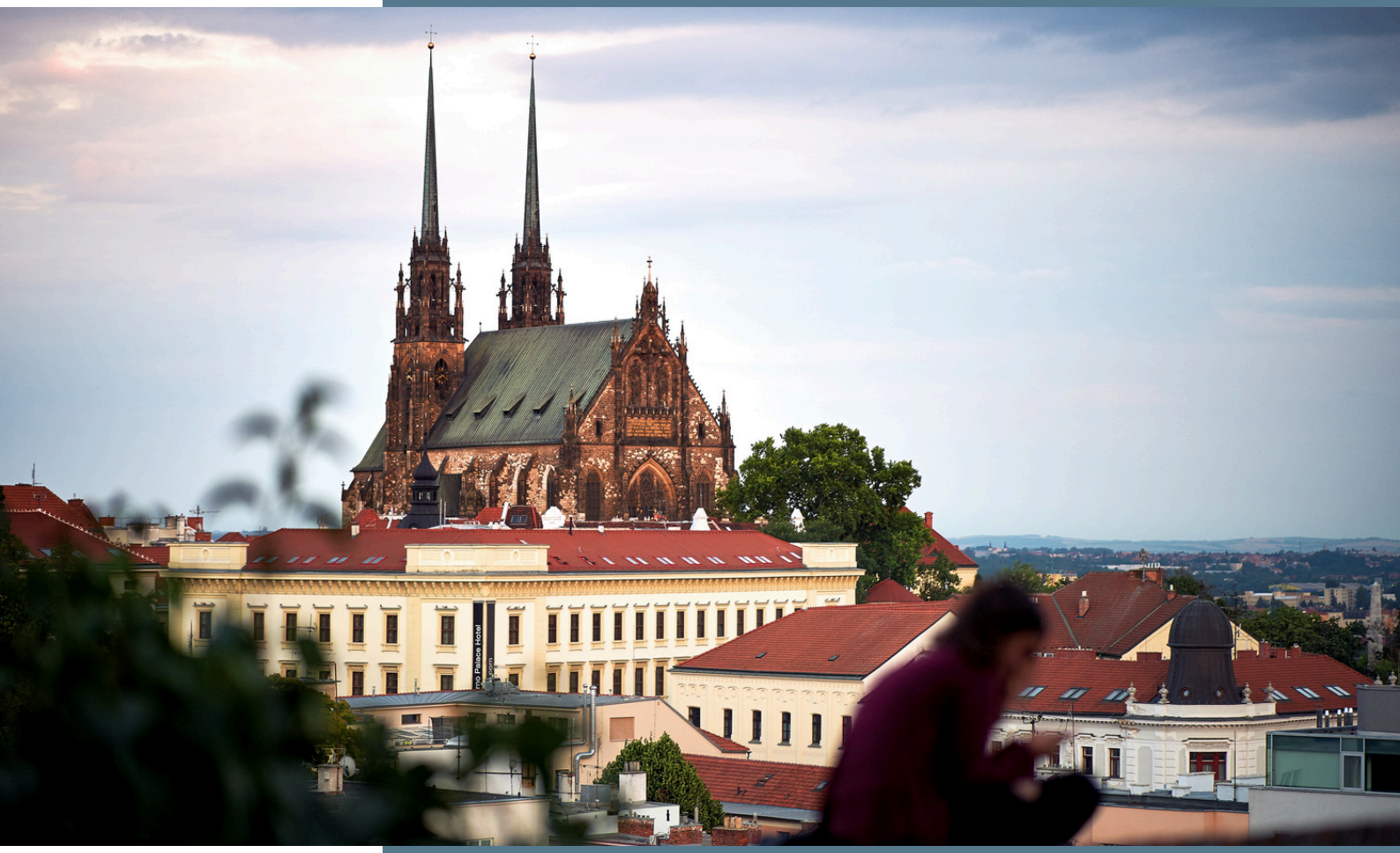

Trends in Natural Products 2024
PSE Young Scientists' Meeting
Phytochemical Society of Europe (PSE)

21st – 24th May 2024, Brno, Czech Republic

# BIOACTIVE NATURAL PRODUCTS IN BIOLOGY, PHARMACY AND COSMETICS

# Book of Abstracts

# Phytochemical Society of Europe

# Trends in Natural Products 2024

# Young Scientists' Meeting

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# BIOACTIVE NATURAL PRODUCTS IN BIOLOGY, PHARMACY AND COSMETICS

# Book of Abstracts

## Editors:

Hana Brožová (Masaryk University, Brno, Czech Republic)
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Lenka Molčanová (Masaryk University, Brno, Czech Republic)
Karel Šmejkal (Masaryk University, Brno, Czech Republic)

# Welcome address

Dear colleagues,

On behalf of the Phytochemical Society Europe and Faculty of Pharmacy, Masaryk University, I would like to express the welcomes and our pleasure to meet you at the conference Trends in Natural Products 2024, Young Scientists' Meeting: Bioactive natural products in biology, pharmacy and cosmetics.

In 2024, YSM is hosted in Hotel Passage, in Brno, Czech Republic. Brno, the second largest city in the Czech Republic located in the South Moravian Region, is renowned for its historical and cultural significance as well as its various economic contributions. Brno is strategically situated at the confluence of the Svitava and Svratka rivers. At the time of the provided information, it boasted a population of approximately 390,000 residents, making it the second-largest city in the Czech Republic, following Prague. The greater Brno metropolitan area encompasses nearly 700,000 people. Brno is located almost in the geographical center of Europe. Brno, the former capital of Moravia, holds a special place in the history and culture of the South Moravian Region.

The aim of the annually organized Young Scientists' Meeting is to give doctoral students and postdoctoral fellows the opportunity to meet their experienced senior colleagues, and to present their research on a broad scientific forum. Beside this, we would like to accent the personal meetings, creation of (sometimes long lasting) contacts and possible networking of students from many countries, we can therefore express the conference as worldwide.

Judging the multicultural and international spirit of conference (almost 150 attendants from more than 20 countries), we hope that this international environment will stimulate the creation of contacts not only on scientific basis, but also socially important, will help to create friends, and will help to strengthen the global community connected by an interest in medicinal plants, natural compounds and their broad utilization in human life.

I would like to thank the mayor of the city of Brno, JUDr. Markéta Vaňková, and the rector of Masaryk University prof. MUDr. Martin Bareš, Ph.D. The conference is held under their auspices.

We wish you all a successful conference and a pleasant stay in Brno. Hopefully, it is not last time that we can meet.

Karel Šmejkal

On behalf of Organisation Committee

May 2024, Brno

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## PL1

# In Nature, nothing is what it seems. Looking for multipurpose bioactive hybrids.

Francisco A. Macías[1,*], Juan J. Merino Fernández, Miriam I. Martínez, Nuria Chinchilla, Francisco J. R. Mejías, Rosa M. Varela, José M. G. Molinillo

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Allelopathy emerged as an alternative to obtain new agrochemicals based on natural products, which would be an alternative to classical herbicides due to the occurrence of weed resistance phenomena[1] and reduce environmental impact.
Our previous experience in this area has allowed us to develop compounds based on sesquiterpene lactones that exhibit high phytotoxic activity against different weeds as potential pre-emergency herbicides. Bioactivity and other properties of natural compounds obtained directly from plants can be increased by preparing derivatives of natural sesquiterpene lactones. More specifically, this is achieved through functionalization by introducing structural changes in the α-methylene-γ-lactone ring with the simultaneous loss of C-13 exocyclic double bond.

Recent years have witnessed significant advances in molecular hybridization to discover new drugs[2]. Inspired by this idea, our group has synthesized hybrids by combining active natural products to achieve a synergistic manifestation of the activities[3]. Hybrids from sesquiterpene lactones and benzohydroxamic acids retain the exocyclic double bond of lactone rings, which taken individually exhibit a good profile of phytotoxic activity.

Those new hybrids were tested in phytotoxicity and cytotoxicity bioassays. Unexpectedly, a decreased activity was observed in comparison to the constituent molecules. Therefore, *in silico* assays were carried out by us using credible databases, revealing a different target: the inhibition of cellular hypoxia. Our synthesized hybrids seem to show a beneficial action to cellular functions, preventing oxygen deficiency. These preliminary results display a new exploration that was not anticipated by our hybrid molecules. Nevertheless, the initial insights highlight the complexity of nature, where nothing is what it seems.

Acknowledgments: This work was supported financially by the Ministerio de Ciencias e Innovación (Project PID2020-115747RB-I00), Spain.

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**PL2**

# Alkaloid derivatives for targeting drug resistance in cancer

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Despite the number of strategies that have been developed in cancer treatment in recent decades, it remains a leading cause of death globally, mainly due to drug resistance. Plants have been playing a key role as source of bioactive compounds, namely anticancer agents. To obtain plant-derived compounds with anticancer activity, we have focused on the phytochemical study of plants capable of producing large amounts of alkaloids, allowing further molecular derivatization. Characterised for bearing diverse and complex scaffolds, alkaloids are commonly characterized with strong biological properties.
Taking advantage of their functional groups, libraries of monoterpene indole and Amaryllidaceae-type alkaloid derivatives have been generated. The ability of natural alkaloids and derivatives as ABC transporter inhibitors, namely as P-glycoprotein inhibitors, in different resistant cancer cell lines has been investigated through chemo-sensitization and functional assays. The antiproliferative activity of alkaloid derivatives has also been investigated in a panel of human cancer cells. Some derivatives showed a selective antiproliferative effect on resistant cells, having a collateral sensitivity effect.

Acknowledgements: Fundação para a Ciência e a Tecnologia (FCT), Portugal (project 2022.05718.PTDC).

## PL3

# Application of computational methods to phytochemical drug discovery

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Natural products and their derivatives have historically been invaluable source for drug discovery. An increasing number of computational strategies are being applied to enhance and speed up natural product-based drug design. Virtual screening has been widely used to identify and optimize bioactive compounds including natural products since it reduces time and costs in drug discovery. Numerous molecular modelling tools are included in the virtual screening process of natural compounds. ADMET and druglikeness filtering, fingerprint and QSAR, shape and pharmacophore screening are among the techniques where ligand-based data is widely used. On the other hand, structure-based methods such as molecular docking, molecular dynamics simulations, and free binding energy calculation have gained momentum with the increasing availability of macromolecular data and high-precision software. Combination of ligand- and structure-based methods and using consensus approaches are currently becoming all the more popular since these approaches have proven to outperform the applications where a single method or approach is used[1]. In the course of our research on Turkish medicinal plants to discover natural hits, we utilized different computational tools to find new α-glucosidase inhibitors[2,3]. We provide brief descriptions and applications of computational techniques and approaches used in rational drug design. Discovery of natural products via these approaches is presented to highlight the importance of computational methods in natural product drug design.

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**PL4**

# Natural skin-whitening agents

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The skin is the largest and primary protective organ of the body, covering its entire external surface and serving as a first-order physical barrier against the environment. The skin tone or 'complexion' ranges from the palest hue to the darkest brown. The colour of the skin is not someone's choice but inherited by an individual. The amount of melanin, which is a tyrosine-derived complex polymer, defines the colour of the skin. In certain regions of the world, 'beauty' is often defined by fair skin colour, hence is the unsatiable quest for skin-whitening or skin-lightening agents that may enhance 'beauty'. This is not a new phenomenon; in fact, skin-whitening attempts started in many civilizations about 200 BCA; for example, the use of honey and olive oil as a method of whitening the skin in Egypt and Greece goes back centuries. From the very beginning, natural products, (organic *e.g.,* plant extracts, or inorganic *e.g.,* mercury), have played a major role as skin-whitening agents. Over the years, several plants, *e.g.,* green tea, pears, rose petal extract, olive oil, pomegranates, peony, *Phyllanthus emblica* and *Rosa roxburghii,* have been identified to have skin-whitening properties and various skin-whitening phytochemicals, *e.g.,* arbutin[1] and kojic acid[2], have been reported in the published literature. Some of these natural products have been formulated into commercially available cosmetic products. This talk will present an overview of skin-whitening natural products available to date and comment on their safety aspects.

Acknowledgements: We gratefully acknowledge Mae Fah Luang University Research Funding (651B02002) and the Reinventing University fund from the Office of the Permanent Secretary of the Ministry of Higher Education, Science, Research and Innovation, Thailand. Lutfun Nahar gratefully acknowledges the financial support of the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and the Czech Agency Grant - Project 23-05474S.

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**PL5**

# Phytochemical inducers of the antioxidant response

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Cancer chemoprevention is the generic term used to describe the process of using a chemical to prevent cancer. The prevention of cancer by such chemicals can generally be broken down into four distinct areas: reducing inflammation, increasing apoptosis, increasing cell defence genes and the prevention of metastasis. Currently, chemicals (both natural and synthetic) with the ability to modulate any one of these cancer promoting processes are classed as cancer chemopreventive agents. At the cellular level, much of the phytochemical modulation of cell defence genes can be distilled down to the effects of one transcription factor, NF-E2 p45-related factor-2 (Nrf2), which binds to the antioxidant response element (ARE) found in the regulatory regions of over 200 genes involved in both cellular protection.

In a quest to discover new phytochemicals with cancer chemopreventive capability, we have utilised a cell-based assay to identify phytochemicals with the ability to activate the transcription factor Nuclear factor-erythroid 2 p45-related factor 2 (Nrf2). In this presentation, our recent efforts to investigate the delicate balance that phytochemicals play in cancer will be considered.

Acknowledgement: Lutfun Nahar gratefully acknowledges the financial support of the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and the Czech Agency Grant - Project 23-05474S.

# PL6

## Plant secondary metabolites in wound healing

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Wound healing is an extremely complicated process that reflects a number of factors, both external and internal. It involves a cascade of events that includes inflammation, neovascularisation, collagen synthesis, granulation tissue formation, epithelialisation and wound remodelling[1]. A number of different cell types participate in this process, starting with fibroblasts, keratinocytes, adipocytes, endothelial cells, but also melanocytes or certain types of leukocytes. At the same time, the wound healing environment is characterized by a very dynamic redox environment, which somewhat extends our thinking about the importance of reactive oxygen species. These have so far been discussed very often in the context of their involvement in immune system responses, but their important role in signalling pathways is emerging[2]. This presentation discusses the possible contribution of different groups of secondary metabolites in the wound healing process with potentially practical applications.

Acknowledgements: Author thanks to the project MUNI/A/1547/2023, “Analysis of (dys)function: from molecules to living organisms”.

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**PL7**

# Phytocannabinoids: Reflexions from a two decade journey

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Modern studies on Cannabis and phytocannabinoids can be traced to seminal investigations carried out in 1940-1970 by Roger Adams in USA, František Šantavý in Czechoslovakia, and Raphael Mechoulan in Israel, the latter also pioneering studies on their molecular targets and clinical applications[1]. Spurred by the identification of cannabinoid receptors and the endocannabinoid system, biological studies on phytocannabinoids have then progressed regularly, culminating in the clinical introduction of Cannabis-based mainstream medicines (dronabinol, nabilone, nabiximol, cannabidiol)[2]. Conversely, phytochemical studies did not progress significantly after the mid-eighties of the past century, mainly because of legal restrictions on the study of Cannabis[3]. Thanks to a EU-sponsored project and the collaboration with plant genetists and pharmacologists, I had the opportunity to start systematic studies on what I considered uncharted areas of the phytocannabinoid chemical and biological space. Two main research lines were pursued, namely, the study of native (acidic) phytocannabinoids, and the one of minor phytocannabinoids. Acidic phytocannabinoids can be obtained in significant amounts by isolation, while many minor phytocannabinoids are only trace constituents, and their biological evaluation required the development of synthetic/semi-synthetic alternatives to isolation. Acidic phytocannabinoids are prone to decarboxylation, and many neutral phytocannabinoids are then easily oxidized under aerobic conditions. A critical part of our phytocannabinoid project was therefore aimed at the study of this “programmed” chemical diversity, and I will provide an outline of the most significant results obtained in exploiting the exuberance of Cannabis as a phytocannabinoid factory for biomedical research.

Acknowledgements: I am grateful to all colleagues and collaborators with whom I shared the excitement of two decades of research on Cannabis and phytocannabinoids

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**PL8**

# The magical story of coffee and its benefits in skincare

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The world of skincare is continuously evolving, with new ingredients such as coffee being utilized for their rejuvenating effects. This plenary talk will explore the ways in which coffee benefits the skin. Coffee is rich in antioxidants, capable of repairing the skin, and shielding it from environmental damage. Utilizing coffee grounds in face scrubs is highly effective as they exfoliate dead skin cells, leaving the skin smoother and more radiant. Additionally, coffee beans contain natural oils that moisturize the skin, making them valuable in various skincare products. Recent research indicates that coffee can stimulate the production of collagen and elastin, proteins crucial for maintaining skin elasticity and firmness. The primary active ingredient in coffee is caffeine, which is known for its anti-skin-cancer properties and helps protect against sun damage.[1] Coffee also contains chlorogenic acid which combats free radicals, prevent premature aging, and preserve youthful skin. Coffee grounds make excellent DIY face scrubs, gently buffing away dead cells for a brighter complexion. These advantages have spurred the development of new coffee-based skincare products designed to combat ageing.

Acknowledgements: We gratefully acknowledge Mae Fah Luang University Research Funding (651B02002) and the Reinventing University fund from the Office of the Permanent Secretary of the Ministry of Higher Education, Science, Research and Innovation, Thailand. Lutfun Nahar gratefully acknowledges the financial support of the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and the Czech Agency Grant - Project 23-05474S.

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## PL9

# Insect molting hormones: from ecological significance to drug discovery

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Ecdysteroids represent the most widely distributed group of steroid hormones that play a highly complex role in nature. In insects, 20-hydroxyecdysone is the so-called molting hormone. In plants, well over 500 of its analogs exist, and large amounts of these compounds are biosynthesized by many plant species as natural pesticides. In mammals, however, ecdysteroids are neither hormones nor toxic compounds: Some are known to act as adaptogenic and anabolic agents, increase resistance to stress, and provide various health benefits. Due to these, a worldwide market of ecdysteroid-containing food supplements, and its possible abuse in competitive sports made 20-hydroxyecdysone included in the watchlist of the World Anti-Doping Agency (WADA).
The wide chemical diversity of ecdysteroids makes these compounds a valuable pool for drug discovery, and their semi-synthetic derivatives may interfere with cell death and survival in either direction, i.e., using different chemical strategies, they can be engineered towards potent antitumor or cytoprotective agents. Our recent studies revealed that certain ecdysteroid-containing nanoassemblies sensitize CNS-originated tumor cells to oxidative stress while protecting the surrounding normal tissues.
The presentation provides an overview of the complex ecological role of ecdysteroids and on our recent developments on their drug discovery perspectives.

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**PL10**

# Getting published in 2024 and beyond: How to adapt to a rapidly changing publishing landscape

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Let's face it, the scientific publishing landscape has changed rapidly over the past years. From print-only to online access, and now to a world where +90% of the papers are found either through a search engine or social media. Interestingly, the process of bringing together your research into a publishable manuscript has not changed that much, but our reading (or media consumption) habits have changed drastically. I am going to show you various tips and tricks that you can use to adapt your manuscripts to the "new publishing world" — It is now more important than ever, not only to get published, but to get noticed.

**PL11**

# Auraptene: a tale of a natural product

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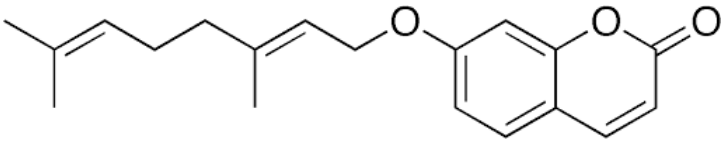
Auraptene (7-geranyloxycoumarin) **1** is the most representative compound of a rare class of natural products, the oxyprenylated phenylpropanoids.



**1**

Despite the fact that it has been isolated for the first time in 1930 from the peels and skin of fruits of *Citrus aurantium* L. (bitter orange), only in the last two decades this secondary metabolite has been subject of intensive research aimed at better characterizing new natural sources, its phytochemical and nutraceutical properties, and to get more insights into its biological activity and therapeutic potential. Like in a fairy tale with a happy ending lasting more than 20 years, auraptene has been characterized among the most interesting and promising active principle that can be obtained from a wide panel of plant sources.
At the very beginning of this "tale", a single easy to handle and high yielding synthetic step made auraptene **1** available in gram scale so that several *in vitro* pharmacological assays could be performed. Thus, it has been found how compound **1** exerted numerous beneficial effects mainly as an anti-inflammatory, anti-cancer, and neuroprotective agent. Moving to *in vivo* tests using suitable and predictive animal models such properties have been confirmed. In parallel, apart from the "classic" plant sources belonging to the Rutaceae family, investigations revealed how auraptene represented an additional component of the phytochemical pool of medicinal, healthy, and food plant species known to have beneficial properties for human health and for the dietary feeding prevention of acute and chronic syndromes. The overall data accumulated over the last 20 years did encourage the performance of a clinical trial in humans using phytopreparations derived from fruits of *Citrus limon* standardized in their composition for auraptene and total flavonoids as a nutraceutical remedy for the prevention of Alzheimer's disease.

**PL12**

# Natural products and synthetic derivatives as anti-coronavirus agents

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Coronaviruses (HCoVs) cause mild, moderate, or severe respiratory illnesses in humans. The COVID-19 pandemic, caused by SARS-CoV-2, has had significant global health and economic consequences. Despite significant advances in understanding the pathology and clinical management of coronaviruses, as well as creating effective vaccines, these viral diseases remain a public health concern due to the risk of recurrent outbreaks linked to the emergence of variants, unsatisfactory vaccination rates in at-risk populations and a lack of specific antiviral agents available against HCoVs. Our overall strategy is to identify pan-coronavirus antivirals, leveraging naturally occurring structural diversity. This presentation will be an opportunity to present certain collaborative work carried out which has highlighted the anti-coronavirus potential of certain substances of natural origin and synthetic analogues.

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**PL13**

# Analysis of agrimony extracts in wound healing

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*Agrimonia eupatoria* L. is an herb of the Rosaceae family, widely used in traditional medicine for its beneficial effects. The HMPC's conclusions on using agrimony medicines to relieve mild diarrhea, mouth and throat inflammation, skin inflammation, and wounds are based on their traditional use in these conditions. In previous studies, we established that *Agrimonia eupatoria* L. water extract (AE) possesses a rich polyphenolic composition, displaying remarkable antioxidant properties. Our research demonstrated its potential clinical utility in preventing and managing cardiovascular complications linked to diabetes and enhancing skin wound healing in rats[1].
These findings led us to extend the evaluation to fibroblasts and keratinocytes, as well as open and sutured wounds in rats, and to formulate a topical preparation to treat minor skin inflammation and small, superficial wounds. The total phenolic liberating capability of prepared gels was realized using a Franz diffusion cell system with different pH conditions. The *in vitro* and *in vivo* experiments revealed that AE extract induced the myofibroblast-like phenotype and enhanced extracellular matrix deposition in both experimental models. Furthermore, the wound tensile strength of skin incisions and the contraction rates of open excisions significantly increased in the AE-treated group.

Conclusion: The present data show that AE water extract significantly improves the healing of open and sutured skin wounds. Further testing in animal models that are physiologically and evolutionarily closer to humans will be necessary.

Acknowledgments: Authors thank Agency VEGA Grant No., 1/0226/22 and APVV-20-0017.

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## PL14

# Natural adenines exhibit an incredible range of biological activities

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The purine ring system (imidazo[4,5-d]pyrimidine) is among the most ubiquitous of all the heterocyclic compounds. Although purine itself has never been found in nature, substituted purines like adenine and guanine or their respective nucleoside/nucleotide derivatives are the most common class of nitrogen heterocycles which play crucial roles in a wide variety of functions of living species. As nucleotides (AMP, GMP), they are the building blocks of nucleic acids (RNA/DNA). They serve as energy cofactors (ATP, GTP), as part of coenzymes (NAD/FAD) in oxidation-reduction reactions, as important second messengers in many intracellular signal transduction processes (cAMP/cGMP, or as direct neurotransmitters by binding to purinergic receptors (adenosine receptors). Therefore, it is not surprising that the analogues of purines have found utility both as chemotherapeutics (antiviral, antibiotic, and anticancer agents) and pharmacodynamic entities (regulation of myocardial oxygen consumption and cardiac blood flow). Since the topic on adenine and its derivatives is obviously too vast and broad to cover it all in a single talk, we will mainly focus on medicinal and pharmaceutical aspects of adenines that are deemed most important based on the recent scientific discoveries. Of particular interesting is their ability to protect cells against various forms of stress and prevent some of the harmful effects of cell aging. For example, human skin fibroblasts cultured in the presence of cytokinin kinetin or *trans*-zeatin retain some characteristics of cells of lower passage. Kinetin is even able to increase the lifespan of invertebrates. The anti-proliferative activity of cytokinin ribosides (through induction of cell cycle block or/and cell death) and bases (through induction of cell differentiation) has prompted studies into their potential utility for the therapy of proliferative diseases like leukaemia, cancer and psoriasis. Furthermore, inhibitors of cyclin-dependent kinases olomoucine, bohemine, roscovitine, indirubin, etc., were inspired also by adenines. Particularly CDK5 inhibitors, as antiangiogenic agents, indicated that structure-activity analyses of $N^6$-substituted adenine derivatives could greatly facilitate the identification of potent new antiangiogenic compounds. In this presentation, the protective effects of phytohormones at molecular, cellular, tissue and organismal levels will be discussed. We would also like to discuss potential application of phytohormones for the treatment of age-related diseases, including neurodegenerations.

Acknowledgements: We wish to thank to Grant Agency of the Czech Republic, grant No. 23-05474S.

# PL15

## Antiadipogenic effects of coumarins from *Citrus x limon*

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The role of fat tissue as an endocrine organ, central to the biosynthesis of various hormones is - next to its close association to several metabolic diseases particularly in those suffering from obesity- a focus for a large body of ongoing research[1]. Given the various reported effects of herbal substances on several organ systems, their effects on adipose tissue are a promising, yet insufficiently explored area of research.
For fermented products of lemon an anti-obesity effect could be shown previously[2]. The aim of this study was to characterize the impact of a non-fermented lemon peel extract on adipogenesis, to identify bioactive compounds and to clarify the underlying mechanism. The study was performed with 3T3-L1 cells, which represent a well-established and widely used in vitro model to study aspects of adipocyte biology[3].
Applying a bioassay-guided approach, we identified coumarin derivatives in the flavedo layer of ripe lemon peels that reduced 3T3-L1 cell differentiation. LC-PDA-ESI-MS analysis revealed one of the active compounds as bergamottin whereas limettin was less potent.
Moreover, we shed light on the mechanism underlying the observed antiadipogenic effect.

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## PL16

# Enhancing oligosaccharide characterization: Leveraging the power of NMR spectroscopy

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The growing interest in diverse carbohydrates stems from their multitude of beneficial health properties, biocompatibility and widespread availability. However, within the realm of natural products, carbohydrates present notable challenges in characterization due to their complex structures. Consequently, the food and pharmaceutical industries require rapid and reliable yet non-destructive analytical techniques for effectively assessing their structures.
NMR spectroscopy stands out as an effective method for rapid and non-destructive analysis, offering detailed and direct structural insights into samples, enabling the simultaneous detection and quantification of byproducts, impurities, and residual reagents. Moreover, NMR spectroscopy, despite being categorized as a relatively insensitive technique, furnishes intricate structural details, especially beneficial in detecting subtle differences among isomeric carbohydrates. Its ability to provide the most comprehensive structural information underscores its significance in analytical chemistry.
This presentation will showcase ongoing projects in our lab, focusing on using NMR for characterizing bioactive oligosaccharides. We will discuss the challenges in characterizing microheterogeneous glycosaminoglycans and delve into their detailed structural analysis during degradation. Additionally, we present a straightforward and non-destructive NMR method for determining the substitution pattern of 2-hydroxypropyl-β-cyclodextrin, a key pharmaceutical excipient oligosaccharide. Lastly, we aim to highlight the utility of NMR in characterizing human milk oligosaccharides, emphasizing its extensive use in distinguishing isomeric structures through techniques like $^{1}H$-$^{15}N$ HSQC NMR.

