Phytochemical Society of Europe

Trends in Natural Product Research

Young Scientist Meeting

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Book of Abstracts

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Welcome address

Dear Colleagues,

On behalf of the Phytochemical Society of Europe we are delighted to welcome you to the 2025 Young Scientists Meeting (YSM) ***Trends in Natural Product Research***. This year, we are honored to host this international event at Faculty of Pharmacy, Wroclaw Medical University in Poland.

As the capital of Lower Silesia, Wrocław is home to over 670 thousnad residents and serves as a beacon of education and research in Poland. Beyond its academic and scientific achievements, Wrocław offers a rich tapestry of cultural and natural attractions. The city's historic Market Square, the serene Cathedral Island, and the Botanical Gardens provide a picturesque backdrop for reflection and inspiration. These spaces not only enhance the city's aesthetic appeal but also underscore its commitment to preserving and celebrating its natural heritage.

In June 2025, Wrocław will host the PSE YSM Trends in Natural Product Research conference. This esteemed event will bring together scientists from around the globe to discuss the latest advancements in natural product research.

The Young Scientists Meeting serves as a dynamic platform for early-career researchers to showcase their work and engage with seasoned scientists across diverse disciplines. This year, the conference attracted over 150 participants from more than 20 countries, featuring 15 plenary lectures, 40 oral presentations, and over 70 posters. These sessions provided attendees with opportunities to exchange ideas, discuss emerging hypotheses, and establish connections that may lead to future collaborations. By facilitating these interactions, the meeting contributes to the growth of a global scientific community dedicated to advancing knowledge and innovation.

While scientific presentations are central to our meeting, the true value often lies in the spontaneous discussions and collaborations that emerge during informal interactions. These face-to-face exchanges are not just about expanding your professional network, they are about building genuine relationships that can lead to meaningful collaborations and long-term partnerships. In a world where digital communication often prevails, these in-person moments are invaluable. They foster trust, spark creativity, and cultivate a sense of belonging within the global community of researchers dedicated to exploring the potential of medicinal plants and natural compounds for human health and well-being.

We extend our sincere gratitude to prof. Piotr Ponikowski Rector of Wroclaw Medical University, for their patronage and support of this conference.

We hope you have a rewarding and enjoyable experience at the conference, and look forward to future opportunities to connect and collaborate.

Adam Matkowski & Sylwia Zielińska

On behalf of Organizing Committee
June 2025, Wrocław

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PL1

The real green chemistry – how plants make specialized metabolites and what we can learn from them

Heribert Warzecha[1,2]

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Plants have evolved an enormous variety of biosynthetic pathways affording formation of diverse chemical structures. These so-called specialized compounds enable the plant to interact with its environment, are vital components of its defense systems, and they are valuable to us as they can serve as fragrances, dyes, or pharmaceuticals. The potential of synthetic biology and metabolic engineering is to allow the design of plants as green bio-factories of fine chemicals, but the requisite platform and technology are still in their infancy. This is mainly due to the complex orchestration of metabolic pathways between cell compartments, cell types, and plant organs.
Exemplified by metabolites derived from tryptophan, we were able to show that simple biosynthetic routes can be grafted into tobacco plants. Moreover, combining genes from various, highly disparate sources spurred the formation of entirely new synthetic pathways leading to new-to-nature plant-generated products, as shown for halogenated indigo-precursors. Modular cloning techniques enabled the assembly of various pathways comprising up to twelve steps, but their evaluation showed that careful design of regulation and localization is needed and tunable expression levels of biosynthetic genes are crucial to unlock their full bio-manufacturing potential. Emerging genome editing technologies can play a crucial role in fine tuning of particular pathways as will be exemplified for a serotonin-derived metabolite.

PL2

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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PL3

Alkaloids from the genus Vinca L. (Apocynaceae): isolation, structural identification, biological activity

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Genus *Vinca* L. (Apocynaceae) is renowned for its rich content of monoterpene indole alkaloids and widespread occurrence in Europe, northwest Africa, and southwest Asia. A total of 202 alkaloids have been identified across five phytochemically investigated species of the genus *Vinca*, categorized into 19 different structural groups, highlighting its significant phytochemical diversity. The majority of the alkaloids possess an indole core in their structure. These alkaloids, including notable compounds such as vincamine and reserpine, exhibit a range of biological activities, including antitumor, anti-Alzheimer, antibacterial, and antihypertensive effects. Within our detailed phytochemical study on aerial part of *Vinca minor* one undescribed indole alkaloid together with twenty-two known compounds have been isolated. The chemical structures of the isolated alkaloids were determined by a combination of MS, HRMS, 1D, and 2D NMR techniques, and by comparison with literature data. The NMR data of several alkaloids have been revised, corrected, and missing data have been supplemented. Alkaloids isolated in sufficient quantity were screened for their in vitro acetylcholinesterase (AChE; E.C. 3.1.1.7) and butyrylcholinesterase (BuChE; E.C. 3.1.1.8) inhibitory activity. Selected compounds were also evaluated for prolyl oligopeptidase (POP; E.C. 3.4.21.26), and glycogen synthase 3β-kinase (GSK-3β; E.C. 2.7.11.26) inhibition potential. Significant hBuChE inhibition activity has been shown by (-)-2-ethyl-3[2-(3-ethylpiperidinyl)-ethyl]-1H-indole with an IC_{50} value of 0.65 ± 0.16 μM. This compound was further studied by enzyme kinetics, along with in silico techniques, to reveal the mode of inhibition. This compound is also predicted to cross the blood-brain barrier (BBB) through passive diffusion.

PL4

Biosynthesis and metabolic engineering of phenylpropanoids for biofuels, bio-based products, and plant and human health

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Phenylpropanoid biosynthesis gives rise to a versatile class of phenolic compounds, including a wide range of small molecules such as benzenoids, coumarins, phenolic acids, stilbenoids, flavonoids, and lignans, as well as complex biopolymers like lignin and aromatic domains of suberin/cutin. These metabolites are essential for plant growth, development, and defense against environmental stresses. Among them, lignin - a major structural component of plant secondary cell walls - has a particularly significant impact on lignocellulosic biomass processability. Over the years, we have focused on uncovering the molecular factors governing phenylpropanoid biosynthesis and pathway organization, as well as on engineering these pathways to produce high-value bioproducts.

Within the phenylpropanoid - lignin biosynthetic pathway, three endoplasmic reticulum-resident cytochrome P450 enzymes - cinnamate 4-hydroxylase (C4H), coumaroyl ester 3'-hydroxylase (C3'H), and ferulate 5-hydroxylase (F5H) - catalyze regio-specific hydroxylation of the aromatic ring in monolignol precursors. These hydroxylation patterns are key determinants of lignin subunit composition and structure. We discovered that, in addition to the canonical electron donor cytochrome P450 reductase (CPR), a specific cytochrome b_5 isoform, CB5D, acts as an essential electron donor to F5H for syringyl (S)-lignin biosynthesis [1,2]. Remarkably, CB5D's functional emergence predates the evolution of F5H enzymes in vascular plants [3], suggesting that the recently evolved angiosperm F5Hs co-opted this ancient electron transfer component to form a modern P450 monooxygenase system for meta-hydroxylation of aromatic rings. Beyond lignin biosynthesis, in collaborative efforts, we identified a suite of key biosynthetic enzymes - including a novel P450 enzyme - responsible for salicylic acid biosynthesis, a well-known plant defense hormone and traditional medicinal compound. This work resolves the long-debated phenylalanine-derived pathway for salicylic acid formation [4].

Finally, by integrating protein engineering and metabolic engineering strategies, we developed a series of novel *O*-methyltransferases. These enzymes not only enhanced the digestibility of woody biomass but also enabled the production of high-value phenolic derivatives. In this talk, I will briefly highlight our recent progress in these areas.

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PL5

Towards better understanding the function of dihydrochalcones in apple: biosynthesis, gene cloning, functional expression, transgenic approaches