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**PL17**

# Integration of liquid-liquid chromatography in natural product research

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Liquid-liquid chromatography (LLC), commonly referred to as countercurrent separation (CCS) or countercurrent distribution (CCD), is a solid-support-free preparative technique that uses the two liquid phases of a pre-equilibrated biphasic system composed of two or more solvents [1]. One of the liquid phases is maintained stationary inside the column with the help of a centrifugal field, while the other (mobile phase) is passed through it. Due to its high adaptability, versatility, and unique features, LLC can successfully separate complex metabolomic mixtures, such as plant extracts. The flow direction and role of the phases can be easily changed during operation, allowing for the implementation of various operating modes. Some of these modes are unique to LLC and are employed to enhance the separation resolution and productivity or reduce the processing time and solvent consumption [2]. The almost limitless variety of biphasic solvent combinations allows users to design a biphasic system tailored to a particular separation. LLC has been shown to be helpful for the purification of >2,500 natural products (NPs) with diverse chemical scaffolds [3]. As applications, numerous examples in which LLC was integrated as a complementary platform preceding the evaluation of the pharmacological activity of NPs will be presented. For instance, the separation of cannabinoids with cosmeceutical properties, the fractionation of coumarins with central nervous system modulatory and trypanocidal effects, the purification of lignans with anti-*Trychophyton* effects, or the isolation of piperamides with anti-*Helicobacter* activity will be discussed.

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**PL18**

# Essentials in the acquisition, interpretation, and reporting of plant metabolite profiles

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Plant metabolite profiling reveals the diversity of secondary or specialized metabolites in the plant kingdom. Specialized plant metabolites constitute a vast class of chemicals posing significant challenges in analytical chemistry. In order to be of maximum scientific relevance, scientific reports in this field must be transparent, make use of standards and reference materials [1], and be based on correctly and traceably identified plant material [2]. This includes: (i) critical review of previous literature and a reasoned sampling strategy; (ii) transparent plant sampling with material documented by vouchers in public herbaria and, optimally, seed banks; (iii) if possible, inclusion of generally available reference plant material; (iv) transparent, documented state-of-the art chemical analysis, ideally including chemical reference standards; (v) testing for artefacts during preparative extraction and isolation, using gentle analytical methods; (vi) careful chemical data interpretation, and (vii) taking all previous scientific knowledge into account in reporting the scientific data. From the current stage of the phytochemical literature, selected comments and suggestions are given. In the past, proposed revisions of botanical taxonomy were sometimes based on metabolite profiles, but this approach ("chemosystematics" or "chemotaxonomy") is outdated due to the advent of DNA sequence-based phylogenies. In contrast, systematic comparisons of plant metabolite profiles in a known phylogenetic framework remain relevant. This approach, known as chemophenetics [3], allows characterizing species and clades based on their array of specialized metabolites, aids in deducing the evolution of biosynthetic pathways and coevolution, and can serve in identifying new sources of rare and economically interesting natural products.

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**PL19**

# Quality, safety and efficacy of natural health product – how HPTLC, bioassays, and KI can contribute to evidence

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In recent years quicker and more thorough examination of natural products and their intricate compositions sourced from nature have been enabled by modern Phytochemical Analysis techniques like Liquid-Column and Gas Chromatography coupled with MS, along with NMR. The aims are still quantification of secondary metabolic analysis and standardization marker quantitation. Yet, even amidst these advancements, the simplicity of planar chromatography remains vital. Modernized into High Performance Thin Layer Chromatography (HPTLC), it retains its importance in pharmaceutical quality control, especially in Ph Eur monographs where it verifies plant material authenticity. However, HPTLC offers more than just quality control—it serves as both a planar solid phase extraction and fractionation tool in a single step. Its versatility extends to integration with MS or bioassays, such as bioautography[1], also known as "effect-directed analysis." Planar Mutagenicity assessment, and anti-fungal efficacy screening, are offering valuable insights and showcase its multifaceted applications in assessing the quality, safety, and efficacy of natural health products.
Moreover, when considering the quality, safety, and efficacy of natural health products, the combined application of Phytochemical Analysis and computational tools and artificial intelligence (AI) for natural products can significantly contribute to building robust evidence. Computational methodologies[2] ensure that data from various sources are synthesized effectively, enhancing the overall evaluation of product quality and safety and computational literature mining tools[3] offer efficacy data of single compounds as well as extracts.

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## PL20 *Dra. Mariola Macías Award*

# Taking Advantage of Allelopathy: Discovery of Natural Products and Design of New Strigolactone Analogues for Weed Management

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Parasitic weeds pose a threat to both crops and ornamental plants. Ineffective control can lead to substantial crop losses, highlighting the need for suitable management strategies. Recent research has notably focused on the species of *Orobanche*, *Phelipanche* and *Cuscuta* genera, which represent ongoing challenges in agronomy due to their persistent seedbank, the difficulty of controlling them after crop infection, and their capacity to evolve new biotypes that expand host species range and/or overcome existing crop resistance. Since agrochemicals based on allelochemicals are promising materials for sustainable weed control, a key approach is discovering accessible compounds capable of stimulating or inhibiting seed germination and seedling growth. Costunolide, a sesquiterpene lactone found in certain plants, presents a unique opportunity for designing, via semi-synthesis, bioactive compounds that emulate the activity of some phytohormones naturally influencing parasitic germination in ecosystems. Following this approach, various collections of strigolactone analogues and mimics were synthesized, which were active in bioassays against parasitic weeds. Their isomeric features led to the discussion of structure-activity relationships, being compared to those of natural strigolactones. Seeking to describe novel allelochemicals with activity on parasitic germination, subsequent screening studies of diverse chemical structures have yielded promising results, notably with compounds such as 2-benzoxazolinone, hydrocinnamic acid, and caffeic acid. Besides, new natural sources of bioactive compounds have been searched and identified, including extracts from safflower (*Carthamus tinctorius*) and *Centaurea cineraria*, which contained active strigolactones and sesquiterpene lactones. This presentation highlights the approaches of long-term research aimed at developing sustainable solutions for parasitic weeds, also helping to describe new models for the development of future agrochemicals based on natural products.Acknowledgements: Work cofunded by PID2020-115747RB-I00, PID2020-114668RB-I00, and RYC-2015-18961 projects, and Plan Propio-UCA, call “Investigadores Noveles, Proyectos para impulsar su Carrera Científica” (PR2023-026). J.G.Z. thanks Univ. of Cadiz for Margarita Salas support (2021-067/PN/MS-RECUAL/CD), funded by the NextGenerationEU program of the European Union. A.M.R. thanks JAE-Intro ICU (JAEIntro2021-5-IAS-02), CSIC.

**PL21**

# CYP1-catalysed bio-activation of the polymethoxy flavone chrysosplenetin

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The potential role of flavonoids, notably polyhydroxy flavones and flavonols, was confirmed in numerous *in vitro* assays, where these compounds showed anti-proliferative and anti-inflammatory activity.

Polymethoxy flavones generally show less activity in *in vitro* tests. However, *in vivo*, they remain in systemic circulation for a prolonged period. In pharmacological terms, their 'area under the curve' or AUC is larger, which means that their clearance rate from the body is slower and the extent of exposure to these compounds is higher.

Previous work in our group has identified a range of polymethoxy flavones that are substrates for the dealkylating enzymes CYP1A1 and CYP1B1. These two enzymes are over-expressed in a range of tumour cell lines. This means that dietary methoxy flavones can circulate in the human body for a prolonged period, causing little overall effect since they have low biological activity. However, only in selected tumour cells, the polymethoxy flavones are converted into polyhydroxy flavones – significantly increasing their anti-proliferative activity.

The polymethoxy flavone chrysosplenetin, a by-product of artemisinin extraction from *Artemisia annua* L., was shown to selectively inhibit the proliferation of CYP-1 expressing tumour cell lines. Our data indicate that chrysosplenetin itself shows little bioactivity, but the CYP1-catalysed conversion products have anti-proliferative activity.

**PL22**

# The challenges in the study of bioactive natural products using comprehensive analytical approaches

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The study of natural compounds from plants in the field of health and medicinal chemistry is full of successful examples leading to the development of many drugs as morphine, digitoxin, quinine and Taxol.[1] Furthermore, in the last years plant have been studied as functional ingredients for the production of nutraceuticals and herbal medicine. The research in this field have strongly evolved thanks to the technical improvement of chromatographic approaches, and the larger availability and easy of use of Nuclear Magnetic Resonance and Mass Spectrometry the application of metabolomics. But, the challenges in this field are still many. Isolation of new compounds, solubility and stability issues of purified compounds, availability of plant material for the extraction and purification processes are some of the "traditional" challenges in this field of research. The large diffusion of HR-MS spectroscopy and the improvement of software give new emphasis to analytical tools with the opportunity to perform dereplication studies in attempt to select more promising plant sources and the possible new compounds. In this talk the need of comprehensive approach combining different techniques comprising High- and Low-resolution Mass Spectrometry[2] as well as Nuclear Magnetic Resonances, bioactivity assays and multivariate data analysis will be described showing the importance in the study of natural bioactive compounds of comprehensive and multidisciplinary approaches.

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**PL23**

# Semisynthetic derivatives of galanthamine and their biological activity

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Galanthamine is a tertiary isoquinoline alkaloid isolated from several plants in the plant family Amaryllidaceae. The idea for developing a medical product from these species seems to be based on the local use of one of these species in a remote part of Europe. Today, galanthamine, especially under its brand names Reminyl® and Nivalin®, is commonly used in the treatment of Alzheimer's disease (AD) as a reversible competitive inhibitor of acetylcholinesterase. Its additional activity, the allosteric modulation of nicotinic ACh receptors, is responsible for its therapeutic benefit in AD.

Since this alkaloid has been isolated in gram amounts from plant material (*Narcissus pseudonarcissus* cv. Carlton), it has been used for the preparation of semisynthetic derivatives. The semisynthetic derivatives of galanthamine were screened for their *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra and other mycobacterial strains (*M. aurum, M. avium, M. smegmatis, and M. kansasii*). The most active compounds were also studied for their *in vitro* hepatotoxicity on the hepatocellular carcinoma cell line HepG2. In general, the derivatization of galanthamine was associated with a significant increase in antimycobacterial activity.

Moreover, newly developed derivatives were also screened on a panel of cancerous and non-cancerous cell lines. Some of the derivatives showed promising cytotoxic activity against tested cell lines.

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**PL24**

# Corn-Cockle phytochemical cocktail – How to make good stuff from old weed?

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Plants from the *Caryophyllaceae* family are widely known for their saponin content. Many of them are also abundant in type 1 ribosome-inactivating proteins (RIP) that are postulated to act in concert with certain saponins in boosting the RIPs toxicity by facilitating cell entry.
For example, an ancient and almost forgotten annual weed - *Agrostemma githago* L., once notorious for contaminating cereal grains, stands out for its notable combination of RIP and triterpenoid glycosides. Nowadays, modern weeding practices have nearly eradicated this plant, to the point where it is now considered endangered.
In addition to the well-known triterpenoid saponins and RIP, there is a high content of flavonoid *C*-glycosides, such as orientin that may render interesting pharmacological properties beyond the saponin/RIP related toxicity.
In history, some records were known on using *A. githago* in both official and folk medicine, but all are scarce and lack coherent information. Currently, the seeds are being marketed for use in blooming meadows in the urban environments.
In this paper, the state-of-the-art in corn-cockle phytochemistry will be presented as well as some outlooks into using biotechnological approach to understand the mechanisms of production and regulation of the major classes of phytochemicals and bioactive proteins. This direction may put the corn-cockle back on stage in pharmaceutical applications.

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OL14 **Valeria Iobbi**
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OL15 **Iasmina Honceriu**
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OL16 **Radim Havelek**
*The antiproliferative activity and apoptosis-inducing mechanism of the Amaryllidaceae alkaloid montanine on A549 and MOLT-4 human cancer cells*

OL17 **Melek Karaaslan**
*Analgesic and anti-inflammatory activity of some Glaucium taxons*

OL18 **Adrian H. Kopf**
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OL19 **Shamee Bhattacharjee**
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OL20 **Janniek Ritsema**
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OL21 **Jana Křoustková**
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OL22 **Chidoh Kootlole**
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OL24 **Hasan Kırmızıbekmez**
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OL25 **Nisa Beril Sen**
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OL26 **Livia Reguengo**
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OL28 **Augustin Bildstein**
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OL29 **Kajetan Grzelka**
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OL30 **Cristina Terenzi**
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OL31 **Maria João Rodrigues**
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OL32 **Senthil Kumar Kanthasamy Jayabal**
*Cosmetic applications of essential oils extracted from indigenous forest species of Taiwan*

OL33 **Jonas Kappen**
*Chasing molecules - a structure elucidation journey*

OL34 **Fabian Alperth**
*Extraction of Spilanthol from Acmella oleracea with Natural Deep Eutectic Solvents*

OL35 **Ceren Öztürk**
*Two new anthraquinones and other secondary metabolites from Asperula sintenisii Achers ex Bornm*

OL36 **Vahap Murat Kutluay**
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*Antioxidant activity and phenolic compounds contents of Tribulus terrestris L.*

**OL1**

# Lignans in wine

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In the plant kingdom, a large number of different specialized metabolites can be found that help the plant in its interaction with the environment. These specialized metabolites include lignans, which serve as protection for the plant against pathogenic microorganisms and herbivores. In humans, lignans are converted to enterolignans after ingestion. Both lignans and enterolignans exhibit antimicrobial, anti-inflammatory, antioxidant, and estrogen-like activities. One source of lignans in the human diet is wine, where, depending on the particular type of wine, lyoniresinol, isolariciresinol, secoisolariciresinol, and to a lesser extent syringaresinol, matairesinol and lariciresinol may be present. To investigate the possibilities of influencing the lignan concentrations in wine, we validated an LC-MS method for the determination of lignans in wine and grapes[1]. Our results show that the content of lignans in wine depends on the variety and vintage, but it can also be increased by using different maturation methods. In concrete, the content of lyoniresinol and syringaresinol depends on the barrel used for wine maturation, while the content of isolariciresinol and secoisolariciresinol can be increased by maturation in contact with the grape seeds and skins[1,2]. Consequently, wines richer in lignans may provide health benefits associated with the intake of these plant metabolites.

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## OL2

# The Receptarium of Burkhard from Hallwyl – from digital indexing historical recipes to pharmacological screening of plant ingredients

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The ethnopharmacological analysis of historical texts offers promising opportunities for drug discovery. Despite the vast amounts of knowledge contained in historical texts few studies have attempted to transfer this information into a modern context. In this work, we present the development of a relational database for indexing recipes contained in the Receptarium of Burkhard from Hallwyl (RBH) (16th century Switzerland) and the subsequent in vitro screening of plants mentioned in this resource.

Recipes in RBH directed at the treatment of skin complaints or topically manifest disease symptoms were recorded in a specially developed relational database consisting of 20 interlinked tables. Focusing on herbal recipes, we explored the mentioned plants and their uses by employing specific literature. Botanical identities of the plant names mentioned were determined and ranked based on their plausibility of being the correct identification. Historical plant uses were interpreted from a modern clinical perspective and the pharmacological basis of the associated diseases elucidated.

The database allows for quantitative and qualitative analysis of individual recipe parameters and targeted identification of candidate plants for screening campaigns. In order to assess the potential of RBH as a resource for pharmacological investigations, a preliminary in vitro screening was conducted. Eleven plants were selected the historical use of which seems to be associated with inflammatory conditions. Hydroethanolic extracts were then tested for cytotoxicity in NIH-3T3 murine fibroblasts and modulation of LPS induced IL-6 and TNF release in human whole blood and PBMC. Promising candidate plants were identified among which *Vincetoxicum hirundinaria* Medik. stands out, with the ethanolic macerate strongly inhibiting IL-6 release in PBMC ($IC_{50}$ 3.6 µg/mL).

Our work illustrates one possible approach to ethnopharmacological data mining of historical texts. We hope that our methodology will contribute to the discussion on solutions for transferring premodern pharmaco-botanical information into a modern context.

## OL3

# Unlocking the powers of *Fragaria vesca*: A revelatory dive into the impact of extraction techniques on seed oil potency and applications

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Wild strawberry (*Fragaria vesca* L.) seed oil (WSO) recovered by two methods – cold pressing (CP) and extraction with supercritical carbon dioxide ($SCO_2E$) – taking into account the different extraction time, was characterized for its composition and quality. The cytotoxicity assessment of WSOs was also carried out using normal human dermal fibroblast (NHDF) cell line[1]. Tocopherol, and total polyphenol contents were significantly higher in WSO recovered by $SCO_2E$, up to 1901.0, and 58.5 mg/kg, respectively in comparison with CP oil. In CP oil the highest content of carotenoids and squalene was determined (123.8 and 31.4 mg/kg, respectively). Phytosterol summed up to 5396 mg/kg in WSO collected in 30 minutes of $SCO_2E$, moreover the highest oxidative stability was found for this oil. All studied WSOs were non-cytotoxic in lactate dehydrogenase (LDH) leaching and sulforhodamine B (SRB) assays, however oils collected by $SCO_2E$ in 15 and 30 minutes were found to be cytotoxic in the tetrazolium salt (MTT) test with the $CC_{50}$ at the concentration of 3.4 and 5.5%, respectively. In conclusion, the composition of WSO indicates that depending on the method of its recovery from seeds can have different bio-potency and various applications.

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## OL4

# Phytochemical investigation and TRPA1/TRPM8 modulation profile of the cannabigerol-rich *Cannabis sativa* L. Chemotype IV

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*Cannabis sativa* L. Chemotype IV derives from a homozygotic $B_0/B_0$ breed of cannabis resulting in the presence of CBG/CBGA being the major phytocannabinoids (PCs), followed by CBD/CBDA (<0.05%)[1]. In our endeavour to explore lesser-known cannabis chemotypes[2], we undertook the first extensive phytochemical investigation of an industrial extract of *C. sativa* chemotype IV. Our previously developed expeditious workflow for the phytochemical analysis of cannabis extracts[3] allowed the isolation of 13 secondary metabolites, thereby enriching the repertoire of the PCs chemical family, with a particular emphasis on the identification of minor constituents pivotal for uncovering novel biological endpoints.

In addition to the expected CBGA/CBG, CBDA/CBD, nine new PCs were isolated and fully characterized by HR-ESIMS and 1D and 2D NMR. Newly isolated PCs included mono- or dihydroxylated CBGA/CBG analogs, a congener with a truncated side chain, cyclocannabigerol B, and cannabifuranols A and B, characterized by unprecedented structural architecture within PCs chemical family.

Isolated PCs were assayed on the receptor channels TRPA1 and TRPM8 unveiling a potent dual TRPA1 agonist/TRPM8 antagonist profile for some compounds. For one of isolated phytocannabinoids, chiral separation of the two enantiomers was carried out and resulted in the discovery of a synergistic effect on TRPA1.

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## OL5

# Unlocking the potential of Macedonian Cannabis wild-types: phytochemical profiling and UPOV descriptors assessment

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The EU Plant Variety Database lists 177 varieties of *Cannabis sativa* L. (hemp) so far. Wild growing Cannabis plants have been detected in Macedonia but their origin remains uncertain in terms of their origin and classification as autochthonous, domesticated, or remnants of cultivated populations.

Our study aimed to evaluate the distinctiveness of Macedonian wild-growing Cannabis plants by conducting morphological and phytochemical characterization. Plants were collected in seed maturation stage from two areas in Eastern Macedonia and seeds were obtained. After successful germination with scarification method, they were outdoor cultivated, along with four commercial Cannabis strains (high CBD (Charlotte's Angel), high Δ9-THC (Bubba Kush x OG Kush), and two 50:50 CBD:Δ9-THC strains). Morphological assessment based on the International Union for the Protection of New Varieties of Plants (UPOV) descriptors was conducted for all cultivated Cannabis samples. Additionally, germination, growth and flower yield were accessed. Phytochemical analysis on cannabinoids followed the German pharmacopoeial monograph for Cannabis inflorescence.

All commercial strains' seeds showed 100% germinability, whereas wild-types germination rates varied from 65% to 20%. The highest flower yield mass from a single plant was obtained from Charlotte's Angel (128.5 g), while the lowest was from Bubba Kush x OG Kush plant (22.5 g). Cannabis cultivated wild-types yield of flowers varied from 104.7-39.9 g. According to UPOV descriptors cultivated wild-type plants were distinguished from the commercial ones. Analysis of cannabinoids in all cultivated wild-type plants exhibited a prevalence of CBD, with total CBD concentrations ranging from 3.43% to 7.61% w/w. The highest CBD concentration of 12.85% w/w was determined in a commercial Charlotte's Angel. Principal Component Analysis (PCA) was performed with four main components that cumulatively explain 99% of the variability of the parameters. The statistical descriptors of the Partial Least Square-discriminatory (PLS-DA) model (R2X=0.852, R2Y=0.856, Q2=0.817) indicate satisfactory predictive capabilities for cannabinoid-based differentiation of the analyzed samples.

This study reveals the UPOV morphological distinctiveness of wild Macedonian Cannabis from commercial strains and underscores its prevalent CBD content, suggesting its potential registration as a distinct Macedonian Cannabis landrace.

**OL6**

# New steroidal saponins from leaves of *Agave bracteosa*: structural characterization and cytotoxic activity

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The genus *Agave* comprises more than 400 species and are known for their various uses and applications, as source of fiber, food and beverages. Currently, species of this genus have been investigated for their metabolic content, and, in particular, saponins are the compounds of main interest. These metabolites play a critical role in the response of plants to different stress situations, and the use of different separation techniques is required, to achieve their structure elucidation or the evaluation of their biological activity. Since no saponins have been described for this species, the current work was addressed to the phytochemical study of *Agave bracteosa* leaves. Dried plant material was extracted and screened through UPLC-QTOF-MS$^{E,}$ NMR spectroscopy for the identification of the main saponins, and cytotoxicity assays. Subsequently, the crude extract was chromatographed and the individual fractions so obtained purified by HPLC, thus obtaining saponins in pure form. Characterization of each compound was carried out using the "HMAI method" (NMR spectroscopy and the HMBC method for aglycone identification), for aglycone and 1D and 2D NMR for sugar chains. In this way, steroidal saponins were elucidated and tested for the cytotoxic activity.

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## OL7

# *In situ* characterisation of plant defence phytohormones and related metabolites *via* advanced mass spectrometry imaging

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Phytohormones are a group of small signalling molecules that play an important role in activating defense-related gene expressions and counteracting exogenous phytotoxic metabolites. Although the presence of defense phytohormones including salicylic acid, jasmonic acid, ethylene as well as their metabolites have been identified in plant immune networks, the metabolomics interactions between host plants and invading pathogens are still unclear. Identification of the large number of low molecular weight compounds resulting from the plant defense response requires an untargeted metabolomic method with the implementation of multiple analyte detection platforms. Plant biotic stress induced by pathogenic microbe infections such as phytobacteria or oomycetes represents a significant problem in crop production[1]. However, current studies focusing on host plant immunity and metabolites involved in the defense response are very limited. We have recently applied label-free desorption electrospray ionization mass spectrometry imaging (DESI-MSI) in high-throughput visualizations of multiple phytohormone species and synthetic phytohormone derivatives from *Arabidopsis* samples[2,3]. DESI-MSI coupled with ion mobility was performed on the untargeted analysis of plant defense-related compounds from *Arabidopsis* leaves pre-inoculated with *Pseudomonas syringae*. Our results demonstrated unique distributions of predicted defense phytohormones with their precursors and metabolites from infected and control leaves. Further segmentation analysis of infected leaf areas established different spatial correlations of endogenous molecules with selected microbial metabolites. This suggests that DESI-MSI is not only able to interpret various biomolecules from both plant host and invasive microbes with up to 200 μm spatial resolution, but also provides insight into plant hormonomics and metabolomics changes under the microbially induced biotic stress.

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# OL8

## Plant triterpenoid saponins – from structure to bioactivity

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Saponins are triterpenoid amphipathic molecules that exhibit detergent-like properties. Due to their potential ability to integrate into membranes with the hydrophobic triterpenoid moiety, it is proposed that they wedge into membranes and thereby disrupt the cell membrane integrity of herbivore pests, causing cell death. These specialized metabolites usually occur in its natural source in a complex mixture of several diverse chemical structures and therefore it has been difficult to determine individual saponin biological activities.
We present a fast and efficient way to fractionate and purify large amounts of single saponins from a complex plant extract via flash chromatography coupled to UV and ELSD. Additionally, we use yeast integrity assays to correlate saponin structure with biological activity. In this way, we elucidate potentially highly bioactive saponins that bear potential for commercially useful biopesticides. Furthermore, these purified saponins allow us to elucidate the general principle of saponin mode of action.
Recently we have employed large unilamellar vesicles in order to test purified saponins and study their behaviour in simple artificial membrane systems composed of different lipid and sterol compositions. We found that non-plant derived sterols make vesicles susceptible to saponin induced pore formation whereas plant sterols protect vesicles from breaking.

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## OL9

# *In vitro* antidiabetic and anticholinesterase potentials of eleven medicinal plants from Lamiaceae naturally grown in Turkey

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Lamiaceae family is one of the largest and most widespread flowering plant families, found notably in the Mediterranean, Central and Minor Asia and is represented with over 7200 species and 240 genera[1]. *In vitro* antidiabetic and anticholinesterase activities of essential oils, methanol, and aqueous extracts obtained from aerial parts of *Origanum vulgare*, *O. leptocladum*, *Nepeta sulfuriflora*, *N. isaurica*, *N. cilicica*, *Thymus cilicicus*, *Ziziphora clinopodioides*, *Cyclotrichium origanifolium*, *Hyssopus officinalis*, *Ajuga chamaepitys*, *Marrubium globosum* growing in Turkey were herein examined. The antidiabetic activity was evaluated against both $\alpha$-glucosidase and $\alpha$-amylase. Anticholinesterase activity was tested against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE)[2]. Among the tested essential oils, *O. leptocladum* and *N. isaurica* exhibited the highest activity against $\alpha$-glucosidase, with inhibitions of 14.39 % and 14.25 %, respectively, while acarbose showed 74.72% inhibition. In terms of extracts, both MeOH and aqueous extracts of *O. leptocladum* displayed significant inhibitions, with 90.10% and 76.22% inhibition, respectively. *T. cilicicus* MeOH extract also showed notable inhibition at 60.05%, surpassing the effect of acarbose. For $\alpha$-amylase inhibition, *C. origanifolium* and *T. cilicicus* essential oils exhibited the highest activities, with inhibitions of 49.54% and 42.67%, respectively, while acarbose showed 67.87% inhibition. Among the extracts, *M. globosum* and *N. cilicica* MeOH extracts displayed the highest inhibitions, with rates of 33.76% and 31.66%, respectively. In anticholinesterase inhibition assays, *C. origanifolium* essential oil showed the highest activity against AChE with 11.84% inhibition, and *O. leptocladum* displayed 17.06% inhibition against BChE. Among the extracts, *O. vulgare* MeOH extract exhibited the best activity against AChE at 29.10%, while *T. cilicicus* showed the highest activity against BChE at 35.44%. Donepezil demonstrated high efficacy in both experiments, from both essential oils and extracts.

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## OL10

# Cellular responses and targets in food-spoilage yeasts exposed to antifungal prenylated isoflavonoids

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Prenylated isoflavonoids are phytochemicals with promising antifungal properties. The highly promising, antifungal prenylated isoflavonoids, glabridin and wighteone were shown to disrupt the plasma membrane (PM) of the food spoilage yeast *Zygosaccharomyces parabailii* in distinct ways. This observation prompted a more in-depth investigation into their modes of antifungal action (MoA) by using advanced molecular tools on spoilage yeasts. Transcriptomic profiling with *Z. parabailii* showed that genes encoding transmembrane ATPase transporters, including Yor1 and genes homologous to the PDR subfamily in *Saccharomyces cerevisiae*, were upregulated in response to both compounds. Gene functions involved in fatty acid and lipid metabolism, proteostasis and DNA replication processes were over-represented among genes upregulated by glabridin and/or wighteone. Chemogenomic analysis using the genome-wide deletant collection for *S. cerevisiae,* further suggested an important role for PM lipids and PM proteins for both compounds. Using lipid biosynthesis inhibitors, we corroborated roles for sphingolipids and ergosterol in prenylated isoflavonoid action. The PM ABC transporter Yor1, and Lem3-dependent flippases conferred sensitivity and resistance, respectively, to the compounds, suggesting an important role for PM phospholipid asymmetry in their MoAs. Impaired tryptophan availability, likely linked to perturbation of the PM tryptophan permease Tat2, was evident in response to glabridin. Last, substantial evidence highlighted a role of the endoplasmic reticulum (ER) in cellular responses to wighteone, including gene functions associated with ER membrane stress or with phospholipid biosynthesis, the primary lipid of the ER membrane. Overall, this study highlights that cellular actions of these two prenylated isoflavonoids share similarities (at the level of the plasma membrane) but also differences. Understanding the mode of action of these novel antifungal agents is essential for their application in food preservation. This study exemplifies the use of genomic tools combined with testing of arising hypotheses to shed new light on the antifungal action of prenylated isoflavonoids.[1]

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## OL11

# Integration of plant-based antimicrobials and magnetic fields for enhanced penetration of pathogenic biofilms

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The escalating challenge of antibiotic resistance underscores the urgent need for novel therapeutic strategies[1]. Plant-derived antimicrobials, with their broad and non-specific modes of action, exemplified by compounds such as 1,8-cineole, thymol, and abietic acid, offer a promising avenue against pathogenic biofilms. However, the protective extracellular matrix of biofilms significantly impedes the penetration of these compounds, limiting their efficacy[2]. In this study, we introduce a novel approach employing a magnetic field with precisely controlled parameters, including frequency and waveform, to disrupt the integrity of the biofilm matrix and alter microbial cell walls. This innovation enables plant-derived antimicrobials to infiltrate biofilms more effectively and exert their antimicrobial action. Our findings suggest that integrating magnetic field exposure with plant-based antimicrobials can significantly reduce the required effective concentration of these compounds by up to several dozen percent, depending on the specific antimicrobial, microbial strain, and magnetic field characteristics. This reduction has promising implications for the pharmacokinetics and pharmacodynamics of antimicrobials designed to combat biofilms, particularly those associated with chronic wounds, pulmonary infections, and osteomyelitis[3]. Our research opens new pathways for the development of more efficient, targeted treatments for biofilm-related infections, highlighting the potential of combining plant-based antimicrobials with magnetic field technology.

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## OL12

# Evaluation of the chemical content and biological activities of "*Orobanche ramosa*" L.

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The *Orobanche* genus, belonging to the Orobanchaceae family, is globally distributed and entirely parasitic in nature. *Orobanche* species have been found to contain a diverse array of compounds, including phenylethanoid glycosides, steroids, terpenoids, organic acids, alkaloids, lignans, and flavonoids. These plants are utilized in traditional medicinal practices across Europe, America, and Africa. Extracts of the plant, collected from agricultural lands and identified, underwent comprehensive LC-MS/MS analysis, quantifying 53 phytochemicals. Antioxidant (DPPH and ABTS assay), enzyme inhibition (anti-tyrosinase, anti-elastase, anti-acetylcholinesterase, and α-glucosidase), and cytotoxic activities were evaluated. The highest total phenol and flavonoid content were observed in the butanol extract at 333.32±5.3 mg $_{GA}$/g $_{extract}$ and 298.31±7.7 mg $_{RE}$/$g_{extract}$, respectively. LC-MS/MS analysis revealed quinic acid (58.619 mg/$g_{extract}$) in methanol extract, protocatechuic acid (3.478 mg/$g_{extract}$) in ethyl acetate extract, and vanillic acid (6.198 mg/$g_{extract}$) in butanol extract among the compounds detected in high amounts. Evaluation of the biological activities of the extracts demonstrated significant ABTS and DPPH radical scavenging effects for ethyl acetate, butanol, and methanol extracts. In cancer cell lines, cell viability was 18% for HeLa-CCL-2 cells treated with ethyl acetate extract at 1000 µg/mL, and 15.51% and 13.86% for COLO 205-CCL-222 cells treated with hexane and ethyl acetate extracts, respectively, at the same concentration. Butanol, hexane, and ethyl acetate extracts exhibited significant tyrosinase inhibitory activity of 99%, 97%, and 91%, respectively, at a concentration of 2500 µg/mL. Additionally, hexane extract demonstrated strong α-glucosidase enzyme inhibition activity (95% at 1 mg/mL concentration).

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## OL13

# The interaction of silymarin with the gut microbiota and its differences with age

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This study evaluates the interaction between dietary silymarin, a flavonolignan complex from milk thistle, and the gut microbiota, highlighting the effect of age on this interaction. The investigation used a combination of next-generation sequencing (NGS), $^{1}$H-NMR, and LC-MS technologies to study the interactions between silymarin and the gut microbiota in healthy young (12–45 years) and healthy elderly (70–80 years) individuals whose microbiota differ.
Twenty microbial silymarin catabolites were identified. The biosynthesis of short-chain fatty acids was found to be suppressed by silymarin. The study also revealed significant age-related differences in silymarin metabolism, with older subjects producing two silymarin metabolites at a significantly higher rate than younger subjects. It was found that the production of these catabolites, which are typical of older donors, was related to the abundance of the genus *Oscillibacter*. In contrast, the genus *Faecalibacterium* was more abundant in the younger group. This was correlated with the degradation of silymarin components. Additionally, the presence of silymarin altered the properties of the intestinal microbiota by increasing its alpha diversity at the order level. These findings highlight the importance of age-specific dietary strategies and contribute to our understanding of the role of the gut microbiome in flavonolignan metabolism. The insights gained from this study provide a basis for the development of personalized nutritional interventions to improve gut health at different stages of life.

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## OL14

# Characterization and sustainable agrochemical application of Vessalico garlic ecotype

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Vessalico garlic ("Aglio di Vessalico"), one of the best-known Italian garlic ecoytpes, is cultivated in Ligurian region, being very appreciated for its shape and aroma, and considered as agri-food excellence of the region. Local farmers use every year garlic cloves of two border French cultivars, Messidor and Messidrôme for sowing, and this fact can affect the "Protected Designation of Origin" registration process. To improve the geographical characterization of Vessalico garlic and to identify its peculiar features in terms of bioactive compounds, LC/MS followed by multivariate data analysis, was firstly applied. Results allowed to differentiate the Vessalico garlic from the French cultivars, and to identify three sub-classes in the accessions of Vessalico ecotype. An NMR-based analytical approach (1, 2) was then applied to identify characteristic chemical markers to classify the product of the farms of the Vessalico geographical area as true Vessalico garlic. Results showed that the content of primary metabolites appeared to be more related to genetic characteristics, while the secondary metabolites appeared to be more related to the area and to the methods of cultivation and harvesting. Self-organizing maps (SOMs) showed that only one homogeneous product of Vessalico appeared well characterized compared to the others. Among the secondary metabolites, *S*-allylcysteine was quantified, leading to the possibility of considering it as a marker to identify Vessalico garlic. Finally, to promote a sustainable agriculture, Vessalico garlic extracts were investigated as possible agrochemicals. All extracts showed ability to deactivate two plant virus infectivity, as a possible consequence of disassembly of the virus coat protein (CP). Molecular docking showed a strong interaction of the sulfur compounds with a high number of residues into ToBRFV CP binding site.

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**OL15**

# Anti-Alzheimer effects of Paradol, mansorin A, and mansonone G in a zebrafish model of Alzheimer's disease induced with okadaic acid

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Alzheimer's Disease (AD) is the most prevalent type of dementia and is still incurable. Given its high socio-economic impact, the World Health Organization stated that novel anti-AD treatments are imperative to discover and develop. Paradol (PD), mansorin A (MA), and mansonone G (MG) are plant-derived phenolic ketones with neuroprotective effects. This study explored the anti-AD effects of PD, MA, and MG using an okadaic acid-induced zebrafish model of AD. First, the animal model was induced by immersion of animals in okadaic acid 10 nM for four days, then treated with PD, MA, and MG, each in three concentrations: 1, 3, and 6 µg/L. The study used several tests to evaluate the effects of these treatments on the animal model. Y-maze and Novel Object Recognition (NOR) tests were performed to assess short-term memory in the experimental groups. The Novel Tank Diving Test (NTT) and Novel Object Approach Test (NOA) were used to evaluate the anxiety-like state. Biochemical analysis assessed the levels of oxidative stress markers and the levels of acetylcholinesterase. *In silico* analysis was performed to investigate some physicochemical and pharmacokinetic parameters. The results indicate the promnesic effects of PD, MA, and MG by enhancing the short-term spatial memory in the animal model, as indicated by the Y-maze task and the short-term recognition memory (NOR test). They also have anxiolytic benefits, according to the results of the NTT and NOA tests. PD, MA, and MG act as antioxidants in the animal model by increasing the catalase and reduced glutathione levels. They also inhibited the acetylcholinesterase activity. The *in silico* analysis indicated that PD, MA, and MG are blood-brain barrier permeant drug-like molecules. In conclusion, through promnesic, anxiolytic, antioxidant, and acetylcholinesterase inhibitory properties, MA, MG, and PD are potential dementia treatments with good bioavailability.

## OL16

# The antiproliferative activity and apoptosis-inducing mechanism of the Amaryllidaceae alkaloid montanine on A549 and MOLT-4 human cancer cells

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There is increasing interest in isoquinoline alkaloids from Amaryllidaceae plants for new anticancer drugs. Montanine-type alkaloids are a less common subclass in this family, yet their specific anticancer mechanisms remain poorly documented. In this study, we screened montanine, manthine, and 15 semisynthetic montanine analogues at a test dose of 10 μM to explore their cytotoxic activities. In the initial single-dose screening, montanine and its derivatives, specifically **12** and **14**, exhibited the highest cytostatic activity. However, native montanine demonstrated the most potent antiproliferative activity. Montanine also displayed significant antiproliferative activity with $IC_{50}$ values of 1.09 μM and 1.26 μM against A549 and MOLT-4 cells, respectively. Investigations on cell death mechanisms revealed ability to induce apoptosis in MOLT-4 cells through caspase activation, mitochondrial depolarization, and Annexin V staining. The Western blot demonstrated that montanine induced the upregulation or activation of pro-apoptotic and cell cycle checkpoint proteins, including p21, Bax, p38 MAPK phosphorylated at Thr180/Tyr182, and Chk1 phosphorylated at Ser345. These findings provide novel insights into the cytostatic, cytotoxic, and proapoptotic effects of montanine alkaloid in lung adenocarcinoma A549 and acute lymphoblastic leukemia MOLT-4 cells.

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**OL17**

# Analgesic and anti-inflammatory activity of some *Glaucium* taxons

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The genus *Glaucium* Mill. (Papaveraceae) is distributed in Europe, Southeast and Central Asia, North Africa and North America with approximately 30 species, and is represented by 11 taxa in Turkey. Plants of this genus stand out especially with the isoquinoline alkaloids they contain. In the content of this study, the analgesic and anti-inflammatory effects of *Glaucium corniculatum* (L.) Rud. subsp. *refractum* (NAB) Cullen ve *Glaucium leiocarpum* Boiss. were evaluated. The ethanolic extracts and alkaloid fractions were prepared using the aerial parts. LC-MS analyses were conducted for the purpose of evaluation of the phytochemical profiles of the total extracts and the alkaloid fractions.

Carrageenan-induced hind paw edema test was conducted on Wistar Albino rats in order to determine the anti-inflammatory activity, while tail flick and hot plate tests were performed on *Mus musculus* Balb/C mice for the evaluation of analgesic activity. For all three experimental models, the animals were divided into twelve groups comprising 6 members in each, namely; control, reference, extract groups (for the doses of 50; 100; 200 mg/kg) and fraction groups (for the doses of 50; 100 mg/kg).

The ethanolic extract of *G. leiocarpum* (200 mg/kag) was found to possess anti-inflammatory activity between the 2nd and 6th hours, while the alkaloid fraction of *G. corniculatum* (100 and 50 m/kg) inhibited the edema between 1st and 3rd hours, although the activity was not found to be in a dose-dependent manner. As the result of analgesic activity tests, it was determined that the ethanolic extract of *G. leiocarpum* showed the highest activity at the dose of 200 mg/kg. The results of the study revealed that the total extract of *G. leiocarpum* exhibited a higher analgesic and anti-inflammatory effects than the alkaloid fractions.

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## OL18

# 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. Due to the harsh environment that seaweeds grow in, they produce a variety of bioactives with unique chemical scaffolds[1].

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)[2]. 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 both 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, such as fucophlorethol A)[3]. This class of unique phenolic compounds shows promise for the valorisation of brown seaweed species.

This work demonstrates the systematic study of seaweeds, including the isolation and characterization of bioactive secondary metabolites. In summary, we have developed a highly sensitive pipeline for non-targeted screening and characterization of antimicrobial compounds in seaweeds, for the valorisation of this abundant marine resource.

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## OL19

# Spice-derived bioactive components inhibit cancer metastasis by targeting cellular signaling pathways

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Spices have been used to impart flavour, colour and taste to cuisines in various countries. The distinct aroma of spices, which provide an ethnic identity to cuisines, is contributed by their constituent bioactive secondary metabolites. Owing to these phytochemicals, the culinary use of spices is complemented by several health-promoting benefits including anti-cancer potential[1]. Cancer is a significant public health challenge globally with metastasis causing 90% of all cancer-related deaths. Therefore, development of anti-metastatic agents is extremely pertinent[2]. We present here the *in vitro and in vivo* anti-metastatic activity of two spice principles, 1,8-cineole (active component of cardamom) and capsaicin (active component of chili peppers) in cancer cells and murine transplantable tumor models. Scratch wound assay, transwell migration assay, gelatine zymography and detection of molecular markers of metastasis and epithelial to mesenchymal transition (EMT) by qPCR, immunofluorescence and immunoblotting were performed.

1,8-cineole and capsaicin respectively inhibited B16 melanoma and AGS gastric carcinoma cell migration and invasion accompanied by a reduction in mesenchymal markers such as Slug, Snail and Vimentin and increase in the epithelial marker E-cadherin. The phytochemicals also downregulated matrix metallo protease (MMP) activities. The results were validated *in vivo* using transplantable tumor models viz. B16 melanoma and 4T1 breast carcinoma. Finally, we also demonstrated that the anti-metastatic activity of 1,8 cineole and that of capsaicin is mediated by targeting the PI3K/Akt/mTOR and TGFβ signalling pathways respectively.

Collectively the results underline the potential of these phytochemicalsas anti-metastatic agents. However, the application of spice active principles in the treatment of cancer is often marked with caution because of their hormetic effect on cancer cells[3]. Therefore, future studies should be directed at assessing the effect of phytochemicals over a wide-range of concentrations alone and in combination with conventional chemotherapeutic agents.

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## OL20

# Antibacterial prenylated (iso)flavonoids from Fabaceae: 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[1]. Very potent antimicrobial diprenylated (iso)flavonoids, however, did not show membrane permeabilization in *Listeria monocytogenes*[1]. 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 ($EC_{10}$).

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**) ($EC_{10}$: 23±7 µg/mL) and neobavaisoflavone (**2**) ($EC_{10}$: 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 ($EC_{10}$) could not be explained by relative hydrophobic surface area, as found for *Listeria monocytogenes*. Conversely, polar surface area was positively correlated to $pEC_{10}$, 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.

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## OL21

# Carltonines from *Narcissus pseudonarcissus* cv. Carlton and lead optimization

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The newly discovered alkaloids, carltonines A-E, were isolated from *Narcissus pseudonarcissus* cv. Carlton. Following comprehensive structure analysis, the pure new compounds were evaluated for *in vitro* inhibitory activity on human acetylcholinesterase (*h*AChE), butyrylcholinesterase (*h*BChE), and propyl oligopeptidase. Notably, they exhibited a selective inhibition of *h*BChE over *h*AChE with excellent $IC_{50}$ values[1].

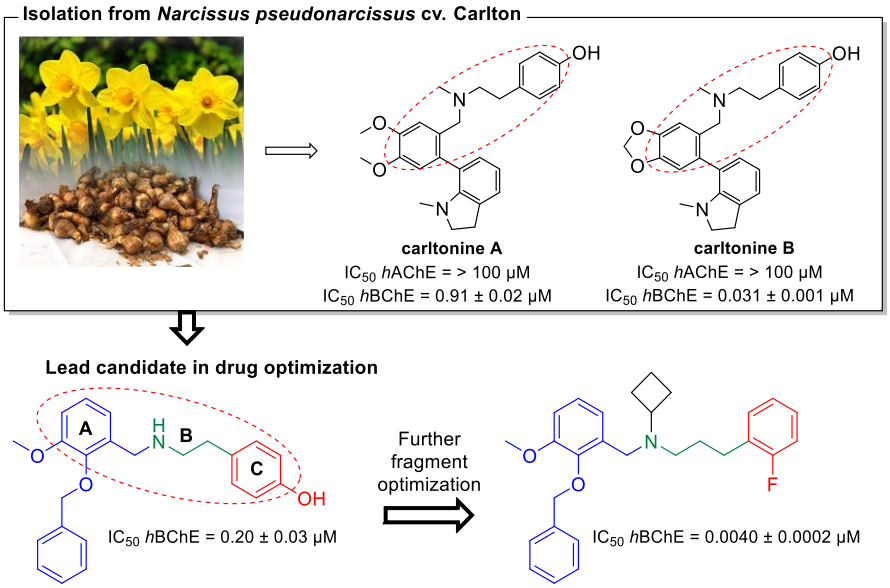


**Figure:** An overview of the workflow in this project. Isolation, followed by structure optimization, yields a molecule capable of inhibiting *h*BChE at a nanomolar concentration.

Indeed, the outstanding activities associated with the relatively simple scaffold motivated us to optimize the structure to achieve enhanced *h*BChE inhibition (Figure). This endeavor resulted in the synthesis of a derivative with $IC_{50}$ *h*BChE = 3.8 ± 0.2 nM[2].

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**OL22**

# NMR-based metabolomics and anti-leukemic activity of plants used in traditional medicine in Botswana

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Throughout the years, there have been great advancements in cancer medicine with an effort to treat leukemia. Regardless of these several immunotherapies, leukemia remains the leading cause of cancer related deaths in the world. Some of these therapies are associated with adverse effects, as well as surfacing of drug resistance. Therefore, continuous search for new compounds remains crucial.
With the help of NMR-based metabolomics, this study aims at identifying compounds from selected Botswana plants and further evaluating their anti-leukemic activity. Through ethnopharmacological knowledge, nine plant species from different families were identified and extracted. The extracts were subjected to NMR analysis for their chemical characterization. Partial purification of the plants' crude extracts using amberlite XAD-4 and XAD-7 was done using water and methanol as eluents. The effects of the obtained methanol fractions on cell viability, cell cycle and cell death in human U937 leukemia cell line were examined using flow cytometry-based assays. Following the treatment, induction of cell death and strong modulation of cell cycle was observed, particularly with fractions deriving from *Maytenus senegalensis*, *Elaeodendron transvaalense*, and *Ozoroa paniculosa*. The NMR profiles showed that these fractions were characterized by the presence of phenolic compounds, terpenoids, and alkaloids. Further experiments are ongoing to obtain pure compounds, including their structures, from the most active extracts.

## OL23

# Selective isolation and concentration of oleuropein and elenolic acid from *Olea europaea* L. aqueous leaves extracts

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The cultivation of olive trees and the production of olive oil generate massive amounts of waste. For this reason, the valorization of by-products deriving from *Olea europaea* L. (Fam. Oleaceae), has been a topic of great interest in recent years[1]. Leaves, the most abundant waste, are already widely known as remedies for infections, hypertension, arrhythmia, intestinal spasms, cancer, and many others and thus their extract is used as main ingredient of several pharmaceutical products. Indeed, they are rich in health-promoting functional components, of which the secoiridoid glycoside oleuropein is one of the most abundant, together with its aglycones hydroxytyrosol (HT) and elenolic acid (EA), the latter less biologically studied until now[2].

To this regard, a microwave assisted procedure was performed to obtain an aqueous extract with a high content in oleuropein. Subsequentially the active compound was selectively isolated through solid phase adsorption (SPA) with the use of lamellar solids, powerful tools to enhance the chemical stability of the molecule, and surely facilitating its use in olive-leaf based phytopreparations. Finally, an eco-friendly, readily available, and reusable catalyst like $H_2SO_4$ supported on silica was applied for the hydrolysis of oleuropein into its two main components. An extract enriched in the monoaldehydic dihydropyran form of EA was obtained by an acid-base work-up and this was later on characterized by GC-MS. A semipreparative scale HPLC method was also validated for the final isolation of EA.

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## OL24

# Unprecedented phenolic compounds from *Prospero autumnale* (L.) Speta with *in vitro* cytotoxic activities

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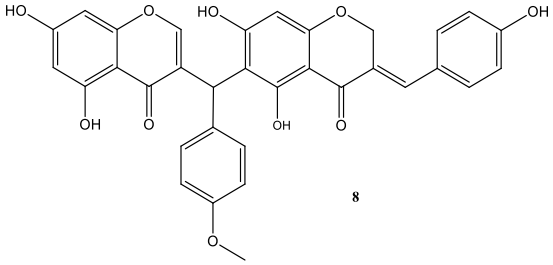
In our previous study, we reported the isolation of six secondary metabolites (**1-6**) from *P. autumnale*[1]. As a part of our ongoing study on the same species, we aimed to isolate further secondary metabolites and evaluate the *in vitro* cytotoxic activities of all compounds. Phytochemical studies on the EtOAc fraction of the EtOH extract prepared from the bulbs led to the isolation of a new homoisoflavonoid, 3*R*-(4′-hydroxybenzyl)-5,6,8-trihydroxy-7-methoxy-4-chromanone (**7**), a new dimeric homoisoflavonoid (**8**) and a novel homoisoflavone-stilbene type heterodimer (**9**). Chemical structures of the isolates were determined by NMR and HR-MS analyses. This is the first report on the isolation of a homoisoflavone-stilbene type heterodimer as well as a dimeric homoisoflavonoid from a natural source. The *in vitro* cytotoxic activities of all compounds (**1-9**) were evaluated against colon (HCT116, LoVo), prostate (DU145, PC3), liver (HEP3B, HEPG2) and breast (MCF7, MDA-MB-231) cancer cell lines by MTS assay. Compounds **1-3** and **9** exhibited potent cytotoxicity towards one or more cell lines ($IC_{50}$: 8.2-36.7 µM). Furthermore, these compounds were shown to induce apoptosis, necrosis and cell cycle arrest showing variation depending on the cancer cells.

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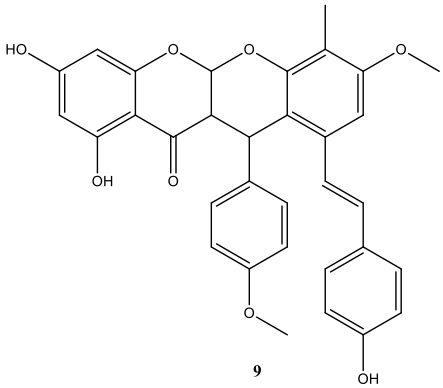


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## OL25

# Comparative phytochemical analysis and bioactivity evaluation of C*astanea sativa*, *Salix* and *Quercus* spp. flower pollen and their bee pollen

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In Türkiye and Slovenia, apitherapy has been traditionally practiced for the prevention and/or treatment of diseases for many years by using various bee products (bee pollen, propolis, honey). Among them, bee pollen is produced from pollen grains found in anthers of flowering plants. Therefore, it should be taken into account that the chemical composition of bee pollen, which is directly linked to its main floral source, is responsible for its health benefits[1]. This study aims to compare chemical compositions and bioactivity profiles of hydroalcoholic extracts of *Castanea sativa*, *Salix* sp., and *Quercus* sp. flower pollen grains and their bee pollen samples obtained from Türkiye and Slovenia. Firstly, the botanical origins of bee pollen samples were evaluated by palynological analysis and HPTLC. After, fingerprinting profiles were comparatively evaluated and the dominant and common compound in all samples was purified by successive chromatographic methods and its structure was elucidated as $N^1$,$N^5$,$N^{10}$-tricaffeoyl spermidine (TCS) by NMR and HRMS analyses. Besides TCS, other compounds rutin, hyperoside, quercetin, quercitrin, isoquercitrin, and myricitrin were determined in flower pollen grains and quantified in all samples by using a validated UPLC method. Then, standardized hydroalcoholic extracts were evaluated for their antioxidant and xanthine oxidase (XO) inhibitory activities by *in-vitro* tests and bioautography analyses (HPTLC-DPPH and HPTLC-XO). *C. sativa* flower pollen and bee pollen showed the highest antioxidant and XO inhibitory activities. Moreover, TCS was found to be the most bioactive compound that may be responsible for the pharmacological activities of the extracts. Standardized *C. sativa* bee pollen extract over TCS could have potential to be used as a food supplement in apitherapy.

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## OL26

# Anti-inflammatory properties of jabuticaba peel bioactive compounds during *in vitro* gastrointestinal and colonic digestion

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Inflammation involves multiple processes in response to harmful stimuli, such as bacterial metabolites (lipopolysaccharide), producing cytokines and pro-inflammatory biomarkers. Therefore, the inhibition of the inflammatory cascade response could attenuate this unbalanced status. Vegetable food rich in polyphenols are correlated to health benefits in many studies, especially for their anti-inflammatory potential. Notwithstanding, the potential effects of bioactive compounds are directly correlated to their bioavailability during digestion. Jaboticaba, also known as "Brazilian berry", is a small fruit with a dark purple skin, normally discarded, which is rich in anthocyanins, tannins and phenolic acids. Despite jaboticaba peel (JP) health benefits being extensively reported in literature, 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 (SHIME) on its phenolic composition, antioxidant capacity and anti-inflammatory effects in RAW 264.7 macrophage cell line. Antioxidant capacity analysis presented a slight divergence but were in unison for JP extract superiority to stomach and, especially, 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, monomeric anthocyanins are degraded in stomach, and in duodenum in a greater extent, but there is 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.