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Phloridzin (phloretin-2´-O-glucoside), a dominant dihydrochalcone (DHC) in apple (*Malus domestica* Borkh.), is synthesized through the 2´ glucosyltransferase activity of MdPGT1. To investigate the role of this enzyme, both conventional RNA interference (RNAi) knockdown and CRISPR/Cas9-mediated genome editing was employed to target the MdPGT1 gene. Transcriptomic and metabolic analyses revealed a shared set of genes involved in phenylpropanoid and flavonoid pathways were regulated in both types of modified lines. However, while knockdown lines exhibited stunted growth and altered leaf morphology, genome-edited lines showed normal growth despite reduced foliar phloridzin levels. Further analysis identified differential modulation of key transcription factors and phytohormone signaling pathways between the two types of lines. Phytohormone profiling revealed elevated salicylic and jasmonic acid levels in the dwarf lines, with no correlation between auxin and ABA and the growth phenotype. Notably, bioactive brassinosteroids were generally upregulated, while gibberellin GA4 was significantly decreased specifically in the RNAi knockdown lines. Expression analysis confirmed transcriptional regulation of genes involved in brassinosteroid and gibberellin interaction, suggesting that the contrasting growth effects following phloridzin reduction might be linked to differential phytohormone modulation. This study highlights the utility of CRISPR/Cas9 in dissecting gene function in phloridzin biosynthesis in apple and reveals potential off-target effects or pleiotropic effects associated with the RNAi approach. A related study focused on sieboldin (3-hydroxyphloretin-4´-O-glucoside), a DHC found in high concentrations in specific wild *Malus* species closely related to domesticated apple. The initial step in sieboldin biosynthesis was previously unknown. By combining transcriptomic analysis and de novo transcriptome assembly of two wild *Malus* species (*Malus toringo* and *Malus micromalus*) with high sieboldin content, two putative 3-hydroxylase candidates were identified. In vivo functional characterization in Saccharomyces cerevisiae demonstrated that CYP98A proteins from the wild Malus accessions could produce 3-hydroxyphloretin, the precursor to sieboldin, when co-expressed with MtorPGT2, a glycosyltransferase involved in DHC 4´-O-hydroxylation. In contrast, CYP98A197-198 genes from *M*. × *domestica* lacked this hydroxylase activity. The active CYP98A proteins from wild accessions is localized to the endoplasmic reticulum and exhibited key amino acid mutations near the ligand-binding pocket, potentially explaining their ability to accept phloretin as a substrate. Screening of a *Malus* germplasm collection using HRM markers for CYP98A genes identified three clusters corresponding to alleles from domesticated and wild species. Furthermore, the presence of CYP98A isoforms identified in *M. toringo* and *M. micromalus* correlated with sieboldin accumulation in other wild and hybrid *Malus* genotypes. This research provides the first in vivo evidence for an enzyme involved in the key hydroxylation step in sieboldin biosynthesis in *Malus* species.

PL6

Cancer chemopreventive potential of Ruta chalepensis from Syria

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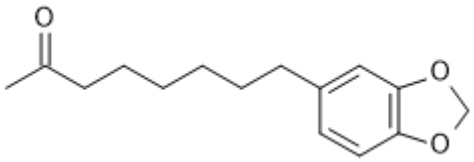
Cancer is one of the leading causes of mortality and morbidity in humans, and it is the third major cause of death in Syria. Before the onset of the conflict in 2011, Syria had an estimated 17,599 new cancer cases in 2009, with 52% of the cases occurring in males and 48% in females, according to the Syrian National Cancer Registry [1]. The most widely used conventional treatment approaches for cancer are chemotherapy, radiotherapy, and surgery, while the modern modalities include hormone-based therapy, anti-angiogenic therapy, stem cell therapies, and immunotherapy [2]. However, cancer chemoprevention is always a preferable option as it is much more cost-effective than the currently available cancer treatment options (Ritchie and Sarker, 2020). A research project was designed to isolate and identify chemopreventive phytochemicals from the methanolic extract of the aerial parts of the well-known medicinal plant Ruta chalepensis, collected from Syria using a bioassay-guided protocol. The noncytotoxic concentrations of the extracts, fractions, and isolated compounds against the human mammary MCF-7 derived reporter AREc32 cell line were determined by the MTT assay, and the ability to induce Nrf2 activation was determined using the AREc32 cell-based luciferase gene reporter assay. The active solid-phase extraction fraction 3 (eluted with 80% methanol in water) of the methanolic extract, which showed a 9.1-fold induction of Nrf2 activation, was subjected to preparative and semi-preparative reversed-phase HPLC for the isolation of eleven compounds including bergapten, chalepensin, chalepin, g-fagarine, 2-[6'-(2H-benzo [d] 1", 3"- dioxoPlen-5"-yl)-hexyl]-hydroquinolin-4-one, kokusaginine, moskachan D, pseudane IX, ribalinium, rutamarin, skimmianine, the structures of which were elucidated by spectroscopic means. Among the purified compounds, moskachan D was the most active compound, with a 7.5-fold induction of Nrf2 activation, suggesting that this compound could be used as an ideal structural template for developing new cancer chemopreventive agents. This study established the cancer chemopreventive potential of R. chalepensis.