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**OL27**

# Pulse Electric Field (PEF) treatment as innovative approach for milking the plant roots; advantage and disadvantage of this technique

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The aim of this work was to develop and optimize PEF treatment for the reversible electroporation from the roots and rhizome of 3 model plants *Iris domestica* (syn. *Belamcanda chinensis* L. DC., Iridaceae) *Scutellaria baicalensis* L. and *Cicerbita alpina* (L.), all cultivated in aeroponic system. In available literature[1,2] is improved that electroporation can be successfully used in extraction of compounds from plant tissue. In our work we expand on these findings and further optimize the treatment parameters. 3 months old aeroponic cultivar of model plants were electroporated using different pulsed electric field strength from 0,2 to 7.5 kV/cm with constant 50 or 100 µs duration of pulse and repetition N 50, 100 and 200 pulses. We successfully extracted secondary metabolites after PEF, treated plants survived this process and it can be regularly repeated on the same individual plants. The most promising variant was when we applied: E = 0.5 kV/cm N = 100 f = 1 Hz t = 100 us **(A)** and E = 0.5 kV/cm N = 200 f = 10 Hz t = 50 us **(B)** For extraction following solvents were used: choline chloride : glucose (1:2) + 30% water; choline chloride : ethylene glycol (1:2); choline chloride : fructose (1:2) + 30% water; choline chloride : saccharose (1:2) + 40% water and tap water. After PEF plants were cultivated in aeroponic systems and observed throughout the next 4 months, compared to control. For HPLC-MS analysis all collected samples were first purified on SPE system to 10 mg/ml concentration. Main disadvantage is frequent infection of aeroponic cultivars with typical pests and frequent fungal and bacterial overfeeding, which must be combated.

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## OL28

# Originality of wild hops from northern France - a chemosensory approach

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*Humulus lupulus* L., or hops, is a key ingredient used in brewing as an antiseptic, bittering agent and flavouring agent. In recent years, brewers have been looking for new hop varieties with original aromatic profiles. In our local context, a study highlighted the genetic and chemical diversity of wild hops harvested in northern France, suggesting that wild hops could constitute a potential resource for the creation of new varieties. The present study aims to confirm their brewing potential using a chemosensory approach. Twenty-nine wild accessions were collected in northern France and replanted *ex-situ* in an experimental hop field. They were compared to 19 commercial varieties and two heirloom varieties. The demonstration of the phytochemical diversity of wild hops was based on the composition of non-volatile compounds, in particular of prenylated phenolic compounds, by targeted and untargeted analyses by UHPLC-UV and UHPLC-HRMS/MS, as well as on the composition of volatile compounds by HS-SPME GC-MS. In addition, experimental beers were brewed and hopped with 13 wild hops from our collection, 14 cultivars and 2 heirloom varieties. Sensory analysis was carried out by an expert panel of 19 people trained in the characterization of hopped beers. Wild hops from northern France showed a lower cohumulone/total alpha acid ratio. In addition, several accessions stood out both in chemical analysis, with high levels of aromadendrene, zingiberene and farnesene derivatives, and in sensory analysis, on specific descriptors such as floral and yellow fruit. Some wild hops were comparable to commercial varieties, showing strong brewing potential.

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## OL29

# Application of Pulsed Electric Fields increases yield of flavonoids and enables their extraction from *Scutellaria baicalensis* Georgi living roots

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*Scutellaria baicalensis* Georgi (Lamiaceae) is an important medicinal herb. Under the name Huang-Qin its rhizome has been used for centuries in Traditional Chinese Medicine. Nowadays it's listed in Chinese, European and British Pharmacopoeias[1]. It produces many phenolic compounds, among which flavonoids (mainly baicalin, wogonoside, baicalein and wogonin) are dominant. Thanks to these substances, root extract of *S. baicalensis* has cytotoxic, anxiolytic, antioxidant and antimicrobial properties[2]. Our aim was to use Pulsed Electric Fields (PEF) in order to extract these secondary metabolites from living roots of *S. baicalensis* grown in aeroponic cultures, and hopefully promote plant growth due to abiotic stress. In our studies we've shown that most plants survive such treatment and keep on growing, reaching size and content of root phytochemicals larger than control[3].

In this experiment 6 months old *S. baicalensis* specimens were electroporated in a special cuvette filled with a conductive solvent. After 3 weeks of observation plants were harvested, measured and dried. In most cases a significant increase in average mass and size of PEF-treated plants' roots was observed. Subsequently methanolic extracts of harvested roots were prepared. We found that the extracts were synonymous quality-wise, but differed in quantity of compounds when compared to control, in some cases exceeding the concentrations found in this group. This highlights the potential of such electrostimulation procedure, which can be further developed and applied to virtually any other plant model after some necessary adjustments.

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**OL30**

# Valorization of legume by-products for pharmaceutical, cosmetic, and nutraceutical applications

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Approximately 5 billion tons of biomass residues are generated annually as agri-food by-products globally. Among them, hulls, shells, peels, squeezing residues, and seed residues are typically discarded as waste, leading to several environmental problems[1]. Today, food by-products generated from industrial processes are considered valuable in pharmaceutical, nutraceutical, and cosmetic fields mostly due to their high content of bioactive molecules, such as polyphenols with a wide range of healing properties[2]. Thus, a new idea of using by-products as a source of bioactive, rather than discarding them, was born to reduce waste generation following circular economy principles. This research aimed to investigate the phenolic composition of different legume waste products supplied by a Romagna agri-food industry such as soy, peas, green beans, and beans to valorise them. A polyphenol-rich liquid extract was obtained from legume samples by a suitable Ultrasound-Assisted Extraction (UAE) method. Then, a thorough analysis of polyphenols in UAE extracts was conducted using UHPLC-DAD-ESI-$MS^n$. After meticulously comparing the phenolic profile of legume waste to that of commercially available final products, it was proved that by-products can be effectively considered a valuable resource. Then, the possible biological activities were explored to assign different applications of each by-product UAE extract. Interesting results were obtained for bean by-products containing glucaric acid derivatives, which have been shown to significantly reduce blood cholesterol levels[3]. Results confirmed that the agri-food by-products can potentially be exploited as a promising source of bioactive ingredients to design new formulations with a wide range of applications in the pharmaceutical, cosmetic, and nutraceutical industries falling within a circular economy perspective.

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**OL31**

# Establishment and elicitation of *Limonium algarvense* Erben callus cultures: Implications for primary and secondary metabolism

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*Limonium algarvense* Erben, a medicinal halophyte, holds significant pharmacological promise, attributed to its rich repertoire of bioactive compounds. The objective of this study was to establish robust callus cultures as a sustainable, *in vitro* model for studying the plant's metabolic responses, particularly focusing on the synthesis and accumulation of primary and secondary metabolites under various elicitation treatments. Callus cultures were initiated from leaf explants on Murashige and Skoog's medium supplemented with 1 mg/L picloram for 4 weeks. Afterwards, callus cultures were subjected to two elicitor treatments, including salicylic acid–SA and yeast extract–YE at 50 and 100 mg/L for four weeks. Water extracts were assessed for their shifts in primary (total soluble sugars and proteins, and proline), and secondary metabolism (total phenolics, flavonoids, and condensed tannins). In addition, a detailed metabolic profiling was conducted using high-performance liquid chromatography with electrospray ionization mass spectrometry (HPLC-ESI-MS/MS). Elicitation induced significant shifts in the metabolite synthesis of elicited cultures compared to controls. While YE50 markedly increased the callus yield, the total levels of phenolics, flavonoids condensed tannins and total soluble proteins, the SA50 led to the highest increase in proline content. Metabolomic analysis identified 10 metabolites, including gluconic acid, pyridoxine, and *p*-coumaric acid, that were found across all cultures. Specific elicitation treatments resulted in unique metabolomic profiles; for instance, uralenneoside and riboflavin were missing in control samples, while SA50 treatment led to the exclusive presence of hydroxybenzoic acid *O*-hexoside and the absence of seven other compounds. This suggests that elicitation can effectively enhance the biosynthesis of primary and secondary metabolites in *L. algarvense* callus cultures, offering great potential for medicinal applications.

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**OL32**

# Cosmetic applications of essential oils extracted from indigenous forest species of Taiwan

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Essential oils, also known as plant volatiles, have garnered attention in cosmetic applications due to their array of beneficial properties, including their aromatic fragrances and potential therapeutic effects on the skin and hair. These oils serve as natural sources of biologically active ingredients, offering a broad spectrum of bioactivities such as antioxidant, anti-inflammatory, dermatoprotection, antiaging, and antimicrobial properties, thus becoming integral components of herbal cosmetic products. Taiwan's rich forest biodiversity hosts over 5000 known native species of flora. Our ongoing research focuses on exploring the skin health benefits of essential oils extracted from indigenous forest species of Taiwan. In our investigations, we initially screened the anti-melanogenic properties of 16 essential oils extracted from native forest species in Taiwan, along with 31 commercial essential oils. Among them, *Alpinia nantoensis* leaf and rhizome,[1] *Calocedrus formosana* wood,[2] *Pogostemon cablin*, and *Glossogyne tenuifolia*, along with their bioactive compounds, exhibit robust melanin and tyrosinase inhibitory activities in skin melanocytes. Furthermore, our studies have unveiled that these essential oils not only inhibit cellular tyrosinase activity but also modulate melanin biosynthesis pathways. Additionally, we have discovered that *Glossogyne tenuifolia* essential oil and limonene from *Alpinia nantoensis* essential oil protect skin keratinocytes from ultraviolet irradiation-induced photodamage and photoaging.[3] Collectively, our findings underscore the potential of essential oils as promising natural sources for the development of skin whitening/lightening and anti-aging agents for cosmetic applications.

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**OL33**

# Chasing molecules - a structure elucidation journey

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The newly discovered natural compound lumnitzeralactone, identified as an ellagic acid derivative, was isolated from the antibacterial extract of the Indonesian mangrove species *Lumnitzera racemosa* Willd. The structure of lumnitzeralactone, characterized by a deficit in protons and a complex condensed aromatic ring system, was unequivocally determined by comprehensive spectroscopic analyses, including high-resolution mass spectrometry (HRMS), 1D $^{1}H$ and $^{13}C$ nuclear magnetic resonance spectroscopy (NMR), and 2D NMR techniques, including the more unusual experiments 1,1-ADEQUATE and 1,n-ADEQUATE. The structural elucidation was further supported by computer-assisted structure elucidation (CASE system utilizing ACD-SE), density functional theory (DFT) calculations, and finally by a two-step chemical synthesis.

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**OL34**

# Extraction of spilanthol from *Acmella oleracea* with natural deep eutectic solvents

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Green chemistry approaches gain more and more importance in the field of natural product research. The use of natural deep eutectic solvents (NADES), eutectic mixtures of hydrogen bond donors and acceptors, as extraction agents for natural products has therefore become a growing field of research[1-3]. For the first time, 20 choline chloride-based NADES were tested in comparison with ethanol as solvents for the extraction of spilanthol from *Acmella oleracea* (L.) R.K.Jansen flower heads, analysed by HPLC-DAD quantification. Preceding UHPLC-DAD-ESI-$MS^n$ analysis as well as isolation using a dichloromethane extract revealed the composition of *Acmella oleracea* extracts and yielded spilanthol along with further main constituents as reference compounds. The effects of water addition, time and temperature on NADES extractions were also investigated. Best results in screening were achieved through extractions with NADES consisting of choline chloride and 1,2-propanediol (1:2 molar ratio, +20% water), comparable to ethanol yields. Choline chloride and methylurea (1:2, +20% water) also showed promising results. These combinations were used for effect testing experiments, in which time had the least effect on spilanthol yield, a raised temperature up to 80 °C increased extraction, and water addition greater than 20% decreased spilanthol extraction. Since non-toxic constituents were used for NADES preparations, promising extraction results could lead to directly applicable formulations, among others for medicinal purposes.

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## OL35

# Two new anthraquinones and other secondary metabolites from *Asperula sintenisii* Achers ex Bornm

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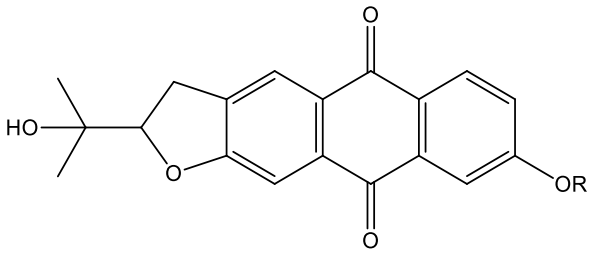
*Asperula* L. is one of the foremost genera of the family Rubiaceae, which comprises approximately 200 species worldwide. It is represented by 41 species in the flora of Türkiye with 49% endemism ratio. Some *Asperula* species have been used as a diuretic, tonic and against diarrhea in Anatolian folk medicine. Previous phytochemical investigations on *Asperula* demonstrated that the genus contains anthraquinones, iridoids, flavonoids and phenolic acids as the main secondary metabolites[1]. *A. sintenisii* Achers ex Bornm is a perennial subshrub endemic to Ida Mountains which has never been studied. In this study, we aimed to isolate and identify the secondary metabolites from the whole plant of *A. sintenisii*. The air-dried and powdered plant material was extracted with MeOH. The crude MeOH extract was suspended in $H_2O$ and submitted to liquid-liquid extraction with *n*-hexane, EtOAc and *n*-BuOH, respectively. Sequential chromatographic separations on EtOAc and *n*-BuOH subextracts by MPLC ($C_{18}$ and $SiO_2$) and Sephadex LH-20 afforded 14 secondary metabolites, including two new anthraquinones (**1** and **2**) along with five known iridoid glycosides (asperuloside, deacetylasperuloside, asperulosidic acid, deacetylasperulosidic acid and geniposide), four flavonoids (quercetin, isoquercitrin, rutin and kaempferol 3-*O*-rutinoside) as well as three phenolic acid derivatives (chlorogenic acid, ferulic acid 4-*O*-β-D-glucopyranoside and rosmarinic acid). The chemical structures of purified compounds were elucidated on the basis of extensive 1D and 2D NMR experiments and HR-MS analysis. Compounds **1** and **2** are rare anthraquinone derivatives bearing a prenyl group cyclized to tetrahydrofuran skeleton. Moreover, geniposide, kaempferol 3-*O*-rutinoside and rosmarinic acid are being reported for the first time from the genus *Asperula*.




**1** R = H
**2** R = β-Glc

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## OL36

# Integrating network pharmacology and experimental validation: *In vitro* assessment of neuroprotective effects and enzyme inhibition studies on *Prunella vulgaris* L. extract and isolated compounds

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*Prunella vulgaris* (PV) has been used traditionally for its anti-inflammatory effects and known to be rich of rosmarinic acid (RA)[1]. RA is frequently researched in recent years for Alzheimer's Disease (AD) treatment. However, the mechanism has not been elucidated yet. This study is focused to predict potential targets-pathways of PV and isolated compounds in AD and support these through *in vitro* assays.

RA, ursolic acid (UA) and rutin was isolated from the aerial parts of PV. RA content, the major compound of PV, was determined through LC-MS analysis as 11.2% (w/w). For network pharmacology studies, target proteins of the compounds retrieved and GO and KEGG pathway enrichment analysis performed. TNF, HIF-1, and TLR signalling pathways, and response to LPS and $H_2O_2$ was determined as possible mechanisms of PV against AD. *In vitro* assays were conducted parallel to network pharmacology studies. SOD and MDA levels in $H_2O_2$-induced SH-SY5Y cells were evaluated and found that extract and compounds reduce oxidative damage by decreasing MDA levels. UA (100 µM) was identified as the most effective compound (reduction of 91%). To determine neuroprotective activity, samples were applied to $H_2O_2$-damaged SH-SY5Y cells and viability significantly increased even at 0.1 µM concentration for all compounds. Rutin showed the highest activity. TNFα levels was investigated on LPS-stimulated BV2 cells, but samples didn't show any significant change. In acetylcholine esterase (AChE) and butyrylcholine esterase (BuChE) inhibition assays, PV and compounds showed significant inhibition, with higher activity on BuChE. RA showed the highest activity for AChE and BuChE ($IC_{50}$ values of 97.5 and 25.3 µM, respectively). Results showed that PV and compounds showed significant neuroprotective activity and also showed AChE and BuChE inhibitory activities that suggests as a potential for treatment of AD.

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## OL37

# Anti-inflammatory properties of biomolecules from the Euganean Thermal District

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The Euganean Thermal District (North-East Italy) is one of Europe's largest and oldest thermal centres. The Italian Health System acknowledges the use of its therapeutic thermal muds as a beneficial treatment for patients with arthro-rheumatic diseases. Cyanobacteria represents one of the most abundant Phyla among the microorganisms that constitute mud's biofilms and are known to produce complex polysaccharides and glycoglycerolipids with potential therapeutic efficacy[1-3]. In this study, we assessed the anti-inflammatory activity of polysaccharides (PS) extracted from muds matured at different temperatures in five spas of the Euganean Thermal District, as well as total lipids (TL) extracted from the three most abundant cyanobacteria strains, cultivated under optimal conditions on a lab scale. PS were characterized by analyzing the FT-IR spectrum, the zeta potential, the monosaccharide composition and the abundance of sulfate groups. TL were characterized by analyzing the fatty acid composition and the relative abundance of lipid classes. Subsequently, we evaluated recovery from an inflammatory state using a zebrafish model of copper sulfate-induced inflammation. Following treatment with our bioactive compounds, we examined the recovery of the developmental delays due to the inflammatory state through morphometric analyses of swim bladder insufflation and operculum bone area. The results indicate that all PSs, extracted from muds matured at different temperatures, possess chemical composition homogeneity and exhibit similar anti-inflammatory activity. Additionally, TL also presents an anti-inflammatory potential. In conclusion, investigating the healing effects of compounds produced by the Euganean mud microbiota represents a crucial step in the scientific validation of the Euganean mud therapies and further supports their potential in the recovery from chronic inflammatory diseases.

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**OL38**

# Antioxidant activity and phenolic compounds contents of *Tribulus terrestris* L.

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*Tribulus* L. genus, belonging to Zygophyllaceae family, has about 20 species, but only *Tribulus terrestris* grows naturally in Türkiye. It is known as "demirdikeni, çobançökerten, çarıkdikeni, or deveçökerten" in Türkiye and used as infusion (5%) to treat kidney stones, as diuretic and strengthener. Due to the traditional use of *T. terrestris* species, many biological activity studies have been performed on the species such as anticancer, antioxidant, antimicrobial, analgesic, anti-inflammatory, antiurolitic, antidiabetic, cardiotonic, aphrodisiac, and antispasmodic etc. Phenolic compounds that are found in plants have significant biological activities. Therefore, it is important to determine the amount of these compounds.[1,2] In this study, amounts of some phenolic compounds were determined on methanol extracts prepared from different parts of *Tribulus terrestris* (flowers, fruits and herb) collected from Dikili, Türkiye. In addition, total phenolic and total flavonoid contents of extracts were determined as gallic acid and quercetin equivalents, respectively. Antioxidant activities were carried out by two methods (DPPH free radical scavenging capacity assay and ABTS radical cation radical scavenging assay).
As a result of HPLC analysis, chlorogenic acid, ferulic acid, gallic acid, and rutin were detected. The highest amount of chlorogenic acid and gallic acid were determined as 0.75% and 0.15% in the extract of the flowers, respectively. The highest amount of ferulic acid and rutin were determined as 0.1% and 0.23% in the herb extract, respectively. Extracts of the flowers also had the highest total phenolic content (35.03 mg GAE/g extract); on the other hand, extracts of the fruits had the highest total flavonoid content (113.22 mg QE/g extract). In terms of antioxidant activity, the herb extract (DPPH: 0.19 mg/mL ($IC_{50}$); ABTS: 30.81 mg TE/g extract) was found to be more active than the extracts prepared from the other parts of the plant.

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**PO1**

# The pharmacological potential of the lichens *Pseudevernia furfuracea* (L.) Zopf. and *Cladonia stellaris* (Opiz) Pouzar & Vězda

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Lichens are symbiotic organisms that are composed of a mycobiont (fungus) and one or more photobionts (algae or cyanobacteria). The products of lichen secondary metabolism represent a unique group of natural organic substances with significant pharmacological activity. The present work evaluates the antiproliferative, antifungal, antiangiogenic and antioxidant effects of lichen extracts *Pseudevernia furfuracea* (PSE) and *Cladonia stellaris* (CLA). The metabolic activity of PSE and CLA extracts at different concentrations was determined by MTS assay on four tumor cell lines. The lichen CLA extract significantly inhibited the metabolic activity of the cells compared to the PSE extract. The antifungal activity of PSE and CLA extracts was tested on three reference yeast strains. *Candida albicans* was the most sensitive to the PSE extract with an average MIC of 0.16%. *Candida parapsilosis* and *Malassezia pachydermatis* showed very good sensitivity to PSE and CLA.e. In terms of angiogenesis evaluation in the CAM model, there was a significant inhibition of vessel density after application of CLA extract at a concentration of 200 µg/ml. Total vessel length was not significantly affected and vessel branching was significantly affected by PSE extract at a concentration of 200 µg/ml. PSE extract showed higher antioxidant activity compared to CLA. Based on the comparison of the chemical shifts of hydrogen and carbon atoms in the $^{1}H$ and $^{13}C$ NMR spectra of the standard and in the lichen extract samples, it can be concluded that atranorin was present in the PSE extract and the presence of usnic acid was confirmed in the CLA extract.

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## PO2

# Effect of subcritical water extraction fractionation on antioxidant activity and total phenolic content of teas and tea-like plants

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Tea (*Camellia sinensis*) and tea-like plants (e.g. maté – *Ilex paraguariensis*) are rich sources of antioxidants. Unfortunately, methods used for their extraction are usually environmentally non-friendly, time-consuming, and with low extraction efficiency. Subcritical Water Extraction (SWE) is a novel green method used for the extraction of natural products that can improve heat and mass transfer, thus increasing extraction yields and efficiency. However, the methodology for SWE extraction of beverage plants is globally not uniform, especially in temperature and extraction time, which strongly affect the extraction efficiency. In this study, 3 different plant beverages (green tea, black tea, maté) were extracted using the SWE method where the extracts were collected in the form of time-separated fractions (1×10 min, 2×5 min and 5×2 min). In addition, as representative of the conventional extraction methods, water infusion was also performed. Subsequently, antioxidant activity (DPPH, ORAC) and Total phenolic content (TPC) were evaluated in all samples to describe the best extraction conditions. The strongest antioxidant activity and highest TPC in green and black tea were found in the 2nd 5-min fraction, where $IC_{50}$ for DPPH was 9.733 µg/mL (green tea) and 16.23 µg/mL (black tea), $IC_{50}$ for ORAC was 6.503 µg/mL (green tea) and 6.4383 µg/mL (black tea), and TPC was 778.53 mg GAE/g (green tea) and 650.55 mg GAE/g (black tea). In the case of maté, the results of DPPH and ORAC testing were not unequivocal, but the highest TPC was also found in the 2nd 5-min faction (540.314 mg GAE/g) and in addition in the 4th 2-min fraction (543.308 mg GAE/g). These findings indicate that the chemical composition of the SWE extracts changes during the extraction time and the fractionation is a powerful tool for achieving the strongest possible extracts.

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**PO3**

# Expression analysis of ribosome-inactivating proteins and genes related to triterpenoid saponin biosynthesis in *Agrostemma githago* L.

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The seeds of *Agrostemma githago* L (corn cockle, Caryophyllaceae) are toxic and poisonings were frequent in the past from contaminated flour. The toxicity of corn cockle is attributed to a toxic two component-system (TTS) which consists of type I ribosome-inactivating proteins (RIPs) and specific triterpene saponins[1]. RIPs are N-glycosylases that inactivate the ribosomal RNA, leading to an irreversible inhibition of protein synthesis and cell death. *A. githago* has been found to produce agrostin[2], the type I RIP, and saponins such as gypsogenin and quillaic acid glycosides. The biosynthesis of agrostin and saponins has not been studied in *A. githago*. To get insight into the mechanisms regulating the TTS, we performed expression analysis of its components in various tissues and organs at the transcription level. The plant material grown in the experimental field of the Botanical Garden of Medicinal Plants (BGMP) at the Wroclaw Medical University (Poland) and from *in vitro* cultures was used. Nucleotide sequences of genes putatively involved in the biosynthesis of triterpenes and these encoding RIP were identified in the *A. githago* transcriptome obtained by RNAseq[2]. Selected sequences were used for PCR primer design and the tissue-specific expression of genes encoding for: RIP, squalene synthase, squalene monooxygenase, β-amyrin synthase as well as several cytochromes P450 and UDP-dependent glycosyltransferases, was studied.
The analysis contributed to an elucidation of the unexplored toxic synergism within plants of the carnation family. In a long run, our investigation may lead to an optimization of triterpene saponin profile in *A. githago* and serve for further research in their biomedical applications.

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**PO4**

# Phytochemical and antibacterial profile of *Perilla frutescens* L.

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*Perilla frutescens* L. is an aromatic medicinal plant widely used in food and as a medicine. Two *P. frutescens* varieties (green and purple) were examined in terms of antioxidant and antibacterial action. Phytochemical analysis was performed using LC-MS, HPLC, and spectrophotometry. Of tested gram-positive and gram-negative bacterial strains, the most susceptible in the microdilution test was methicillin-resistant *Staphylococcus aureus* (MRSA; MIC/MBC 2.5 mg/mL). The antibacterial effect against MSSA/MRSA was further examined using microcalorimetry (Fig. 1). In antioxidant activity testing by the DPPH method, the purple variety of perilla was 3-fold stronger than the green one ($IC_{50\ DPPH}$ 7.7 µg/mL). Phytochemical analysis revealed the presence of phenolic compounds (phenolic acids and flavonoids), among which rosmarinic acid dominated (30 – 50 mg/g dry leaves extract). Perilla leaves rich in polyphenols showed strong antioxidant and moderate antibacterial action.

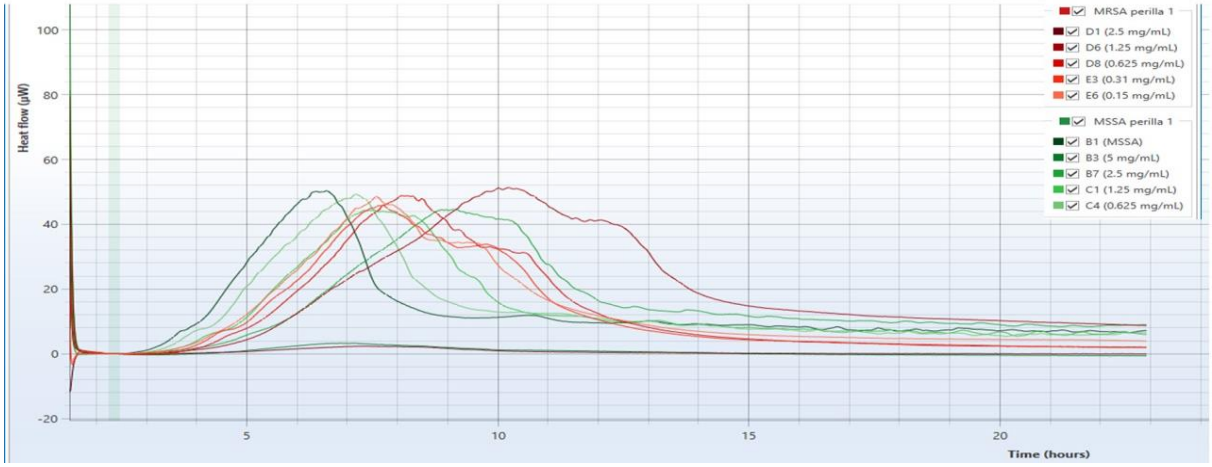


Figure 1. Heat flow of MSSA (green) and MRSA (red) nontreated/treated by perilla water extract determined by microcalorimetry.

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**PO5**

# From koala snack to anti-microbial attack: Potency of 1,8-cineole against drug resistant microorganisms

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The escalating resistance of microorganisms to antibiotics poses a formidable challenge to global health, necessitating the exploration and validation of novel antimicrobial agents beyond antibiotics. In this context, plant-derived compounds, notably essential oils, emerge as potent alternatives due to their broad-spectrum antimicrobial properties. These oils, characterized by their complex compositions of multiple constituents in varying proportions, may offer effective strategies against antibiotic-resistant pathogens. Interactions among the constituents of essential oils contribute to their overall antimicrobial activity, which may differ significantly from the effects of their individual components.

Our preceding investigations have shown that eucalyptus essential oil, predominantly comprising 1,8-cineole, which accounts for 80% of its composition, substantially inhibits the proliferation of a range of pathogens. This observation propelled a focused analysis on the antimicrobial activity of 1,8-cineole against reference strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* employing the microdilution method.

The study revealed that staphylococcal cells showed more than 90% reduction in 1,8-cineole concentrations between 100 and 25 mg/ml. Complete inhibition of *C. albicans* was achieved at a minimal concentration of 25 mg/ml, with a 50% reduction of cells at 1.6 mg/ml. However, 1,8-cineole exhibited no activity against *P. aeruginosa* within the tested concentration range.

The results underscore 1,8-cineole's potent inhibitory effect on the growth of *S. aureus* and *C. albicans*, highlighting its potential as a promising candidate for developing treatments targeting infections caused by these pathogens. Opting for a singular, well-defined component such as 1,8-cineole rather than the complex essential oil might offer advantages in terms of reduced side effects and circumventing the challenges associated with essential oil standardization. This study contributes to the burgeoning field of antimicrobial research, offering a foundation for further exploration of plant-derived compounds in the fight against antibiotic resistance.

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**PO6**

# Using mass spectrometry for study of salicylic acid metabolism in plants

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Plant immunity is defined as the capacity of plants to prevent or ward off biological attacks by pathogens. It involves the recognition of the pathogen by specific receptors and the triggering of signalling pathways leading to processes that help plants to defend themselves. The defence signalling is mediated by cross-communication of groups of plant hormones. Salicylic acid (SA) is one of the most pronounced plant hormones involved in control of immunity and defence mechanisms in plants. Defence related SA accumulation comes from its biosynthesis, transport and possible release from some of its metabolites. The involvement of SA biosynthetic pathways as well as extend of SA metabolism in various plant species are still under investigation. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is one of the most commonly used analytical technics in plant hormone quantification. The selectivity and high sensitivity enables to track typically low concentrations of phytohormones. The comprehensive multiclass phytohormone LC-MS/MS profiling methods usually include only SA (as the active compound) from the group of SAs. LC-MS/MS methods for determination of defence related phytohormones focus on SA, or some on its biosynthetic precursors or metabolites. In this study, we focus on identification of new SA metabolites and development of liquid chromatography mass spectrometry methods for targeted, semi- or untargeted analysis of biosynthetic precursors and metabolites of SA in plants.

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**PO7**

# Quantification of Yang cycle intermediates and related compounds by LC-MS/MS in plants

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The amino acid methionine represents a fundamental compound in plant metabolism. Through a set of methionine-recycling reactions, collectively called "Yang cycle", and the compounds arising from them, it influences essentially all plant processes. Its first metabolite, S-adenosylmethionine (SAM) represents one of the most used substrates for enzymatic reactions in plants and mammals, providing the role of a methyl donor. In plants, SAM is used for synthesis of 1-aminocyclopropane-1-carboxylic acid, which is converted to an indispensable gaseous plant hormone ethylene, and serves also as a precursor in polyamine biosynthesis.
Accurate determination of the levels of Yang cycle metabolites is therefore very important in understanding plant ontogenesis and various aspects of plant life, however verified profiling methods are lacking. Quantification of these highly polar compounds presents a challenge due to their low retention on reversed-phase columns, as well as the chemical instability and low abundance of some of these metabolites. In this study, we have developed an UHPLC-MS/MS method for simultaneous determination of these metabolic intermediates based on isotope dilution. To improve retention and ionization efficiency, amino groups were derivatized and the chromatographic separation was achieved using a C18 column with water and acetonitrile containing formic acid as mobile phases. Multiple reaction monitoring mode was employed for qualitative and quantitative detection of the target metabolites.
In addition to analysis of different plant biological samples, we have further used the method to study the effect of exogenous methionine on endogenous levels of these compounds, as well as the changes in metabolism upon stress treatments.

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**PO8**

# Functionalization of goat cheese with biomass and extracts from the halophyte species *Helychrisum picardii picardii*

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In the food industry, additives play a vital role in extending the shelf life of products, facilitating mass production, and enabling distribution across global markets. However, the use of synthetic additives has been associated with an increased risk of health issues, including allergies and cancers. There is a growing global trend towards healthier diets, favoring foods with natural preservatives or ingredients. Commonly, such enhancements are applied to familiar foods like cheese. This study introduces biomass and leaf infusions from the medicinal halophyte *Helichrysum picardii*, harvested in Southern Portugal, into goat cheese production both post- and pre-coagulation. The cheeses were assessed for microbial safety, physicochemical properties, and antioxidant activity over 8 days at 4°C. The addition post-coagulation resulted in a less yellow cheese, and the use of biomass led to a higher dry extract percentage than other methods. The pH was lower when additions were made post-coagulation. Interestingly, the introduction of plant material did not affect the cheese's antioxidant capacity. It did, however, increase microorganism and fungal colony counts at 30°C, likely due to the plants' microbial quality. Despite this, all cheese samples met legal standards for the examined pathogens. While this study demonstrated that the addition of plant material can alter the physicochemical characteristics of cheese without impacting its antioxidant properties, it also led to an increased presence of microorganisms, underscoring the importance of ensuring the microbiological quality of the natural additives used.

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**PO9**

# How ecotype influences the nutritional profile of the edible halophyte *Sarcocornia perennis* cultivated under saline conditions?

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Halophytes, plants thriving in saline conditions, are known for their rich nutritional and bioactive profiles, offering numerous health benefits. The cultivation of halophytes presents a strategic solution to combat the effects of climate change, particularly addressing issues such as freshwater scarcity and soil and water salinization. These plants can be used directly as fresh vegetables with unique flavors or processed into functional food additives that confer health benefits. *Sarcocornia* A.J. Scott is a perennial halophyte suited to harsh saline environments and is valued in gourmet cooking for its unique flavor and crunchy texture. This study evaluated how different *Sarcocornia perennis* ecotypes affect the plant's nutritional content when grown under greenhouse conditions with saline irrigation. Two ecotypes were chosen based on their distinct habitats and growth traits. Cuttings taken in January 2021 in Ria Formosa (Algarve, Portugal) were rooted and potted in a coco peat and perlite mix and irrigated with brackish well water. After 30 days, plants were harvested at a marketable size of 10-15 cm and evaluated for proximate composition and minerals contents. Both ecotypes showed similar proximate compositions; however, mineral content varied, with Sp.E1 showing higher Na (103.06 mg/g DW), Mn (85.23 µg/g DW), and Zn (30.06 µg/g DW). In contrast, Sp.E2 was richer in K (21.89 µg/g DW) and Pb (3.12 µg/g DW). These findings imply that selecting the right *Sarcocornia* ecotype is crucial for optimizing the nutritional value of the biomass for saline agriculture.

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**PO10**

# Comparison of antibiofilm efficacy of selected azole antifungals and plant essential components in *Candida albicans* strains

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The ability to form a biofilm is one of the important factors of virulence in *C. albicans*, which usually leads to the emergence of resistance to commonly used antifungal agents in clinical practice[1,2]. The present study is aimed at comparing the antibiofilm effectiveness of three azole antifungals (miconazole, itraconazole, clotrimazole) and three components of plant essential oils (eugenol, cinamaldehyde, carvacrol). For the antifungals tested, the planktonic cells of *C. albicans* showed the highest susceptibility (30 strains, 100%) to miconazole (MIC 0.03-0.06 µg/mL) and itraconazole (MIC 0.03 µg/mL), followed by clotrimazole (MIC 0.03-1 µg/mL, 29 strains, 96.7%). Regarding the tested plant essential oil components, the best antifungal efficacy was found with carvacrol (MIC 50 µg/mL), followed by cinnamaldehyde (MIC 50-200 µg/mL) and eugenol (MIC 200-800 µg/mL). All three tested antifungals (miconazole, itraconazole and clotrimazole) were ineffective against adhered cells (0 h biofilm) and mature biofilm (48 h biofilm) of *C. albicans*. Out of the three plant essential oil components tested, carvacrol reached the highest activity against adhered cells (MIC 100 µg/mL), then cinnamaldehyde (MIC 100-400 µg/mL) and eugenol (MIC 400-800 µg/mL). In mature biofilm, the best antibiofilm activity was observed for carvacrol and cinnamaldehyde, both MIC 400-800 µg/mL, followed by eugenol (MIC 800 µg/mL). The obtained results indicate that the components of plant essential oils could play an important role in the fight against biofilm formation in *C. albicans*.

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**PO11**

# Study of antimicrobial activity of *Sorbus domestica* L. leaf extract against *Staphylococcus aureus*

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*Sorbus domestica* L. (Rosaceae) is a tree cultivated in vineyards and gardens for its apple or pear-shaped fruits. Nowadays, it is almost forgotten, and its therapeutic potential is still not efficiently described. This study aimed to explore the antimicrobial properties of the leaf water extract of *S. domestica* against methicillin-susceptible *Staphylococcus aureus* (MSSA). The minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations were determined by the conventional broth-microdilution method (BMD). The antibacterial mechanism against MSSA was studied *via* a new approach of isothermal microcalorimetry (IMC) measuring the metabolic activity of bacteria. Phytochemical analysis was performed to determine the polyphenolic compounds of the extract. Broth-microdilution method showed MIC 10,0 mg/ml and MBC over 20,0 mg/ml. By the IMC, the antimicrobial activity of the extract, shown in the graphical part of this abstract, was evident. All the measured concentrations (10,0 – 1,25 mg/ml) delayed the time to peak and decreased the total heat, compared to growth control, from which the concentration 10,0 mg/ml showed the most significant effect (Fig. 1). These results showed, that the leaf extract of *S. domestica* has some antibacterial potential against MSSA evaluated by BMD and IMC methods. Moreover, with the IMC method, it was possible to notice antimicrobial activity in 8 times lower concentrations than BMD.

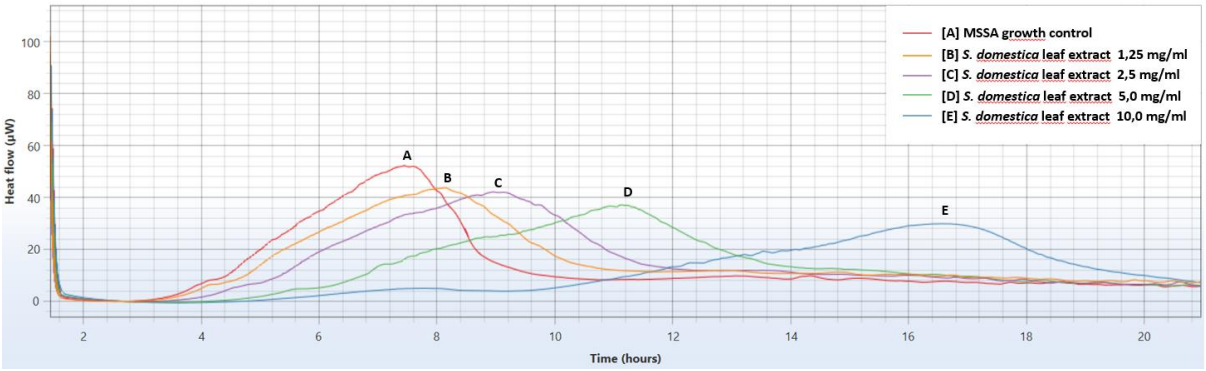


Figure 1 The influence of *S. domestica* leaf extract on growing curves of MSSA expressed as heat flow in time.

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**PO12**

# Chromatographic separation of xanthones from *Maclura pomifera*

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Research on biologically active substances focuses, among other things, on monitoring biological activity as a function of the chemical structure of the tested compounds. Substitution of the basic skeleton and its modification often play a major role.

Xanthones are a remarkable group of natural secondary metabolites occurring in only a few families and having interesting pharmacological effects. For example, *in vitro* cytotoxic or antitumor activity [1], cholinesterase inhibition [2] or antioxidant effect [3] have been described. The stem and root bark of *Maclura pomifera* (Raf.) C.K. Schneid. (Moraceae) was selected as a suitable source for their isolation. The root bark was extracted with methanol and subsequently separated. Ethanolic extract of stem bark was firstly fractionated by liquid/liquid extraction and *n*-hexane, chloroform, and ethyl acetate fractions were obtained. Individual extracts and subsequently selected fractions were separated by chromatographic methods.

Column chromatography, preparative TLC, and semipreparative HPLC led to the isolation of ten xanthones (prenylated and unprenylated), two of them were new compounds. TLC, HPLC, MS, and 1D and 2D NMR experiments were used to identify these compounds. The highest yields were for toxyloxanthone C and osajaxanthone from stem bark, and macluraxanthone from root bark.

The isolated xanthones will be subjected to biological activity screening mainly focusing on anti-inflammatory, antioxidant or cytotoxic effects for future potential therapeutic applications. Due to the isolation of compounds with differently modified substituents, their set will also help to obtain information on the structure-activity relationship.

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## PO13

# Saccharides in *Polygoni cuspidati rhizoma et radix* decoction as biologically active compounds stimulating the proliferation of gingival fibroblasts

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Rhizomes of *Polygonum cuspidatum* were used in Traditional Chinese Medicine (TCM) for centuries. Currently, it is considered as pharmacopoeial raw material in Europe and China. Scientific literature indicates that this raw material is a rich source of compounds with anti-inflammatory and antioxidant activity. Latest study (1) of our research team showed that extracts from rhizomes of *Polygonum cuspidatum*, especially the water decoction possess wound healing stimulating activity. Decoction significantly stimulated human gingival fibroblasts to proliferate, migrate, and increase the synthesis of collagen III. These results confirm the traditional use of a decoction of this raw material in the treatment of inflammation and wounds mouth. The aim of this research project was to test the hypothesis that saccharides were responsible for the observed high activity of the decoction. For this purpose water decoctions were prepared from the raw material. A gradient precipitation of saccharides from aqueous solutions was performed using appropriate concentrations of ethanol. The decoctions, different fractions of saccharides and the residue after isolation of saccharides were subjected to *in vitro* MTT assays on human gingival fibroblasts. All the above samples were subjected to phytochemical analysis using FTIR (Fourier Transform Infrared Spectroscopy), thermogravimetry, HPLC-MS (HPLC/DAD/ESI-HR-QTOF-MS method) and HPLC with RI refractometric detector for further separation and identification. The highest increase in fibroblast viability was observed for the oligosaccharide fraction, but the decoction and other fractions also showed statistically significant activity. Phytochemical studies confirm the abundance of saccharides in the most active fraction. The isolation of saccharides with a studied chemical structure that exhibit wound-healing stimulating properties provides opportunities for their use in regenerative dentistry. Our reports confirm the validity of the traditional use of decoctions as oral rinses and identify saccharides as an important group of compounds with significant biological activity.

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## PO14

# Susceptibility of intestinal bacteria involved in colorectal cancer pathogenesis to extracts from Philippine medicinal plant species and plant-derived compounds

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Colorectal cancer (CRC) is the second most deadly cancer with 0.9 million deaths worldwide in 2020[1], with China and the US having the highest estimated number of new cases[2]. Gut dysbiosis is one of the factors associated with an increased risk of developing CRC. Various phytochemicals and their synthetic analogs (*e.g.*, quinoline derivates), together with traditionally used herbs, have been found to inhibit gut pathogenic microorganisms[3]; however, their effect on CRC associated microorganisms has not been determined yet. Therefore, we tested *in vitro* growth-inhibitory effects of 12 compounds (berberine, bismuth subsalicylate, carbadox, ferron, 8-hydroxyquinoline, chloroxine, nitroxoline, olaquindox, salicylic acid, sanguinarine, tannic acid, and zinc pyrithione), and 10 ethanol extracts obtained from medicinal plant species traditionally used in Philippines to cure gastrointestinal disorders (*Acalypha grandis*, *Artocarpus blancoi*, *A. camansi*, *A. elasticus*, *A. lakoocha*, *Citrus microcarpa*, *Crescentia cujete*, *Dysoxylum blancoi*, *Euphorbia thymifolia*, and *Merremia peltata*), and six antibiotics against CRC-causing pathogens (*Bacteroides fragilis*, *Clostridium septicum*, *Esherichia coli*, *F. necrophorum*, *F. nucleatum*, *P. anaerobius*, and *Streptococcus bovis*) using broth-microdilution assay. In this study, all compounds and plant extracts shown a certain degree of antimicrobial effect with minimum inhibitory concentrations (MICs) ranging from 0.5-512 µg/ml. Carbadox (MICs = 0.5-32 µg/mL), nitroxoline (MICs = 8-16 µg/ml), zinc pyrithione (MICs = 4-32 µg/ml) and chloroxine (MICs = 4-64 µg/ml) were the most active. Plant extract of *A. lakoocha* bark (256 µg/ml) inhibited growth of *E. coli*, *A. elasticus* leaves (512 µg/ml) inhibited growth of *S. bovis*. Leaves extract of *A. blancoi*, *A. elasticus* (256 µg/ml) and *D. blancoi* (bark) (512 µg/ml) significantly inhibited bacterial growth of *S. bovis*. *E. coli*, *C. septicum*, and *S. bovis* were the most susceptible bacteria with MICs ≥ 4 µg/ml. These findings indicate that studied chemicals and plant-extracts have potential to inhibit growth of microorganisms associated with CRC development.

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# PO15

## Antiproliferative diterpenes from three *Plectranthus s.l.* species

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The genus *Plectranthus s.l.* (Lamiaceae) has been used in traditional medicine for centuries, particularly in the treatment of various digestive, respiratory, urogenital, and dermatological disorders. Phytochemical studies revealed a production of phenolic compounds and terpenes as major secondary metabolites. Among them, structurally diverse, highly oxygenated diterpenes are the most prominent group which gathered considerable scientific interest due to their promising biological effects, especially antimicrobial, antiprotozoal, and cytotoxic activities. Abietane diterpenes from subclasses of royleanones, hydroquinones and quinone methides were shown to be the most potent antiproliferative and/or cytotoxic agents.
In our current study, we focused on exploring the methanol extracts derived from the aerial parts of *C. comosus*, *C. forsteri* 'Marginatus', and *P. ciliatus*. We isolated fourteen diterpenes from these extracts, which belong to the abietane, *ent*-clerodane, and *ent*-kaurane classes. Three of them, including 3-*O*-acetylornatin G, 3,12-di-*O*-acetylornatin G, and ornatin B methyl ester, were identified as new natural products. The isolated compounds were examined *in silico* for their physicochemical and early ADME parameters. Their antiproliferative effects were investigated *in vitro* using human breast (MDA-MB-231 and MCF-7), cervical (HeLa), and glioblastoma (U-87 MG) cancer cell lines. The royleanone and hydroquinone abietanes exhibited the most potent antiproliferative activity against all cancer cell lines tested, particularly against glioblastoma cells, with $IC_{50}$ values ranging from 1.1 to 15.6 µM.

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**PO16**

# *p*-Topolin and its mesylate as possible active components of skin care products

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Purine-based phytohormones are involved in the regulation of many processes in plants, including senescence and are therefore of interest in skin care. The best known is kinetin (KIN; $N^6$-furfurylaminopurine) that has antioxidant, cell regenerative and anti-ageing properties and is already used in dermatological preparations[1]. Structurally close to KIN *is para*-topolin (*p*T; 6-(4-hydroxybenzylamino)purine). Recently, *p*T has been shown to have beneficial effects on skin structure, wrinkles, roughness and non-inflammatory acne lesions[2]. *p*T use in skin care is limited due to its poor water solubility. Therefore, a much more water soluble mesylate salt of *p*T (*p*TM) was prepared.
In this study we compared biological activity of *p*TM with the parent substance *p*T. KIN was used as a standard component used in practice. The basic safety characteristics for using substances in dermatological preparations, particularly (photo)stability and (photo)toxicity were evaluated. Further effect on the signalling pathway controlled by Nrf2 transcription factor that modulates cell antioxidant capacity, and UVA-photoprotective properties were studied. Results will be discussed at the poster presentation.

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**PO17**

# Iron and copper chelation by benzoic acids and their effect on metal catalyzed Fenton reaction and hemolysis

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Flavonoids are secondary plant metabolites that are a common part of our diet and their consumption has positive health effects at least according to epidemiological studies. However, flavonoid can have both antioxidant and prooxidant effects[1,2]. The biological relevance of such effects is disputable, as flavonoids are massively metabolized into small phenolics by microbiota before reaching systemic circulation. One class of these metabolites are benzoic acids. It is possible that the biological properties attributed to flavonoids are, in fact, caused by their metabolites[3].

This analysis included investigation of the interactions between benzoic acids and transition metals iron and copper. The chelating activity of benzoic acids was pH dependent. From the tested substances, only 3,4-dihydroxybenzoic acid demonstrated a strong capacity for chelating iron ions. The same compound also demonstrated the capability to reduce ferric and cupric ions. The screening of copper chelation activity with hematoxylin method showed that 3,4-dihydroxybenzoic acid demonstrated the highest copper-chelating activity, again. Subsequently, the determination of the effect on copper-induced lysis of rat red blood cells was used to verify the antioxidant or pro-oxidative action of the tested compounds. None of the tested compounds exhibited the ability to protect rat erythrocytes from copper toxicity. Only 2,4-dihydroxybenzoicacid and 3,4-dihydroxybenzoic acid were able to decrease the production of hydroxyl radicals arising during the metal-catalyzed Fenton reaction at pH 4.5 and 7.5. These results showed significant differences in the anti- and prooxidant activities of the tested substances based on experimental conditions.

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**PO18**

# Evaluating of anti-quorum sensing activity of natural phenolic compounds

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Inhibition of quorum sensing, bacterial cell-to-cell communication, represents a very attractive target for novel antibacterial compounds. Using a light-producing biosensor strain *Vibrio harveyi* MM30, we tested 16 natural compounds: 7 cannabinoids, 7 arylbenzofurans and 2 flavonoids for anti-quorum sensing activity. There are two main genetic circuits responsible for the regulation of quorum sensing behaviour in *V. harveyi*: AI-1 and AI-2. While AI-1 is regulated by strain-specific molecules, AI-2 production is common among various species of bacteria. The mutant strain *V. harveyi* MM30 has knockout genes for production of AI-2 but is still able to react to exogenous AI-2. Therefore, we added AI-2 containing medium to its culture and investigated the direct activity of subinhibitory concentrations of our substances on the AI-2 receptor. For comparison, we measured the activity of the same compounds, but without adding AI-2, so we obtained the results also for the AI-1 inhibitory activity. According to the results of this experiment, we selected 3 compounds and added them at various subinhibitory concentrations to the culture of methicillin-resistant Staphylococcus aureus (MRSA). After the spent media was added to the culture of *V. harveyi* MM30, we obtained the information on the activity of these compounds on AI-2 production in the MRSA strain.

**PO19**

# Auxin metabolism: unravelling novel pathways and metabolites by liquid chromatography-mass spectrometry analysis

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Proper plant growth and development are intricately regulated by a group of bioactive compounds known as plant hormones (phytohormones). These substances function optimally within a narrow concentration range, necessitating strict regulation of their levels in plant cells and organs. This regulation primarily occurs through biosynthesis, metabolism and transport mechanisms. Among the diverse groups of phytohormones, auxins were the first to be identified owing to their profound effects on plant tropisms. Two essential endogenous auxins are indole-3-acetic acid (IAA) and phenylacetic acid (PAA). These compounds exhibit biological activity only in their free, unconjugated form, as enzymatic reactions produce metabolites that serve as temporary storage and transport forms, as well as degradation products. This conversion occurs either through irreversible oxidation or reversible conjugation with amino acids and sugars. So far, the metabolism of PAA and IAA appears quite similar, as the same enzymes catalyse the synthesis of identical conjugates.

In recent years, many new discoveries have been made in the field of auxin metabolism, expanding our knowledge about conjugates and catalysing enzymes. However, most of this research is connected to IAA and *Arabidopsis thaliana* as a model plant. Therefore, we aim to broaden the understanding of metabolism of two endogenous auxins (IAA and PAA) across various land plant species and their organs. By employing liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) we identified novel IAA and PAA endogenous metabolites. Following experiments involved complex IAA and PAA metabolite profiling in various land plant species and their organs and elucidation of enzymes responsible for these reactions utilizing bacterial enzymatic assays.

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**PO20**

# Yeast glucan particles – bioactive drug vehicles

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Yeast glucan particles (YGPs) are promising agents for the delivery of biologically active compounds as drugs. YGPs possess their own biological activities and can act synergistically with their cargo. β-Glucans, the main component of yeast cell walls, can modulate both innate and adaptive immune responses via their recognition by innate immune cells and activated immune cells then promote immune responses.
Glucan particles protect payloads from the harsh conditions in the gastrointestinal tract and can interact with the intestinal immune system, they might increase the bioavailability of their cargo. Immune cells associated with Peyer's patches in small intestine can then use the lymphatic system to distribute the cargo of the YGPs to various organs of the reticulo-endothelial system, such as the liver, the lung, the spleen, and the kidney, or into inflamed sites and tumours. These features make YGPs suitable candidates for the oral delivery of diagnostic or therapeutic compounds.

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## PO21

# Liquid matrix volatilization methods for susceptibility testing of respiratory bacteria to volatile agents in liquid and vapour phase

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Respiratory infections belong to the leading causes of morbidity and mortality throughout the world. Inhalation therapy is possible way of treatment when the active agents are delivered directly to the site of infection in respiratory system[1]. Plant volatile agents such as essential oils (EOs) and their constituents are of great potential for inhalation due to their volatility. However, lack of knowledge on antimicrobial activity of their vapours is a main obstacle for their practical use. Due to the high volatility and hydrophobicity of plant volatiles, conventional laboratory methods of antimicrobial susceptibility testing face specific problems. Recently, broth micro- and macro-dilution volatilization assays have been developed in our laboratory for the evaluation of the antimicrobial potential of volatiles in liquid and vapour phase[2,3]. With aim to validate an appropriateness of both methods, the antibacterial activity of plant-derived volatiles namely 8-hydroxyquinoline, β-thujaplicin, thymohydroquinone, thymol, thymoquinone and EOs obtained from *Alpinia oxymitra* pericarp, *A. elegens* leaf, *Cymbopogon citratus* aerial part, and *Trachyspermum ammi* seed was evaluated against respiratory bacterial pathogens *Haemophilus influenzae*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. As a result, all volatiles tested have shown a certain degree of antibacterial effect with minimum inhibitory concentrations (MICs) ranging 2–512 and 8–1,024 µg/mL in liquid and vapour phase, respectively. 8-hydroxyquinoline possessed the highest antibacterial potential against *S. aureus* in the liquid phase (MIC = 2 µg/mL). Vapours of 8-hydroxyquinoline and thymoquinone were the most active against *S. aureus* and *H. influenzae*, respectively (both MIC = 8 µg/mL).
In summary, presented assays are suitable for simple and rapid susceptibility testing of bacterial pathogens to volatiles in the liquid and vapour phase. They allow a cost- and labour-effective high-throughput screening using commercially available microtubes or microplates. Both methods have been validated for a development of applications for treatment of respiratory infections based on volatile antimicrobials.

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**PO22**

# Bioactive 3D supramolecular coordination architectures of bile acids

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Natural chiral hydrophobic cavity/pocket containing structures are important for many biological functions (*e.g.,* catalytic metalloenzymes or transport proteins). To mimic these natural systems and mechanisms, development of artificial supramolecular systems (*e.g.*, cages or macrocycles) from chiral natural molecules is desirable. Coordination-driven self-assembly is a well-established method to build hollow metallosupramolecular (MSM) structures. However, utilization of natural-molecule-based ligands (L) to construct MSM systems was greatly unexplored until recently, when first bile acid (BA)-based MSM macrocycles $Pd_3L_6$ were introduced and studied in our group.[1,2] Additionally, utilization of BAs provides possibility for potential biomedical application of the MSM systems because of their inherent transportation ability and enterohepatic selectivity.[3] Therefore, our latest study explores novel BA-based *tris*-pyridyl ligand and its adaptive self-assembly with $Pd^{2+}$ resulting in selective formation of octahedral $Pd_6L_8$ (3.6 nm diameter, 6.3 kDa) or first-ever cuboctahedral $Pd_{12}L_{16}$ (5 nm diameter, 12.6 kDa) MSM cages by regulating the reaction kinetics. These large, Pd-containing, highly charged (+12 or +24), and water-soluble cages display anticancer activity and enhanced absorption when employed *in vitro* on the hepatospheroids of HepG2 cell line.

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**PO23**

# *Satureja montana* and *S. kitaibelii* essential oil variability – chemotaxonomic approach

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*Satureja montana* L. and *S. kitaibelii* Wierzb. ex Heuff. are aromatic and medicinal plants used in traditional medicine for different purposes, mostly for urinary, digestive and pulmonary disorders. These uses are due to the plants' proven antimicrobial and antioxidant properties. However, although both species possess medicinal properties, they have different chemical profiles of essential oils, resulting in different modes of action. Individual essential oils (EOs) were isolated by simultaneous hydrodistillation and extraction (SDE) method from 17 natural populations, 14 belonging to *S. montana* and 3 to *S. kitaibelii*. These natural populations inhabit open rocky arid habitats, though the climate differs from the Mediterranean to more continental. GC/MS and GC-FID analyses show intra- and interpopulation variability of *Satureja* EOs. In most populations from *S. montana*, phenolic compounds (carvacrol and thymol) dominated the profile, while in *S. kitaibelii*, those were non-phenolic compounds (limonene, linalool, geraniol and *cis*-sabinene hydrate). Populations of both species that grow deeper in the continent with more arid climates are dominated by non-phenolic compounds in EO, with no or very little presence of phenolic compounds (< 1%). While dominant compounds did not differ between continental *S. montana* and all of the studied *S. kitaibelii* populations, CDA analysis showed a clear separation of these two species based on their entire EO profile. These results suggest that the phytochemical profile of essential oils could be used as a taxonomic marker for differentiation between these two species.

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**PO24**

# The alkane profile as a significant tool for the chemotaxonomy of the genus *Clinopodium* L.

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Alkanes are essential components of the plant cuticle, positioned in the epicuticular layer. Their importance for chemotaxonomy comes from their wide distribution in plants, chemical stability and easy isolation. Previous studies have shown that alkanes can be excellent markers at the species level in some very complex groups but are restricted to higher taxonomic levels in others. The taxonomic problem of the genus *Clinopodium* L. is that formerly separate and morphologically distinct genera have been merged into a single genus. This study aims to evaluate the variability of alkane profiles in the genus *Clinopodium* and to assess whether alkanes can contribute to solving this taxonomic problem. Alkanes were isolated from 24 populations of ten *Clinopodium* taxa from the Balkans. Most of the tested populations comprised three pooled samples to characterise the variability of each population better. The solvent was *n*-hexane and the samples were analysed by GC/MS and GC/FID. Along with 15 *n*-alkanes commonly found in cuticular waxes, a rare series of *iso*- and *anteiso*-alkanes were also detected in a significant percentage. Tritriacontane (*n*-C33) had the highest concentration in 52 of 62 samples. In the remaining samples, hentriacontane (*n*-C31) was the most abundant alkane and all of these taxa are from the former genus *Acinos* Mill. In former *Acinos* taxa, all populations showed intrapopulation variability concerning the dominant alkane (*n*-C33 or *n*-C31), separating them from the other studied taxa. Discriminant analysis (DA) with the taxa as groups showed a clear separation into five groups. *n*-C33, *n*-C31 and *iso*-C31 showed the greatest influence on the separation of the taxa. The DA test also showed that the alkane profile is an excellent marker for delimiting the former genus *Calamintha* Mill species, a particularly morphologically complex group. These results suggest that alkanes could be used in the taxonomy of the genus *Clinopodium*.

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**PO25**

# Anticholinesterase activity of methanolic extract of *Amorpha fruticosa* flowers and isolation of putrescine and spermidine derivatives

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*Amorpha fruticosa* L. is a leguminous shrub indigenous to North America and later introduced to Asia as a windbreak and to Europe as an ornamental shrub. Previous phytochemical studies revealed that it was rich in rotenoids, stilbenes, flavanones, and isoflavones, some of which exhibited significant cytotoxic, antidiabetic, and antimicrobial activities.[1] The methanolic extract of flowers exhibit interesting inhibition activity of cholinesterases. The isolation of substances responsible for this activity was the goal of our work.

Ethyl-acetate extract was separated on silica gel by column chromatography, then selected fractions were purified by means of semi-preparative HPLC with a UV/VIS detector. Five putrescine and spermidine derivatives were obtained from ethyl-acetate extract of the flowers of *A. fruticosa*. Isolated compounds were identified by UV, HRMS, and NMR. After evaluation of the results of spectroscopic methods and comparing them with the literature, the substances were identified as two derivatives of putrescine: mongolicine A (or $N^1$-(*E*)-$N^6$-(Z)-di-*p*-coumaroylputrescine) and $N^1$,$N^6$-(*E*)-di-*p*-coumaroylputrescine; and three derivatives of spermidine: safflospermidine B (or $N^1$-(*E*)-$N^5$,$N^{10}$-(*Z*)-tri-*p*-coumaroylspermidine), $N^1$,$N^5$-(*Z*)-$N^{10}$-(*E*)-tri-*p*-coumaroylspermidine and the last compound was obtained in the largest amount and based on the spectroscopic data, it was determined as „all *trans*“ isomer - $N^1$,$N^5$,$N^{10}$-(*E*)-tri-*p*-coumaroylspermidine.[2]

*A. fruticosa* contains several promising substances and should be further investigated. The anticholinesterase activity of isolated compounds was measured. The interaction of the studied compounds with the target enzymes was studied using molecular docking. Isolated phenolamides have specific functions in plant development and defence, as metabolic intermediates, and final products.[2] These compounds were isolated from *A. fruticosa*, Fabaceae for the first time.

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## PO26

# Exploring structure-activity-relationships of insect deterrent saponins

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Brassicaceae plants are widely distributed around the world and have extremely high economic value. *Plutella xylostella* is a highly insecticidal resistant pest that only feeds on Brassicaceae plants, causing $4-5 billion in annual economic losses. Previous research our group identified a triterpenoid saponin called hederagenin cellobioside that prevents feeding by *P. xylostella*[1]. The genus *Barbarea* is the only known Brassicaceae plant that accumulates saponins. *B. vulgaris* has been divided into two different ecotypes with different saponin profiles, where the G-type is resistant to most crucifer pest insect and the P-type susceptible. Saponins are 2,3-oxidosqualene derived amphipathic triterpenoid glycosides that can help plants prevent herbivores consumption by affecting cell membranes and imparting a bitter flavor[2]. The oxidosqualene cyclase (OSC) LUP2 mainly produces lupeol, and is high expressed in insect-susceptible *B. vulgaris* P-type plants, whereas the OSC LUP5 produces β-amyrin and α-amyrin, and is high expressed in resistant G-type plants[3]. *B. vulgaris* can therefore be developed into an ecological model system to metabolic engineer and evaluate saponins potential as biopesticide. Saponins' toxicity is determined both by the triterpenoid backbone structure and the glycosylation pattern and if it is hydroxylated at C23. To determine structure-activity-relationships for saponins toward *P. xylostella* larvae, *LUP5* was overexpressed in the two genotypes and *CYP72A552* (C-23 hydroxylation) was knocked down in G-type. We found *LUP5* overexpression in the P-type and silencing of *CYP72A552* in the G-type changed the saponin profiles and the resistance level to *P. xylostella*.

In conclusion, using metabolic engineering, we showed that the saponin profile in *B. vulagrais* can be altered, and that this significantly affects its resistance level to feeding by *P. xylostella* larvae. Knowledge of the structure-activity-relationships of saponins towards agricultural economically important insect pest is important for the future development of biopesticides for the Green Transition and for providing biosolutions for a more sustainable food and feed production system.

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**PO27**

# Steroidal molecules as building blocks in supramolecular and material chemistry

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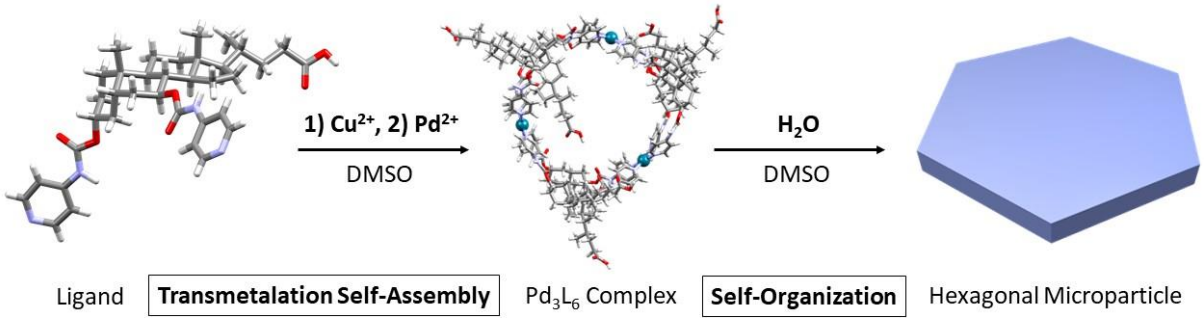
Bile acids (BA) belong to a group of triterpenoid steroidal molecules. The BAs are the main component of bile. Once the bile is excreted into the small intestine, the BAs form nanosized mixed micelles with lipophilic nutrients mediating their digestion, transport through ileal membrane, and distribution within the enterohepatic circulation.[1] Moreover, the BAs have intriguing physicochemical properties such as molecular rigidity, amphiphilicity, chirality, and possessing hydroxyls and carboxylic acid groups they provide a wide range of possibilities for chemical transformations aiming at various, mostly pharmacy-related, applications.[2] In our approach, we also set a goal to understand their behavior from the metallo-supramolecular point of view. We utilized the BAs as building blocks of coordinating ligands which can form large supramolecular self-assemblies. We have mastered the preparation of many large coordination species and further advanced to control the formation of higher self-organized structures, micro- or nanoparticles (Figure),[3] which opens an exciting area of intriguing applications.

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**PO28**

# Assessment of polyphenol variability, flavonoids, phenolic acids, pigments, and antioxidant activity across two growth stages of ginkgo leaves

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Ginkgo leaves are esteemed for their abundance of specialized metabolites, including flavonoids, terpenoids, and trilactones, known for their manifold medicinal properties like anti-inflammatory, antiviral, antifungal, and anticancer effects. Nonetheless, the composition of these metabolites is subject to various factors such as plant variety, growth stage, and environmental conditions during cultivation[1]. This study aimed to compare the levels of phenols, flavonoids, phenolic acids, pigments, and antioxidant activity in Ginkgo leaves harvested in May and October. The analysis revealed significant differences in all phenolic components, with higher concentrations of polyphenols and flavonoids found in October leaves. Conversely, May leaves exhibited higher levels of phenolic acids and antioxidant activity. While these findings suggest the promising potential of autumn leaves as a source of bioactive compounds, further research is warranted to thoroughly explore the phytochemical composition of Ginkgo leaves during the autumn season.

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**PO29**

# Assessment of cytotoxicity and antioxidant capacity of *Ranunculus arvensis* L. and isolation of its flavonoids

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The genus *Ranunculus* L. (Ranunculaceae) is represented by 84 species in Flora of Turkey and is recorded to be used for the treatment of muscle and joint pain, rheumatism, psoriasis, wounds, and edema. It is also used worldwide for asthma, fever, and gout, as well as skin disorders in homeopathic preparations. There are species recorded to be used against various types of cancer in Traditional Chinese Medicine.

This study aimed to evaluate the cytotoxic and antioxidant capacity of *Ranunculus arvensis* L. and isolate its secondary metabolites. The methanolic extract of the whole plant was successively partitioned with *n*-hexane and *n*-butanol. The *n*-butanol sub-extract was fractionated by polyamide column chromatography which yielded 8 fractions. The methanol and *n*-butanol extracts along with mentioned fractions were tested for their cytotoxicity by MTT assay on the A549 cell line and for antioxidant capacity by the ferric reducing antioxidant power (FRAP) and the cupric reducing antioxidant capacity (CUPRAC) assays. Only one fraction, which was not sufficient for the isolation studies, displayed moderate activity with 49,21 ± 2,37% inhibition at a dose of 200 µg/ml. Two of the fractions exhibiting antioxidant capacity were used for further chromatographic separation, allowing the isolation of isovitexin, hyperoside, isoorientin, and gossypitrin as well as a mixture of isoorientin-2"-*O*-[6"'-*O*-*E*-*p*-coumaroyl-beta-D-glucopyranoside] and isoorientin-2"-*O*-[6"'-*O*-*E*-*p*-feruloyl-beta-D-glucopyranoside]. These metabolites were isolated from *R. arvensis* for the first time.

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## PO30

# LC-(HR)MS/MS method to determine cucurbitacins in Cucurbitaceae – Survey in Dutch retail market

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Cucurbitacins (CBCs) constitute a group of oxygenated triterpenoid substances, commonly found in the family of Cucurbitaceae. They are predominantly known for their bitterness and toxicity, yet some exhibit positive bioactivities. Several human-poisoning incidents from CBCs have been reported over the last decades. In 2018, two women developed acute toxicity symptoms upon consumption of a pumpkin soup made of bitter pumpkin and had hair loss weeks later [1, 2]. Furthermore, fatal cases in Germany (2015) [3] and Twente, the Netherlands (2021) potentially linked to CBCs ingestion were reported. Breeding has minimized the CBCs content in edible species but crossbreeding with bitter/wild types (for example ornamental pumpkins) or uncontrolled growth can strongly elevate concentrations. The aim of the study presented herein was to develop a method to detect CBCs in the fruits of three species of Cucurbitaceae (i.e. zucchini, pumpkin and cucumber). Additionally, the study aimed to perform a market survey analysing the presence of CBCs in products obtained from local (super)markets and homegrown products. A straightforward method for the determination of nine CBCs was developed. A simple extraction of CBCs from the food matrices followed by detection and quantification using LC-MS/MS was employed. The method was intra-day validated, showing acceptable precision and recoveries. The obtained LOQs in the two matrices were in the range of 10-25 µg/kg. Fifty samples were analysed, from retail, local market and homegrown origin, including five ornamental pumpkin samples and four samples of bitter zucchini, three of which were from an intoxication case. The majority of samples were from sweet varieties and contained negligible CBC levels. In contrast, ornamental pumpkin and bitter zucchini samples exhibited significant CBC concentrations, reaching up to 1850 mg/kg fresh weight. High-resolution mass spectrometry identified two new CBCs in these samples. Because ornamental pumpkins and bitter zucchini cannot always be easily visually discriminated from sweet varieties, accidental consumption cannot be excluded.

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**PO31**

# Chasing molecules - interdisciplinary approaches in modern structure elucidation

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The newly discovered natural compound lumnitzeralactone, identified as an ellagic acid derivative, was isolated from the antibacterial extract of the Indonesian mangrove species *Lumnitzera racemosa* Willd. The structure of lumnitzeralactone, characterized by a deficit in protons and a complex condensed aromatic ring system, was unequivocally determined by comprehensive spectroscopic analyses, including high-resolution mass spectrometry (HRMS), 1D $^{1}H$ and $^{13}C$ nuclear magnetic resonance spectroscopy (NMR), and 2D NMR techniques, including the more unusual experiments 1,1-ADEQUATE and 1,n-ADEQUATE. The structural elucidation was further supported by computer-assisted structure elucidation (CASE system utilizing ACD-SE), density functional theory (DFT) calculations, and finally by a two-step chemical synthesis.

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**PO32**

# The study of the antibacterial activity of agrimony against gram-positive bacteria

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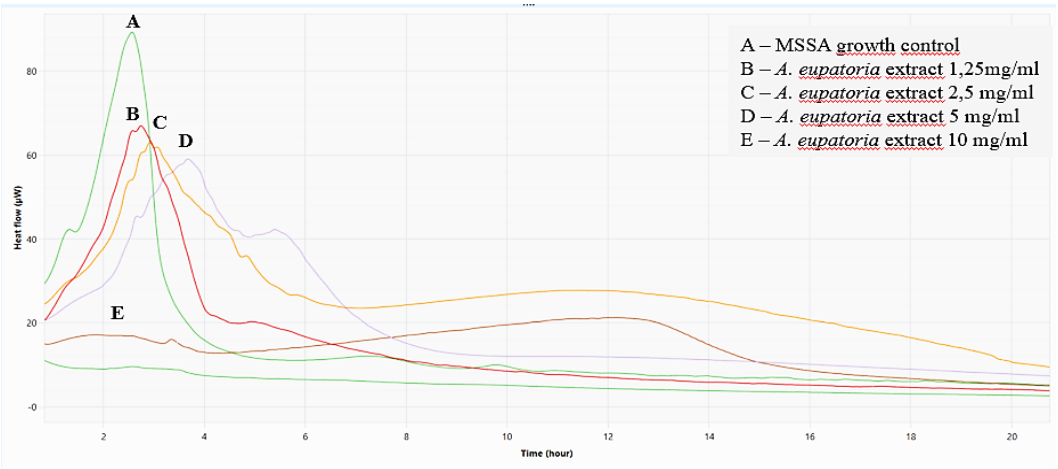
*Agrimonia eupatoria* (Rosaceae) is a well-known perennial herb widely spread in Europe. Its secondary metabolites comprise tannins, flavonoids, phenolic acids, and triterpenoids. Agrimony is traditionally used to treat minor skin inflammations and its secondary metabolites are known for their antimicrobial effects. Still, only a limited number of studies have been conducted in this field. This study aims to explore the antibacterial properties of agrimony methanol extract against gram-positive bacteria using isothermal microcalorimetry (IMC). All biological processes generate or absorb energy in the form of heat. Metabolically active bacteria produce heat that corresponds to their growth rate. IMC measures the heat of biological processes that are directly related to the speed at which the given process progresses. A phytochemical analysis of the extract was also performed. Except for IMC, the conventional broth microdilution method (BMC) to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) has been used. As shown in Fig. 1, the metabolic activity of MSSA decreases alongside the increasing agrimony concentration. All observed parameters (heat flow, total heat, and time to peak) in bacteria exposed to extract, were reduced. The results of both methods (IMC and BMC) confirm the antimicrobial properties of agrimony.

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**PO33**

# Isoquinoline alkaloids in *Corydalis cheilanthifolia* cultured in vitro

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Isoquinoline alkaloids are specialised metabolites produced by poppy plants (*Papaveraceae*). *Chelidonium majus* is a well-known medicinal plant, widely used in folk medicine and known for its high content. A less well known but no less rich source of isoquinoline alkaloids is the fern leaf corydalis - *Corydalis cheilanthifolia*. Techniques are being developed to efficiently extract bioactive substances such as isoquinoline alkaloids under laboratory conditions, using the possibilities of growing medicinal plants in glass and applying classical biotechnological procedures. Isoquinoline alkaloids have the potential to be used as effective drugs without side-effects, with major implications for the prevention and treatment of many inflammatory and cancerous diseases. The structure of natural compounds can be difficult to reproduce by chemical synthesis, and only in their original form can they exhibit valuable biological properties.

The rationale for using a biotechnological approach lies in the greater control and chance of isolating and pinpointing the factors at work that are crucial in regulating the biosynthesis and accumulation of plant metabolites such as isoquinoline alkaloids.

The aim of this research project is to evaluate the biosynthetic pathways for isoquinoline alkaloids in plants and to increase the efficiency of their extraction. The identification of potential enzyme candidates involved in the biosynthesis of isoquinoline alkaloids in the plant *C. cheilanthifolia* and the in vitro cultivation of selected clones is the first step of the project.

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**PO34**

# *In vitro* culture and phytochemical profiling of *Agrostemma githago* and *Gypsophila elegans*

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Plants from the *Caryophyaceae* family are a rich source of valuable phytochemicals known for their medicinal but also preventive properties against f. ex. cardiological or civilization diseases. The main groups of compounds found in *Caryophyllaceae* species are saponins, phytoecdysteroids, polyphenolic compounds including flavonoids, and N-containing compounds. *Agrostemma githago* was once a noxious grainfield weed causing mass poisoning due to its toxic metabolites while *Gypsophila elegans* is an ornamental plant native to Asia and Europe. Despite the specialized compounds both species contain ribosome inactivating proteins type 1 (RIP1) agrostin and gypsophilin, respectively, a specific class of plant protein toxins.
The aim of the study was to 1) verify if the compounds of interest can be produced under the in vitro conditions of *A. githago* and *G. elegans* in organ and/or tissue culture, as well as 2) determination of conditions allowing stable growth and efficient production of specialized compounds.
The explants obtained from aseptic two-week-old seedlings were cultured on MS media supplemented with plant growth regulators (PGR): kinetin (KIN), benzylaminopurine (BAP), 2-isopentenyladenine (2-iP) and indole-3-acetic acid (IAA) in different concentration.
The effect of PGR on the secondary metabolite profile of both species was monitored using chromatographic techniques (HPLC-DAD, UHPLC-MS). Flavone C-glycosides orientin and vitexin were identified in in vitro grown shoots as well as saponin derivatives including gypsophilin ones. The influence of PGR on the growth and morphology of both species was also recorded on the number and length of main and axillary shoots per explant, biomass production, and the spontaneous vitrification occurrence.
In conclusion, *A. githago* and *G. elegans* in vitro culture are a feasible and responsive model for induction of growth and morphogenesis as well as for specialized compound biosynthesis.

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**PO35**

# Oregano and red wine: an antioxidant combination that leads to synergy

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*Origanum vulgare* L., known as oregano, is a popular culinary herb. It is a frequent ingredient for flavoring in Mediterranean cuisine. However, in addition to its taste and smell, which are given by the content of essential oil, it is a plant with a wide potential of biological activities. Red wine is known for many as an integral part of a South Europe dinner and a rich source of polyphenols. As the oregano and red wine combination in meals is quite common, we decided to study mutual interaction in five antioxidant assays with different mechanisms of action. Lyophilizates of red wine (cuvée Blaufränkisch and Dunaj, Matula 2021, BFD) and water infusion from oregano leaves (OV) were mixed in three ratios: 1:1, 1:10, and 10:1. Rosmarinic acid was used as a positive control. Combination index (CI) was used for quantification of interaction, where CI =, < or > 1, which means additive, synergic or antagonistic reaction. In ABTS and DPPH assays, where hydrogen atom transfer is the main mechanism of action, synergy was observed in BFD:OV 1:1 (ABTS: CI = 0.68; DPPH: CI = 0.76) and 10:1 (ABTS: CI =0.72; DPPH CI = 0.52) combination, while 1:10 combination was slightly antagonistic (ABTS: CI = 1.33) or nearly additive (DPPH: CI = 1.06). In the FRAP assay, where the main mechanism of action is based on single electron transfer, the mixture exhibited slight synergy in all combination ratios (BFD:OV 1:1 CI = 0.91; 10:1 CI = 0.90; 1:10 CI = 0.86). Synergy was observed also in Fenton reaction based assays with $H_2O_2$ with 1,10-phenantroline (BDF:OV 1:1 CI = 0.75; 10:1 CI = 0.38; 1:10 CI = 0.67) an salicylic acid (BDF:OV 1:1 CI = 0.85; 10:1 CI = 0.66; 1:10 CI = 0.46). Phytochemical analysis of total polyphenols in both lyophilizates was performed spectrophotometrically, where BFD has 7.7% and OV 18.4% of total polyphenols. Even though the research brought several questions to the interpretation of the interaction, the presence of different synergistic combination of BFD and OV confirms that mixtures of antioxidants are more active, as if they are alone.

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**PO36**

# Evaluation of Antidiabetic Properties of Roselle plant extracts (*Hibiscus sabdarifa* L.)

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The Roselle plant (*Hibiscus sabdariffa* L.) produces various substances in its metabolism that have potential antidiabetic properties. As these metabolites are characterized by different polarity and extractability into different solvents the experiment was designed for three types of solvent (water, methanol, chloroform). In the prepared extracts, selected substances (total polyphenols and total anthocyanins) which are assumed to be co-carriers of the observed antidiabetic activity were quantitatively determined. The highest level of total polyphenols expressed as gallic acid equivalent (10.96 ± 0.13 mg/g) and anthocyanins expressed as cyanidin-3-glucoside (0.28 ± 0.01 mg/g) were detected in the aqueous extract. Antioxidants contribute to the protection of the pancreatic β cells from oxidative damage and at the same time reduce the prevalence of diabetes complications. That is why the antioxidant activity of the extracts was evaluated using two methods. In the DPPH assay the aqueous extract showed the highest antioxidant activity (0.40 ± 0.03 μmol Trolox®/μl) and in the FRAP assay the methanolic extract (58.67 ± 0.92 FRAP/ml). By inhibiting α-amylase, the amount of resorbable glucose from the intestine is significantly reduced, which limits the development of postprandial hyperglycemia in diabetics. The water extract of roselle plant was *in vitro* able to inhibit α-amylase (70.63 ± 1.59%). The prepared aqueous extract thus provides great potential in the complementary treatment of patients suffering from diabetes.

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**PO37**

# Evaluating the anti-inflammatory potential of prenylated (iso)flavonoids from *Ficus cyathistipula* in an *in vitro* model of inflammatory response

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In recent years, there has been renewed interest in natural treatments and plants as potential sources of new drugs for the treatment of inflammatory diseases. Prenylated phenolics, a diverse group of natural compounds found in various plants, are known for their significant anti-inflammatory potential. Therefore, roots of *Ficus cyathistipula* Warb. (Moraceae) were subjected to extensive chromatographic separation leading to the isolation of twelve prenylated (iso)flavonoids of which five compounds were isolated from plant material for the first time.
Their potential anti-inflammatory effect was investigated in an *in vitro* study. The first screening was conducted on the human leukemia monocytic cell line THP1-Blue™ NF-κB stimulated with lipopolysaccharide. The anti-inflammatory potential was evaluated as the inhibition of NF-κB, one of the key pro-inflammatory transcriptional factors, using the QUANTI-Blue™ colorimetric enzyme assay.
Results demonstrated that seven prenylated (iso)flavonoids showed the same or better effect on the inhibition of the NF-κB signaling pathway compared to the standard drug prednisolone. The inhibition of nuclear translocation of NF-kB, as one of the potential mechanisms of action, was investigated in the two most active compounds. The obtained results opened the door for further study of the anti-inflammatory potential of these compounds.

**PO38**

# Antimicrobial activity of two orange varieties essential oils for the valorisation of peel wastes

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*Citrus* fruits are one of the most important commercial crops cultivated all over the world. These fruits have attracted attention for their medicinal value. *Citrus* fruits contain a wide range of bioactive components, and by-products obtained from *Citrus* waste are recognized as a renewable source of valuable substances for application in the pharmaceutical industry [1]. Essential oils are natural plant constituents known for their antimicrobial, antifungal and antioxidant properties [2]. In this study, essential oils from two citrus species (*Citrus sinensis* "Navel orange" and *Citrus sinensis* "Tarocco") were investigated for their antimicrobial activity against plant infecting agents. Fruits were collected at the ripening stage. After squeezing the juice, the orange peels were removed and dried in the sun. The peels were cut into small pieces and subjected to hydro-distillation using a Clevenger apparatus. The essential oils were dried over anhydrous magnesium sulphate and stored in sealed vials until the characterization by GC/EI-MS analysis. The identification of components was based on the comparison of their mass spectral fragmentation pattern with databases. Subsequently, a screening of the biological activity of the essential oils was conducted on various bacterial and fungal strains, including those known to infest numerous varieties of fruits, vegetables, and ornamentals. This analysis aimed to study and compare the efficacy and potential impact of the two essential oils on the development and growth of the tested pathogens. The results of this screening provide valuable insights into the antibacterial and antifungal activity of the two *Citrus* essential oils and their potential utility as agrochemicals.

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**PO39**

# Constituents of hawthorn (*Crataegus* spp.) and their ability to inhibit intestinal α-glucosidases

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Hawthorn (*Crataegus* spp., Rosaceae) is an old traditional and popular herbal medicine that contains a broad spectrum of substances (e.g., flavonoids, triterpenic acids, amines or phenolic acids) and has been used for the treatment of different cardiovascular diseases[1]. In traditional Chinese medicine, it has been administered for the treatment of hyperglycaemia for a long time. Even though several studies have confirmed the antidiabetic effects of *Crataegus* extract, the mechanism of action is not known. A possible clue can be α-glucosidase, the main enzyme catalysing the final step of carbohydrate hydrolysis to glucose in the digestive system. Inhibition of this enzyme is associated reduction in glucose absorption and glycaemia[2, 3].
For this reason, several major constituents of hawthorn were tested for their ability to block α-glucosidases. Both yeast and mammalian (rat) glucosidases were used. While triterpenic and phenolic acids showed a rather mild effect, flavonoids and especially flavonoid glycosides were potent inhibitors. The most effective compound was spireoside, which inhibited α-glucosidase more effectively than the registered drug acarbose, which was chosen as the standard agent. There were significant discrepancies between yeast and mammalian (rat) intestinal α-glucosidase inhibition. Yeast α-glucosidase does not appear to be a suitable model to predict effect in humans.

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**PO40**

# Anti-inflammatory activity and phytochemical profile of different plant material from *Rhododendron luteum* Sweet – comparative analysis

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Terpenes are plant secondary metabolites known for their anti-inflammatory potential. *Rhododendron luteum* Sweet (RL) was previously reported to present anti-inflammatory activity.[1] This species is a deciduous shrub that grows to a height of 4 m with oblanceolate leaves and yellow, exquisitely scented flowers.[2]

The present research investigates the terpene profile and anti-inflammatory activity of ASE (accelerated solvent extraction) extracts obtained from RL leaves, flowers, fruits, and woody stems. Samples were purified using SPE (solid phase extraction) before the chromatographic analysis. LC-ESI-MS/MS (liquid chromatography with electrospray ionization coupled with triple quadrupole mass spectrometry) analysis revealed the presence of pentacyclic triterpenes and phytosterols. Samples were also subjected to in vitro anti-inflammatory activity assays using spectrophotometric methods. $IC_{50}$ (half maximal inhibitory concentration) values for investigated extracts, as a measure of their potency in inhibiting the specific activity of enzymes lipoxygenase, hyaluronidase, and xanthine oxidase, were determined. Additionally, the mode of lipoxygenase and xanthine oxidase inhibitory was determined. Significant differences in enzymes inhibition were observed depending on the tested sample.

The rich abundance of compounds known for their pharmacological potential and the observed high anti-inflammatory activity of the extracts obtained from various RL organs encourage further studies of this species. Based on the obtained results, it can be concluded that *R. luteum* is a valuable source of biologically active pentacyclic terpenes and phytosterols.

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**PO41**

# Optimization of extraction of bioactive pentacyclic triterpenes and polyphenols from rose roots

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Rose roots contain several groups of bioactive metabolites e.g. triterpenes, flavonoids, and tannins and constitute a source of herb medicines described in Chinese Pharmacopoeia. In Asian medicine, they have been used e.g. to treat diabetes, diarrhoea, pain and chronic inflammatory diseases. However, in Europe, rose roots are almost unused and treated as crop waste. Few experimental studies indicated the high pharmacological potential of extracts from this plant material. They were found to demonstrate antioxidant, antiproliferative, anti-inflammatory and antihyperglycemic properties (Giménez et al., 2015). Therefore, this study aimed to determine the optimal conditions for obtaining pentacyclic triterpenes and polyphenols from *Rosa rugosa* root using relatively simple, cheap and eco-friendly methods.
Ultrasound-assisted extraction (UAE; 30 min, 60°C) and 16 extractants (different water/methanol/acetone ratios) were tested. The content of metabolites in the samples was determined using LC-ESI-MS/MS and spectrophotometric methods. Moreover, antioxidant and enzyme inhibitory activity was assayed. It was revealed that the acetone, acetone-methanolic and acetone-water or methanol-water extract had the highest triterpene and polyphenol content, respectively (130.24 mg/g DW and 63.76 mg/g DW, respectively). However, the highest extraction yield was obtained using a mixture of solvents: water, acetone, methanol. The strongest radical scavenging activity was presented by extracts where the eluent contained water and more than 60% of organic solvent (134.29-152.35 µg of Trolox/mg). The highest glucosidase inhibitory ability was observed for sample obtained with 50% methanol. LC-MS/MS analysis revealed large amount of catechin (17.14 mg/g DW) and other bioactive polyphenols e.g. quercetin, hyperoside, isoquercetin, quercitrin, as well as, gallic, chlorogenic, protocatechuic, *p*-coumaric and salicylic acid.

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**PO42**

# α-Glucosidase inhibitory activity and phytochemical profile of fruit extracts of Czech *Sorbus* endemics

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*Sorbus* L. *sensu lato* (Rosaceae) comprises over 250 species widespread throughout the Northern Hemisphere.[1] However, taxonomy is very complicated due to the ability of apomixis and hybridization of *Sorbus* species. The landscape of the Czech Republic provides a unique environment that enabled the creation of several endemic microspecies that are sometimes referred as *Karpatiosorbus* or *Aria* species based on the latest taxonomic revision of *Sorbus* s.l.[2] Unfortunately, these endemic species are overlooked and we know nothing about their content compounds and biological activities so far. Therefore, this study is focused on the comparison of the phytochemical profile of Czech *Sorbus* endemics with emphasis on their α-glucosidase inhibitory activity. Fruits collected in the Dendrological Garden, Průhonice were extracted with ethanol, and crude extracts were further partitioned with *n*-butanol and water to remove carbohydrates and other polar compounds. *n*-Butanol fractions were analyzed by HPLC-DAD, HPLC-ELSD, and $^{1}$H NMR to compare their phytochemical profile and to reveal the compounds responsible for α-glucosidase inhibitory activity because *n*-butanol fractions of all evaluated *Sorbus* s.l. species displayed greater inhibition of α-glucosidase than that of acarbose used as a positive control. Results of phytochemical profiling revealed triterpenes as dominant compounds in *n*-butanol fractions of all evaluated *Sorbus* s.l. species and therefore very probably responsible for bioactivity.

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**PO43**

# Screening of *Glycyrrhiza* species for anti-inflammatory phenols

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Roots and rhizomes of *Glycyrrhiza* species (Fabaceae) have long been used for their anti-inflammatory, expectorant, and antimicrobial effects.[1,2] Our research is dedicated to screening and selection of different *Glycyrrhiza* taxa for further isolation of bioactive compounds with with an emphasis on prenylated phenols. Plant material was obtained by cooperation with Mendel University, Faculty of Horticulture. Raw material from total of thirteen *Glycyrrhiza* varieties including six species (*G. glabra, G. uralensis, G. pallidiflora, G. foetida, G. yunnanensis,* and *G. echinata*) was extracted with ethanol. Extracts were fractionated by liquid-liquid extraction (n-hexane, chloroform, ethyl acetate and water). All fractions were then analysed by HPLC-DAD and ELSD. Chloroform and ethyl acetate fractions were tested for cytotoxicity and inhibition of NFκB on THP-1 cells. The major compounds from *G. uralensis* were isolated by flash chromatography and purified by semipreparative HPLC. HPLC-DAD analysis has not revealed any apparent intraspecific difference in content of phenolic compounds. Structures of isolated compounds will be elucidated by various spectral methods. Identified compounds will undergo further bioassays for anti-inflammatory effects.

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**PO44**

# Phlorotannins from brown algae *Ecklonia radiata* (C.Agardh) J.Agardh – isolation, structural elucidation, and antiproliferative properties

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*Ecklonia radiata* (C.Agardh) J.Agardh (Lessoniaceae) is a brown algae found abundantly in the warm-temperate parts of South Africa, Australia, and New Zealand[1]. It is a rich source of phlorotannins, which are polyphenolic compounds formed through the polymerization of phloroglucinol (1,3,5-trihydroxybenzene). The carbon to carbon and carbon to oxygen linkages of phloroglucinol units form various types of phlorotannins, such as phloroeckols, phloroethols, fucols, and fucophlorethols. Phlorotannins possess several bioactivities, including anti-herbivory, antioxidant, anti-inflammatory, antimicrobial, antiproliferative, antidiabetic, radioprotective, adipogenic, antiallergic, and antiviral, demonstrating promising potential in areas such as food, nutraceutical, and pharmaceutical industry[2].
The dried water-ethanolic extract of different parts of *E. radiata* was separated by liquid-liquid extraction. Ethyl acetate portion was subsequently separated using column chromatography and semi-preparative RP-HPLC. Identification of the isolated compounds was carried out using UV and IR spectroscopy, HRMS, and NMR spectroscopy. The antiproliferative activity of the isolated compounds was tested in cancer cell lines THP-1, MCF-7, and DU-145 using WST-1 analysis, and subsequently, cell cycle analysis was performed.

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**PO45**

# *Agrostemma githago* as a rich source of flavonoid-C-glycosides and triterpene saponins

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In the past *Agrostemma githago* (corn-cockle) occurred in large quantities in Eurasian fields. The seeds of this plant ripen at the same time as cereal seeds, so they were collected together and then ground into flour. Due to the toxicity of *Agrostemma githago* seeds, poisoning has occurred after eating flour products containing corn-cockle seeds. It is now known that mainly ribosome-inactivating proteins (RIPs) are responsible for seeds toxicity. The development of techniques for cleaning seeds before sowing has resulted in a radical decrease in the presence of *Agrostemma githago*. In 2016, it was placed on the Polish red list in the NT (near threatened) category. Despite the toxicity of the seeds, which caused *Agrostemma githago* to disappear from the natural environment, it is known from historical data that the root and herb of this species were used in folk medicine in the past. In Traditional Chinese Medicine (TCM) the root of *A. githago* was known as Mai Xian Weng (麦仙翁). Due to limited data on the phytochemistry of the herb (data limited to the saponins) and root of *A. githago* (no data in scientific databases), detailed phytochemical analyzes were performed.

Detailed phytochemical analysis (HPLC-DAD-MS, NMR) of extracts, fractions and isolated compounds revealed that the *Agrostemma githago* herb is a rich source of flavonoid-C-glycosides and triterpene saponins, while the root is a saponin raw material. Flavonoid-C-glycosides have a wide spectrum of bioactivity, including antioxidant, anti-inflammatory, antidiabetic, anticancer, hepatoprotective, antiviral, antibacterial, antinociceptive and immunomodulatory, which puts *Agrostemma githago* herb in a new light. The high content of biologically active compounds in raw materials may justify their traditional use in folk medicine and encourages further research to determine the effect on the human body.

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**PO46**

# Determination of PPAR gamma agonistic activity of mimulone and diplacone in nanoparticle formulations

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Geranylated flavonoids from *Paulownia tomentosa* have previously displayed potential antidiabetic activity via inhibition of protein tyrosine phosphatase 1B and α-glucosidase.[1]
This study aimed to determine whether these substances can also affect glucose metabolism via activation of the PPARγ pathway *in vitro*, to compare the data for the compounds alone and the same compounds in different nanoparticles, and to determine the most effective nanoparticle delivery system with the least possible loss of biological effect of the active substance after encapsulation.
The geranylated flavanones tested, mimulone and diplacone, were isolated from the unripe fruits of *Paulownia tomentosa* (Thunb.) Steud. (Paulowniaceae).[2]
First, the effect of the compounds on the viability of the PPARγ$_2$ CALUX® (U-2 OS) cell line (BioDetection Systems BV, Netherlands) was measured using WST-1 assay kit (Roche, Switzerland). Nanoparticle formulations were prepared using the lipid film hydration technique and detergent methods. Particles of a specific size, concentration, and surface charge were prepared using Dynamic Light Scattering and MADLS (Multiangle Dynamic Light Scattering). After 24 hours of incubation, their ability to induce PPARγ-mediated luciferase expression was assessed using the PPARγ$_2$ CALUX® reporter gene assay.[3]
Mimulone (2.5μM) showed partial PPARγ-agonistic activity (3.9-fold, $p \leq 0.0001$) as the compound itself (the maximum response compared to rosiglitazone at conc. of 2.5 μM was 16.8%). Cyclodextrins proved to be the most effective nanoparticles (with a loss of 10.5% of mimulone biological activity), the anionic liposome formulations were also active but with a loss of 46% of mimulone activity. Diplacone showed no statistically significant effect, either as a substance itself or as a component of nanoparticles.

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**47**

# Formulation with three herbs exhibits antiviral activity against highly pathogenic influenza a virus in chickens

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Influenza, particularly caused by Influenza A viruses (IAVs), poses significant health risks worldwide, affecting vulnerable populations such as young children, pregnant women, and the elderly. Despite advancements in medical technology and the development of antivirals, the fight against flu continues, highlighted by the 2009 H1N1 pandemic resulting in up to 575,400 deaths in the U.S. alone. Traditional methods to combat IAVs, including antiviral medications and vaccinations, face challenges due to the virus's rapid evolution, leading to resistance. Moreover, zoonotic avian influenza viruses (AIVs) present new public health risks, with control measures like mass culling causing economic and ethical concerns. This study explores a multicomponent and multitarget (MCMT) approach, derived from traditional Chinese medicine (TCM), which combines different medicinal plants to lower the chance of resistance development[1].

We investigated the antiviral effects of three herbal formulation consisting of *Angelica dahurica*, *Pinus densiflora*, and *Curcuma longa*, known for their respective antiviral properties, against IAV H1N1[2,3]. Our findings show promising results, particularly for a selected combination enhanced with ascorbic acid and chitosan, demonstrating significant anti-influenza activity in chickens infected with the highly pathogenic avian influenza (HPAI) A H5N6. This study suggests the potential of MCMT strategies in developing effective antiviral treatments against IAVs and AIVs, offering a novel approach to addressing current limitations in influenza management.

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## PO48

# Evaluation of reference gene expression stability by quantitative real-time PCR in *Agrostemma githago*

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Quantitative real-time polymerase chain reaction (qPCR) is widely used for gene expression studies, with high specificity, sensitivity and repeatability. However, for precise and reliable expression analysis using the relative quantification approach, a selection of a stable reference gene is a prerequisite to accurately normalize the data. Expression of internal reference genes can vary considerably during developmental stages and under different experimental conditions[1].

For *Agrostemma githago* L. (corn cockle, Caryophyllaceae), there are no reports of stable reference genes. In order to study the expression of genes related to natural product biosynthesis and regulation of specialized metabolism, we aimed at selecting most stable reference genes across all studied plant tissues and growing conditions.

Based on previous studies[2,3], 10 frequently-used housekeeping genes: *actin*, *translation elongation factor 1*, *glyceraldehyde-3-phosphate dehydrogenase*, *tubulin α*, *tubulin β*, *ubiquitin*, *histone H3*, *elongation factor 1-α*, *elongation factor 1-β*, *eukaryotic translation initiation factor 5A*, and *serine/threonine protein phosphatase 2A* were selected for evaluation as potential reference genes in *A. githago*. The integrated expression stability of potential reference genes was established with the RefFinder tool (https://blooge.cn/RefFinder/), which integrates following computational statistical methods: the geNorm, NormFinder, BestKeeper, and the comparative Δ-Ct method. The results showed that the expression stability of candidate genes varies in different sample pools, but allowed for successful selection of three most adequate reference genes.

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**PO49**

# Synthesis and screening of carltonine mimetics for selective butyrylcholinesterase inhibition

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Butyrylcholinesterase (BChE) assumes a pivotal role in the pathophysiology and progression of Alzheimer's disease (AD). As AD progresses, acetylcholinesterase levels decrease by over 90%, while BChE levels rise to 165% of their normal level, suggesting a compensatory mechanism. We are exploring innovative Alzheimer's drug candidates, focusing on Amaryllidaceae alkaloids carltonine A and B. Our research group has previously isolated these compounds, revealing their significant selectivity for human BChE (*h*BChE)[1].
This study encompasses the design, synthesis, and *in vitro* evaluation of compounds exhibiting *h*BChE inhibitory potential, ranging from micromolar to low nanomolar scale. Compounds demonstrating the most significant inhibition underwent theoretical validation for their potential to penetrate the CNS, using the BBB score algorithm, corroborated by *in vitro* PAMPA results. Additionally, the safety profiles of these compounds were assessed using human neuroblastoma and hepatocellular carcinoma cell lines. Significantly, compounds **87** (*h*BChE $IC_{50} = 3.8 \pm 0.2$ nM) and **88** (*h*BChE $IC_{50} = 5.7 \pm 1.5$ nM) have emerged as exemplary inhibitors. A crystallographic study was conducted to elucidate the binding mode of the most potent inhibitor. Distinguished by its novel scaffold, compound **87** emerges as a promising lead for tackling mid to late-stage AD challenges[2].

Acknowledgements: The study was supported by Charles University SVV 260 662.

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## PO50

# Colon-targeted delivery of pomegranate and blackcurrant seed oils exhibiting antitumor activity

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Components of vegetable oils, including polyunsaturated fatty acids (PUFAs) and sterols, can inhibit colorectal cancer cells. Pomegranate seed (PSO) and black currant seed (BCSO), laden with various PUFAs, sterols and tocopherols with antitumor activity, are good candidates for preventive and supportive anticancer agents in chemotherapy. Encapsulation of oils using cross-linking with alginate and chitosan may enable colon delivery of unaffected oil components and extend their retention time in the colon due to mucosal adhesion of chitosan.
We aimed to investigate the composition and in vitro anticancer potential of pomegranate (PG) and blackcurrant (BC) seed oils extracted by cold-pressing, and to test the oxidative stability and release under simulated gastrointestinal conditions of oils encapsulated by cross-linking with alginate and chitosan or its oleoyl-derivative.
The GC analyses of fatty acids and sterols, and UPLC analyses of tocopherols and carotenoids were performed. Oil stability was assessed using PV, AV and DSC. Chemopreventive potential of 1 – 5% oil-in-water emulsions against LoVo, LoVo/DX and CCD 841 CoTr cell lines was investigated using MTT assay, fluorescence microscopy of the apoptotic cell number, Rhodamine-123 test and cellular antioxidant analysis (CAA).
PG and BC oils were encapsulated by drip feeding into a $CaCl_2$ solution the alginate based emulsion (5% Na alginate and 20% oil) with further chitosan/oleoyl chitosan coating. The effectiveness of encapsulation was verified using FTIR and NMR.
BC was characterized by high tocopherol (1649 mg/kg) and carotenoid (483 mg/kg) amounts and higher increase in apoptotic cells and Rhodamine-123 accumulation than in case of PG. High punicic acid (63%) and phytosterol (6427 mg/kg) contents in PG were accompanied by a stronger influence on the cytotoxic effect of doxorubicin on cancer cells. Encapsulation significantly enhanced PG and BC oxidative stability and enabled safe in vitro simulated gastrointestinal transit into the colon.

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**PO51**

# Quality assessment of *Boswellia serrata* extract- containing food supplements on the Hungarian market

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*Boswellia serrata* extract is applied in the management of osteoarthritis as a food supplement[1]. The quality of food supplements is scarcely monitored in Hungary. Moreover, the limited access to the drug is a red flag in terms of product quality. The European Pharmacopeia's quality parameters are based on the content of marker compounds in drug (AKβBA: acetyl-11-keto-*β*-boswellic acid and KβBA: 11-keto-*β*-boswellic acid); whereas, commercial extracts are characterized by their total boswellic acid content (TBA). The latter parameter is not specific enough to ensure the possible adulteration of the products[2]. The AKβBA and KβBA content of the drug should be not less than 1%, respectively[2]. For extracts EFSA recommends 2.53%-3.68% of AKβBA and 2.35%–3.57% of KβBA content. The TBA should not be lower than 65% determined by titrimetry[3].

In our research seven frankincense containing products were purchased in Hungarian pharmacies. The quality of the obtained products was screened applying various instrumental analytical methods (HPLC-PDA, LC-MS, $^1$H-NMR). Four major boswellic acids (AKβBA, KβBA, AβBA: acetyl-*β*-boswellic acid, βBA: *β*-boswellic acid) were quantified, as well as the $^1$H-NMR fingerprint spectra of the products were compared to a USP grade reference extract. Result of the chromatographic measurements was interpreted based on the USP-NF. The sum of KBA and AKBA was calculated based on the labelled amount of extract in the food supplements. In four out of seven products miniscule amount of boswellic acids (the sum of AkβBA and KβBA was 0.00%–0.30%) as well as the lack of characteristic $^1$H-NMR fingerprint were observed. No quality issues had been risen in three product based on boswellic acid content; whereas, in the suitable food supplements, similar boswellic acid profile was observed: AKβBA: 5.88%–7.07%; KβBA: 3.09%–4.12%; βBA: 10.38%–22.42%; AβBA: 3.49%–8,87%.

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**PO52**

# 2D- NMR chemical investigation of bioactive fractions of *Clinopodium nepeta*

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*Clinopodium nepeta* subsp. *glandulosum* belongs to the Lamiaceae family is known to be a polymorphic and fragrant plant with traditional uses including medicinal herbs for anti-spasmodic, diuretic, stimulant, and tonic purposes (Debbabi et al., 2020). Several studies regarding essential oils have been revealed a high content of oxygenated monoterpenes, such as pulegone, piperitenone and piperitenone oxide (Boškailo et al., 2022) as well as their antimicrobial, anti-candida, antioxidant, and insecticidal activities (Debbabi et al., 2020). Despite several investigations regarding essential oils and their biological activities, at the best of our knowledge, still little is known about chemical composition in term of polar compounds present in crude extract. Based on these considerations, the aim of the present study is to investigate the polar extracts of *C. nepeta* subsp. *glandulosum* in term of metabolic composition and biological activities. The current work was addresses to investigate six extracts of *Clinopodium nepeta subsp. glandulosum* through biological assays and NMR analysis, in order to furnish information about the specialized metabolite content. The hydroalcoholic extract was the most promising one in term of antioxidant and enzyme inhibiting activities, reporting a DPPH radicals inhibition of 48.07 ± 0.21 mg TE/g and a AChE inhibitory capacity of 2.66 mg GALAE/g, respectively. As highlighted by exhaustive 2D-NMR investigation hydroalcoholic extract was dominated by resonance of flavonoidic glucosides and triterpenoidic compounds, whose fully characterization was reach only after a liquid-liquid separation and chromatographic purification. Through deeply 2D-NMR analysis, acacetin glycoside derivatives, flavonol glycosides, ursolic acid and 2,4-di-t-butylphenol were highlighted as principal component of more bioactive extract.

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**PO53**

# Nature *versus* cultivation: exploring the phytochemical diversity and bioactive potential of the halophyte *Plantago coronopus* L.

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The resilience and nutritional profile of halophyte plants, particularly *Plantago coronopus* L., a member of the Plantaginaceae family, highlight their potential for sustainable agriculture in saline-prone environments. *P. coronopus* thrives in the Mediterranean, North Africa, and the Middle East, offering a rich source of proteins, vitamins, minerals, and fibres, along with various bioactive compounds with promising therapeutic applications. With the escalating interest in halophyte cultivation due to their adaptability to harsh saline conditions, this study delves into a comparative analysis of PC's bio-properties and chemical variability in nature *versus* greenhouse conditions. Methanol extracts were evaluated for their DPPH and ABTS radical-scavenging activities, copper and iron chelation, ferric-reducing antioxidant power, and enzymatic inhibitory properties towards AChE, BuChE, α-glucosidase, α-amylase, and lipase. The extracts were also evaluated for their total phenolics, flavonoids, and condensed tannin contents, followed by the chemical profiling of bioactive compounds using Ultra-Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry/Mass Spectrometry (UPLC–HR–MS/MS). Generally, samples collected from nature exhibited higher radical-scavenging, metal-chelation, ferric-reducing, and enzyme-inhibitory properties, as well as higher total contents of phenolics, flavonoids, and condensed tannins. Moreover, chemical profiling distinguished the metabolomes of wild and greenhouse samples. Out of the 331 compound extract features, 211 showed significant differences between the wild and greenhouse samples. Library spectral matches allowed the annotation of 23 statistically significant features. Trehalose, skimmine/isomer, and 13, 16, 19–docosatrienoic acid were more abundant in greenhouse samples. The remaining twenty features showed a significant increase in wild extracts of *P. coronopus*, most of which belonged to cinnamic acids and derivatives and prenol lipids. The implications of our findings contribute to refining halophyte cultivation practices, enhancing sustainable agriculture in arid regions, and investigating *P. coronopus* as a viable source of health-beneficial bioactive compounds.

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**PO54**

# Isolation and identification of compounds from *Schisandra chinensis* fruit and effect of dibenzocyclooctadiene lignans on gap junction intercellular communication

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*Schisandra chinensis* belongs to 50 basic herbs of Chinese medicine[1]. It is a deciduous, shrub-like, dextrorotatory creeper without tendrils. Dibenzocyclooctadiene lignans are the most important active substances from *S. chinensis*., which have a biological and pharmacological activity.

An investigation of compounds of the fruit of *S. chinensis* led to the isolation and structural determination of eight dibenzocyclooctadiene lignans and arisantetralones A and C never isolated from *S. chinensis* before. We isolated new sesquiterpene 7,7-dimethyl-11-methylidenespiro[5.5]undec-2-ene-3-carboxylic acid. Gap junction intercellular communication (GJIC) allows communication between cells and the exchange of small molecules through connexin-based gap junction channels[2]. Selected dibenzocyclooctadiene lignans were tested to evaluate their effect on gap junction intercellular communication in WB-*ras* cells. This is the first report on GJIC for dibenzocyclooctadiene lignans.

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**PO55**

# Effect of short-term apple consumption on fecal metabolites: A pre-post interventional study using NMR analysis

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Apples are an important source of dietary fibre, vitamins K, C, B6, and phytonutrients. Prolonged apple consumption has demonstrated potential anti-cancerous, anti-diabetic, anti-obesity, and anti-inflammatory properties, with many of these benefits being attributed to the fruit's phytonutrients or dietary fibre[1]. Nuclear magnetic resonance (NMR)-based metabolomics is a powerful method for capturing a snapshot of 40-60 abundant metabolites in biological samples. Meanwhile, NMR metabolomics offers insights into an individual's dietary and digestive processes in the colon and the composition of their gut microbiota[2]. Our objective was to explore the changes in the faecal metabolites associated with increased apple intake. Based on existing research, we anticipated an increase in short-chain fatty acids (SCFAs) and metabolic by-products of dietary phenolics, such as phenylacetic acid derivatives.
Fifteen participants submitted stool samples before and after a three-day apple consumption intervention. These samples were analysed to identify and quantify changes in faecal metabolite concentrations. Univariate analysis showed a moderate decrease of methanol (FC=0.35, p=0.016, FDR=0.339), but an increase of propionate (FC=1.19, p=0.029, FDR=0.339), valine (FC=1.24, p=0.026, FDR=0.339), fumarate (FC=1.24, p=0.040, FDR=0.339), glycine (FC=1.18, p=0.047, FDR=0.339), and aspartate (FC=1.20, p=0.045, FDR=0.339) following the intervention. These changes were not statistically significant after adjusting for multiple comparison corrections. Interestingly, methanol levels decreased, which contrasts with the assumption that pectin fermentation will lead to the opposite effect. The marginally significant changes in faecal metabolites post-intervention suggest that short-term dietary intake has a minimal effect on the stool's metabolic profile. The most substantial differences are attributed to individual variances, which remain constant and do not fluctuate with such short-term dietary adjustments. Further research is needed to understand the impact of diet on the gut microbiota's composition.

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**PO56**

# Extraction of cannabinoids, volatile terpenes, and polyphenols from *Cannabis sativa*: subcritical water vs. supercritical $CO_2$ and organic solvents

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The utilization of hemp (*Cannabis sativa*) has garnered significant attention due to its diverse applications across pharmaceuticals, food, and cosmetics industries. Employing a $2^3$ full factorial Design of Experiments approach, we investigated subcritical water extraction as a sustainable extraction technique (1) for obtaining hemp extracts (var. 'Futura 75') as an alternative to supercritical $CO_2$ extraction (2), ultrasonic extraction (organic solvents, water), Soxhlet and high-pressure ethanol extraction and hydro-distillation.

In the extraction with subcritical water, we confirmed statistically significant models for the yield, the antioxidant activity (determined by the DPPH and ABTS tests), and the content of polyphenolic compounds (determined by the FC test) 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 key parameters. 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 (temperature was a key parameter) content as the extracts obtained with other methods.

Essential oil and condensed Clevenger water fraction were found to be significantly different, the former containing predominantly sesquiterpenes (82.51%) and oxygenated sesquiterpenes (9.12%), while the latter was rich in oxygenated monoterpenes (34.97%) and non-terpene compounds with carbonyl groups (45.16%).

In conclusion, subcritical water extraction presents a promising method for obtaining novel hemp extracts with relatively high antioxidant activity and favorable yield.

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**PO57**

# Antimicrobial properties of phenolics against fusidic acid-resistant MRSA in wound healing

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*Staphylococcus aureus* is a pathogen responsible for skin and soft-tissue infections. The emergence of methicillin-resistant *S. aureus* (MRSA) leads to antibiotic treatment failure, including topical antibiotics. Plant-derived natural compounds, especially prenylated phenolics, provide a significant source of potent antimicrobial agents[1]. Firstly, the antimicrobial activity of eight compounds was evaluated against six MRSA clinical isolates resistant to fusidic acid. The minimum inhibitory concentrations (MICs) were assessed using the microdilution method. The synergistic potential with fusidic acid was measured by the checkerboard titration technique, and the fractional inhibitory concentration (FIC) described synergy (FIC $\leq$0.5), additive effect (0.5< FIC $\leq$1); and indifference (1< FIC $\leq$4). Prenylated phenolics inhibited the growth of MRSA in a range of 4–16 µg/mL. MIC of fusidic acid proved resistance of all clinical isolates (MIC $\geq$2 µg/mL). Kuwanon E, U, morusin, and Diels-Alder adducts kuwanon G and albafuran C in combination showed additive effects against at least one MRSA isolate. Kuwanon C with the antibiotic revealed additive effect against five MRSA strains and represents the most effective combination. Thymol and epigallocatechin gallate showed higher MIC ($\geq$32 µg/mL) but, in combination with fusidic acid, revealed partial synergism against at least three MRSA strains. No synergism was observed for any combinations of selected compounds with fusidic acid. To sum up, prenylated phenolics represent prospective antimicrobial agents against MRSA, and their anti-inflammatory and antioxidant effects could further support wound healing.

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**PO58**

# *Agrostemma githago* (common corn-cockle) seeds as a new source of unsaturated fatty acids

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*Agrostemma githago* L. (corn-cockle) is a segetal weed, which in the past occurred in large quantities in Eurasian fields. It was regarded as a significant toxicological agent, due the high ribosome-inactivating proteins (RIPs) and saponins content. To date, most studies on this taxon have focused on above groups of specialized metabolites.

Among neglected constituents of *A. githago* fatty acids may be listed. Our studies show that seeds of corn-cockle could be used as a novel source of oil for polyunsaturated fatty acids.

The oil was isolated from seeds, using hexane as a solvent. In the next step, the transesterification was performed. The compositions of the seed oils and essential oils were determined using a gas chromatography–mass spectrometry (GC–MS) instrument.

The most important constituents, found in tested sample are: benzyl alcohol, dodecyl acrylate, 14-methyl-pentadecanoic acid methyl ester, 9,12-Octadecadienoic acid (Z,Z) methyl ester, and 9,12,15-octadecatrienoic acid methyl ester.

Further analysis and optimalisation of isolation process could help to utilize oil of *A. githago* in cosmetic, food and pharmaceutical industries.

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**PO59**

# Anti-inflammatory and antioxidant activity of dibenzocyclooctadiene lignans from *Schisandra chinensis*

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*Schisandra chinensis* (Schisandraceae) is a medicinal plant used in traditional Chinese medicine to treat many diseases. It grows wild in China, Korea, Japan, and Eastern part of Russia[1]. Dibenzocyclooctadiene lignans are the main substances with biological and pharmacological activity. These lignans stimulate liver regeneration, inhibit hepatocarcinogenesis, and suppress lipid peroxidation[2]. Dibenzocyclooctadiene lignans showed anti-HIV, antioxidant, and anti-inflammatory activities[3].

Examination of compounds from *S. chinensis* fruits has led to the isolation and structural determination of eight dibenzocyclooctadiene lignans and two aryltetralones. Furthermore, we isolated new sesquiterpene 7,7-dimethyl-11-methylidenespiro[5.5]undec-2-ene-3-carboxylic acid. These compounds were isolated by extensive chromatographic separation on normal and reversed phase and identified by comparison of their $^{1}H$ and $^{13}C$ NMR spectra, MS, CD, and IR spectra with the corresponding literature.

Selected dibenzocyclooctadiene lignans were tested to examine their cytotoxicity (using the WST-1 assay), anti-inflammatory activity (in LPS-stimulated monocytic leukemia THP-1 NF-κB cells monitoring their anti-NF-κB activity), and antioxidant activity (using CAA assay). The WST-1 experiment led to establishing a non-toxic concetration of 10 μM for furhter biological assays. Only some dibenzocyclooctadiene lignans have shown antioxidant activity. The highest antioxidant activity was demonstrated by (-)-gomisin J ($p < 0.001$). This activity corresponded to approximately 50% of the activity of quercetin ($p < 0.001$), which served as a positive control. The anti-inflammatory activity was detected for (−)-gomisin N ($p < 0.0001$) and (+)-γ-schisandrin ($p < 0.0001$) at a concentration of 10 μM. The activity of these compounds was higher than that of the positive control prednisolone ($p < 0.0001$) at a concentration 2 μM [1].

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**PO60**

# Phytochemical profile of wild and cultivated *Nigella damascena* species collected in Sicily (Italy)

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*Nigella damascena* L. is an annual herbaceous plant with a Euro-Mediterranean distribution range, widely used in the food, pharmaceutical and cosmetic industries, since these plants' unique phytochemical composition directly relates to their virtues[1]. The current work was addressed to a comparison between the aerial parts of the same plants of *N. damascena*, both wild (WND) and cultivated, with white flowers (CWND) and blue flowers (CBND).
Plant material for each sample type (WND, CWND, and CBND) was subjected to a preliminary analysis using a metabolomics approach[2]. A phytochemical investigation of the three methanolic extracts was implemented by employing a combination of NMR spectroscopy and LC-MS analysis to identify the metabolites present in the extracts. Furthermore, a partial purification of the extracts was conducted by dissolving them in water and partitioning in ethyl acetate to concentrate the compounds of interest before analysis.
The study emphasized the distinct phytochemical composition of *N. damascena* extracts, uncovering the presence of different phytochemical compounds, including triterpene saponins, glycosylated flavonoids and other metabolites. The plants, when exposed to different growth stress conditions, were characterized by distinctive chemical profiles. Notably, the relative abundance of glycosylated triterpenes in the extracts was higher in the WND and CBND extracts compared to CWND. Overall, the chemical analysis provided insights into the diverse array of phytochemical compounds present in the *N. damascena* extracts, highlighting the plant's rich chemical profile and potential pharmacological significance. Subsequent studies will aim to evaluate the biological activity of these new potentially bioactive compounds.

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**PO61**

# How flavonoid-C-glycosides are made in the *Caryophyllaceae*? – a contribution to biosynthesis-related genes discovery

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Plants from the *Caryophyllaceae* family are widely known for their saponin content. However, previous studies have shown a significant quantity and variety of flavonoid C-glycosides, which have several medicinal properties. An HPLC analysis of *Agrostemma githago* L. confirmed the presence of orientin, isoorientin, vitexin and isovitexin.

C-glycosides are less known than the more common O-glycosides and we know even less about their biosynthesis in the Caryophyllaceae. This study aimed at expanding knowledge about the crucial steps of flavonoid C-glycosides biosynthesis and its regulation at the molecular level and was focused on identifying previously unknown C-glycosyltransferases. The plant material from *A. githago* was obtained from field cultivation and *in vitro* cultures. Nucleotide sequences of homolog genes involved in the plant flavonoid biosynthetic pathway were identified by RNAseq – including general phenylpropanoid pathway, through flavones pathway (*CHS*, *CHI*, *F3'H*, *FSI*/*FSII)*, to hypothetical C-glycosylflavones biosynthesis with *F2H*/*CYP93G* and, currently unknown, *CGT*. Selected sequences were employed for PCR primer design and gene expression analysis. Metabolic profiling of flavonoid C-glycosides was performed concurrently using LC-qTOF-MS.

The discovery and characterization of the mechanisms of transcriptional regulation of the C-glycoside biosynthetic pathway contribute to a significant increase in knowledge about this group of flavonoids. Their considerable importance in the prevention of many lifestyle diseases allows us to expect that the previously poorly understood aspect of the properties of medicinal plants among the *Caryophyllaceae* would finally find an appropriate place in their multi-directional use.

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# PO62

## Oncostatic activities of plant nutraceuticals in rodent models of breast carcinoma

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Comprehensive oncology research suggests an important role of phytochemicals or whole plant foods in the modulation of signaling pathways associated with anticancer action. The original data of our working group demonstrated the oncostatic efficiency of low-dose mixtures of phytochemicals present in specific herbs, spices, or fruits in rodent models of breast carcinoma. The goal of this study was to assess the anticancer activities of *Hippophae rhamnoides* L. using rat and mouse breast carcinoma models. *H. rhamnoides* (as a fruit peel powder) was administered in the diet at two concentrations of 0.3 % (w/w) and 3 % (w/w) during the whole experiment in chemopreventive rat and therapeutic 4T1 mouse models.

In the chemopreventive model, sea buckthorn significantly decreased the HG/LG ratio vs controls. This result was associated with increased expression of caspase-3 and Bax/Bcl-2 ratio, and increased levels of histone chemical modifications i.e. H4K16ac and H4K20m3. Moreover, sea buckthorn decreased expressions of VEGF, Ki67, MDA, CD44, and EpCam when compared to control samples. In addition, the 4T1 mice model showed a decrease in tumor volume by 48 % (and a decrease by 45 % in mitotic activity index) after sea buckthorn treatment vs control tumors.

The present study demonstrated significant chemopreventive and therapeutic activities of *H. rhamnoides* against experimental breast carcinoma *in vivo*.

Acknowledgement: This study was supported by the grant VEGA 1/0045/23.

**PO63**

# Environmentally friendly solid-liquid extraction for the recovery of polyphenols from almond okara

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The plant-based milk industry represents the largest plant-based food sector today, with an estimated annual global growth rate of 15.5% from 2022 to 2029. The reasons for this increase are dairy milk allergy, lactose intolerance and the vegan lifestyle. However, like the manufacturing processes of other plant-based drinks, almond beverage production generates considerable quantities of a solid byproduct known as “okara”. Despite its potential valuable content of bioactive components, such as polyphenols, lipids, dietary fiber and proteins, the studies on almond okara are scarce.
Our objective is therefore to valorize almond okara, with the aim of promoting a sustainable approach with zero agro-industrial waste and producing functional ingredients for the nutraceutical and cosmetic industries (functional foods, food supplements …).
Almond milk was produced by soaking and blanching almonds for a defined time, then wet grinding them with ultrapure water at a ratio of 1/9 (*w/v*). The mixture was then filtered and the freshly obtained okara was used for characterization and evaluation.
Aqueous and ethanolic solid-liquid extractions were carried out on almond and almond okara. The effect of several parameters was studied (solid-to-solvent ratio, solvent concentration, temperature, extraction time). Our major objective is to study the influence of these parameters on the recovery of polyphenols as well as on the antioxidant activity. We analyzed the effect of these parameters on total polyphenol content (TPC), tannin content (TC) and total flavonoid content (TFC), as well as on the quality of the extracts by determining the antioxidant activity.
Results obtained showed that the polyphenol contents of the almond okara are relatively close to the values of the almond. In addition, our results showed a significant antioxidant activity of this by-product. These results confirm our theory that almond okara is a very promising by-product that could be implemented at several industrial levels.

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**PO64**

# Identification of plant phenolics as novel PPARγ agonists and hypoglycemic agents

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There is a great need in finding new hypoglycemic agents as the number of diabetes mellitus patients is rising sharply[1]. The objective of this study was to explore PPARγ agonism and hypoglycemic activity among plant-derived phenolic compounds. We started with a preselection using *in silico* molecular docking, and later, the selected compounds were investigated using *in vitro* cell culture-based assays: PPARγ luciferase reporter gene assay[2] and PPARγ protein expression (by western blot analysis). Moreover, the ability of the selected compounds to induce GLUT4 translocation in cell culture and lower blood glucose levels in chicken embryos was determined.
Among the thirty-six plant phenolic compounds, moracin M showed the highest hypoglycemic effect in an in ovo experiment followed by mulberrofuran Y and diplacone. Neither moracin M or mulberrofuran Y showed a conclusive effect on enhancement of GLUT4 translocation or agonism on PPARγ. On the other hand, diplacone increased GLUT4-GFP signal with greater agonism towards PPARγ. Thus, we believe that compounds moracin M, mulberrofuran Y, and diplacone are suitable for further experiments in antidiabetic research and elucidation of their mechanisms of action.

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# PO65

## Bioactive small molecules from Mediterranean plants as ligands for DNA secondary structures

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Mediterranean plants have long been used by traditional medicine to treat various disorders. They contain a wide range of bioactive molecules with distinct chemical structures showing pharmacological activities[1]. Experimental evidence demonstrated that bioactive molecules play a crucial role in anticancer therapy by targeting and interfering with biological processes responsible for tumor growth and progression. Several studies demonstrated that cancer development is strictly associated with structural changes of the genomic DNA that adopts alternative secondary structure. In particular, the main gene expression regulator G quadruplexes (G4s) DNA play an important role in the cancer progression and their stabilization by specific binders as natural ligands may open promising avenues for potential therapies against cancer. Phytochemicals may act as ligands for DNA secondary structures in target cells, interfering with their regulatory activity and acting as potential anti-tumor drugs [2,3]. In the aim to identify bioactive molecules able to interact specifically with genomic G4s, different Mediterranean plant extracts were screened by using modern NMR-based metabolomics methodologies allowing faster and improved discovery and characterization of potential biomolecules. In particular, our methodological approach, based on the combination of advanced in-mixture NMR experiments with an *ad hoc* phytochemical strategy allowed us to isolate different secondary metabolites such as carexanes, lignans, alkaloids, flavonoids. Interaction studies between the isolated pure metabolites and the G-quadruplex-folded DNA regulatory regions of the human *K-RAS* oncogene are currently in progress in our labs. Targeted delivery studies on the most promising ligands will also be performed using halloysite nanotubes as drug carriers.

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**PO66**

# Five new tamarixetin glycosides from *Astragalus thracicus* Griseb., including some substituted with the rare 3-hydroxy-3-methylglutaric acid and their collagenase inhibitory effects *in vitro*

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Along with the known kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-[6-O-(3-hydroxy-3-methylglutaryl)]-β-D-galactopyranoside (**1**), five new flavonoids, containing the rarely isolated aglycon tamarixetin, were isolated from methanolic extract of the endemic Balkan species *Astragalus thracicus* Griseb. Three of the new compounds are substituted with the untypical for genus *Astragalus* 3-hydroxy-3-methylglutaryl residue (HMG). The compounds were identified as tamarixetin-3-*O*-α-L-rhamnopyranosyl-(1→2)-[6-O-(3-hydroxy-3-methylglutaryl)]-β-D-galactopyranoside (**2**), tamarixetin-3-*O*-(2,6-di-*O*-α-L-rhamno-pyranosyl)-β-D-galactopyranoside (**3**), tamarixetin 3-*O*-β-D-apiofuranosyl-(1→2)-β-D-galacto-pyranoside (**4**), tamarixetin-3-*O*-β-D-apiofuranosyl-(1→2)-[6-*O*-(3-hydroxy-3-methylglutaryl)]-β-D-galactopyranoside (**5**), and tamarixetin-3-*O*-β-D-apiofuranosyl-(1→2)-[α-L-rhamno-pyranosyl-(1→6)]-β-D-galactopyranoside (**6**). Selected compounds from *A. thracicus* were tested for to evaluate their anticollagenase activity. The greatest effect was observed for quercetin-3-*O*-β-D-apiofuranosyl-(1→2)-β-D-galactopyranoside, possibly due to a presence of ortho-dihydroxy arrangement of flavonoid ring B. The effect on collagenase and elastase was further evaluated also by *in silico* study and the test compounds showed some level of *in silico* interaction.

**PO67**

# Vincarubine: Unraveling a cytotoxic mechanism against cancer cells

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The naturally occurring monoterpene *bis*-indole alkaloids vincristine and vinblastine are well-known and established chemotherapeutics used in the conventional treatment of certain types of cancer. They act as cytotoxic agents mainly by disrupting microtubule dynamics in cells. Another naturally occurring alkaloid with a similar structure, vincarubine, has been isolated from aerial parts of *Vinca minor* in this study. Given its structure similarity, an initial screening of vincarubine's cytostatic and cytotoxic activity on a panel of nine tumor and one non-tumor cell lines was performed. Vincarubine significantly inhibited cell growth at 10 μM, more so than the positive standard doxorubicine at 1 μM. The $IC_{50}$ values for these cell lines were determined with a average of 3.5 μM. These results encouraged a deeper focus on its mechanism of action. The effects of vincarubine on cell viability and proliferation in A549, MCF-7, A2780, and MRC-5 cell lines were further studied using xCELLigence method in real time. The impact on the cell cycle in A549 cell line suggested that vincarubine perturbs proliferation in the G1 phase, contrary to vincristine. The docking study into the active site of tubulin showed weaker interaction as well, implying the involvement of different mechanisms. The study on activation of caspase-3 and -7 indicated that vincarubine induces apoptosis, particularly in Jurkat and SAOS-2 cell lines, at concentrations of 5 and 10 μM. Additionally, vincarubine influence on the level and organisation of proteins such as actin, tubulin, paxillin, and vinculin was determined through epifluorescence imaging of A549 cells, further suggesting a distinct mechanism of action from vincristine and vinblastine. This study highlights the importance of exploring naturally occurring compounds for cancer therapy, as evidenced by vincarubine's specific mechanism of action and its potential to offer a new therapeutic approach.

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## PO68

# Semi-targeted metabolomics of *Selaginella* plants based on LC-MS coupled with bioactivity assay for identification of biomarkers

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The single genus *Selaginella* P. Beauv. (Selaginellaceae) is an important plant resource with renowned for its diverse applications in food and medicinal chemistry. *Selaginella* plants are recognized for their abundance in bioactive biflavonoids (BFVs). Although the presence of BFVs has been documented in numerous species, a comprehensive metabolomic and bioactivity assessment of various plant species in Thailand remains absent. Biochemometrics, which integrates chemical profiles with biological data, serves as a robust methodology for analyzing and identifying bioactive compounds within intricate mixtures such as fractions or crude extracts. This study aims to establish semi-targeted metabolomic profiling of *Selaginella* plants from different regions in Thailand through a methodology centered on LC-MS and bioassay-guided approaches. HPLC analysis of EtOAc extracts from *Selaginella* plants revealed several BFVs, notably amentoflavone, which is extensively acknowledged for its pharmacological properties. The disparities in metabolite profiles among *Selaginella* species from diverse geographical locations were scrutinized using multivariate analysis. Unsupervised analysis of this dataset showcases the efficacy of the analytical workflow in discriminating between different *Selaginella* species. Coupled with supervised analysis, a roster of metabolites responsible for the anti-inflammatory activity under investigation was delineated. This study serves as a valuable tool for expeditious dereplication and identification of known or unknown BFVs. It offers potential for amalgamating data from multiple stages of fractionation and bioassays into a cohesive analysis framework.

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**PO69**

# The analysis of the *in vitro* flax plant with tocopherol-pathway gene *VTE2* overexpression

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The flax is one of the oldest plants domesticated by human and having unique characteristics. It provides fibers for the textile industry and the oil used in the food, cosmetic, chemical and pharmaceutical industries[1]. Flax seed oil contains a large number of biologically active components. One of these components are tocopherols, which are synthesized exclusively by plants. In plants, tocopherols function to protect cell membranes from oxidative stress. In humans, tocopherols from food play important role in the prevention of neurodegenerative, cardiovascular diseases and cancer. The antioxidant properties of these components come from the ability to reduce free radicals, before their interaction with the biological membrane[2]. In this work we overexpressed in the flax plants the *vte2* gene, which encodes homogentisate phytyl transferase from *Arabidopsis thaliana*. The enzyme produces the first substrate in the tocopherol pathway and is considered as a key enzyme in the synthesis of tocopherols[3].

This report shows the analysis of the transgene plants. The introduced gene was confirmed by PCR; the tocochromanols were determined by ultra-high performance liquid chromatography (UPLC). The terpenoid pathways activity was checked by real-time PCR (MEP, MEV, carotenoids, tocochromanols) and the content of the main metabolites formed in these pathways checked by UPLC (tocochromanols, carotenoids, chlorophylls). Also, the effect of the modifications performed on the levels of metabolites of the tocopherol synthesis pathway also reflected the antioxidant potential of the plant extracts. The resistance of transgene plants against fungal infections using *Fusarium culmorum* and *Fusarium oxysporum* was also examined.

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**PO70**

# Biotic stress as modulator of isoquinoline alkaloid profile in *Chelidonium majus* in hydroponic and *in vitro* cultures

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*Chelidonium majus* L. metabolic response to biotic stress was investigated using elicitation wih *Pseudomonas luteola* and *P. aeruginosa* as well as the stress signaling mediator methyl jasmonate (MeJa). *In vitro* shoots cultured on MH3 solid media under white light (WL) or photosynthetic active radiation (PAR), as well as callus cultured on MH3 medium with kinetin, NAA, and 2,4D were treated with lyophilized *P. luteola* cells [0.02, 0.2% suspension] and MeJa [100 µM]. In the hydroponic system, *P. aeruginosa* [1, 0.2, 0.02%] and MeJa [100 µM] were used. Following the application of biotic elicitors, the isoquinoline alkaloid profile was analyzed using chromatographic techniques (HPLC, LC-MS/MS). PAR illumination resulted in higher content of allocryptopine and coptisine in *in vitro* shoots treated with biotic elicitors as well as MeJa, compared to untreated plant material and WL. In the callus cultures, *P. luteola* [0.2%] influenced the content protopine and chelerythrine, compared to untreated plants. Conversely, a lower bioelicitor concentration [0.02%] was more effective for sanguinarine increase. However, the ultimate outcome in both *in vitro* culture types, shoots and callus, may also be affected by various culture parameters, including light exposure and the developmental phase of the plant. In the hydroponic cultures, the application of *P. aeruginosa* [0.2%] was most effective in boosting the content of protoberberine and benzophenanthridine derivatives such as coptisine, berberine, chelidonine, sanguinarine and chelerythrine. Nevertheless, in all experiments, the influence of MeJa on isoquinoline alkaloid content was most noticeable. This investigation highlights the need for further research into the specific effects of microbial strains, including optimal concentrations and exposure durations, on both *in vitro* and *in vivo* grown plants. Such studies are crucial for elucidating the mechanisms of action and optimizing the production of specialized metabolites.

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**PO71**

# Anti-Inflammatory Effect and Cytotoxic Properties of *Glaucium flavum* Extracts

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*Glaucium flavum* Crantz, commonly known as yellow horn poppy (Papaveraceae) is a coastal herb indigenous to i.a. Europe, Northern Africa (Macaronesia). Due to interesting metabolic profile, *G. flavum* extracts derived from organ cultures *in vitro* were investigated for their potential anti-inflammatory and cytotoxic effect. *G. flavum* shoots were cultured on MH3 solid media supplemented with kinetin (KIN) at concentrations of 2.5, 4.5, and 7µM, along with indole-3-acetic acid at a concentration of 0.5 µM. Methanolic extracts were tested for their cytotoxicity and the effect on IL-1, IL-8, and TNF-α secretion in human neutrophil model. For comparative analysis methanolic and ethanolic extracts from roots, stems with leaves and flowers of plants cultivated in two botanical gardens (Kraków and Lublin) were used.

The extracts tested demonstrated no cytotoxic effects on neutrophil cells. Their capacity to inhibit secretion varied with concentration and was distinct across different extracted organs and specific cytokines. For TNF-α, the stem extract from Lublin exhibited the most potent inhibitory effect at a concentration of 1 µg/ml. Regarding IL-1 and IL-8, the extracts showing the greatest efficacy were from *in vitro* shoots grown on MH3 medium containing 7 µM of KIN and from shoots on non-supplemented 1/2 MH3 medium, each at 1 µg/mL, respectively. Further investigation is essential to identify the phytochemical profiles of these extracts and to indicate the group of compounds directly contributing to the observed bioactivities.

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**PO72**

# Bug vs Bug: Use of *Galleria mellonella* larvae to test activity of Essential Oils and their components against drug-resistant microbes

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*Galleria mellonella,* an invertebrate model organism, presents a valuable alternative to traditional mammalian infection models, offering several advantages for scientific research. This model bypasses the necessity for bioethics committee approval, due to its invertebrate status, and provides a cost-effective and efficient solution for cultivation. *G. mellonella* larvae are notable for their production of antimicrobial compounds and the circulation of hemolymph, supported by diverse cell lines, which are instrumental for research purposes.The increasing emphasis on reducing the use of higher animals in laboratory settings and the shift towards alternative model organisms underscore the significance of *G. mellonella* in studying bacterial pathogenicity. A pivotal attribute of *G. mellonella*, particularly in researching human pathogens, is its ability to be cultured at 37˚C, paralleling some fundamental aspects of the human immune response both structurally and physiologically. This makes *G. mellonella* an exemplary model for cytotoxicity evaluations and for determining the efficacy of antimicrobial agents, including antibiotics and disinfectants. Our study introduces an innovative approach by utilizing the external integuments of *G. mellonella* larvae as a platform for inducing localized infections associated with biofilms, aiming to assess the effectiveness of antimicrobial treatments. We explored the use of thyme essential oil (T-EO) as a potential antibiofilm agent against bacterial biofilm-related infections. Remarkably, the application of T-EO proved to be non-toxic to the larvae and significantly enhanced their survival rate when infected with Staphylococcus aureus, from 48% to 85%. These findings highlight T-EO's potential as an effective antimicrobial agent for biofilm-associated infections and affirm the utility of the *G. mellonella* model in mimicking the conditions of multicellular organisms, offering promising avenues for future antimicrobial research.

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