Moskachan D

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PL7

Nitrogen-containing derivatives of flavonoids and terpenoids as multidrug resistance reversers in cancer

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Cancer remains a leading cause of death worldwide. Successful cancer chemotherapy has been hampered by multidrug resistance (MDR). Several mechanisms have been identified in the MDR phenotype, including drug efflux by ABC transporter proteins, namely P-glycoprotein (P-gp), which has been playing a critical role in MDR. A number of strategies has been addressed to overcome MDR, including the development of P-gp inhibitors.Natural products, such as flavonoids and terpenoids, have emerged as a promising alternative for the development of effective MDR reversers, due to their remarkable chemical diversity and bioactivity. Aiming at optimizing the structures of flavonoids and terpenoids for reversing MDR, structural modifications have been performed, namely by introduction of nitrogen-bearing moieties, combined with aromatic substituents, which have been considered as important features in the interaction with P-gp.

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PL8

Mediterranean diet and the gut-brain axis

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Recent studies have indicated that our gut health and diet affect a range of health conditions, including those linked to the brain. The Mediterranean diet, which is rich whole grains, fruits, and vegetables, can slow down memory loss and lower the risk of diseases like Alzheimer's and Parkinson's. In Parkinson's, gut bacteria may influence the disease's progression, and probiotics or dietary changes could help manage symptoms. Mental health too can be linked to gut bacteria; poor gut health may contribute to anxiety and depression, and eating the right foods or taking probiotics could help improve mood. Research has shown that specific diets—like the Mediterranean diet—may help protect brain function as we age. This has triggered a wide interest into how diet, gut bacteria, and brain health are all connected. A complex web of connections has come into view; our gut – or by extension, even our whole body – can be considered as an ecosystem. Our gut is the physical environment in which our dietary intake interacts with microbial and human cells. It will take a concerted effort and multidisciplinary collaboration to untangle the many strands to make up the whole network.

PL9

Citrus phytochemicals as modulators of lipid metabolism

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Modern lifestyle characterised by overnutrition, physical inactivity, and stress, has led to prevalence of obesity and obesity-induced diseases world-wide within the last decades. In context of the development of novel therapeutic concepts for treatment of overweight and obesity, the interest for plant-based dietary supplements with positive effects is enormous. *Citrus* species represent major fruit crops worldwide, more than half of the global production refers to oranges. Studies on Citrus flavonoids, essential oils, coumarins and pectins, among others, will be discussed concerning their anti-obesity potential. In our on-going studies on anti-adipogenic compounds from lemon peels we could show that 5-geranyloxy-7-methoxycoumarin has a similar mode of action to bergamottin [1,2] whereas limettin has a lipolytic activity [3]. In order to explore the quantities of coumarins in lemon varieties, we recently analysed 14 accessions after ethanol and NADES extraction. Interestingly, using NADES composed of choline chloride and an acidic hydrogen bond donor, artefact formation due to hydrochlorination of coumarin epoxides could be seen [4]. Furthermore, polar extracts showed a remarkable variety of HMG-substituted flavonoids, such compounds seem to be competitive inhibitors of HMG-CoA reductase [5].

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PL10

The Multipurpose Health Benefits of Elephant Apple (*Dillenia indica* L.)

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Elephant apple (*Dillenia indica* L.), commonly known as "Chalta," is a tropical fruit gaining attention for its exceptional nutritional value and potential health benefits. This Talk explores its multifaceted properties, highlighting its role as a nutritional powerhouse and a potential natural remedy. Elephant apple is rich in vitamin C, dietary fibre, potassium, and B vitamins, and it promotes overall health and well-being. Its high antioxidant content, including flavonoids, combats oxidative stress and enhances immune function. Moreover, emerging research suggests that elephant apple has anti-inflammatory and antidiabetic properties, making it a valuable dietary addition, especially for managing blood sugar levels. Traditionally this plant has been used to treat digestive issues and kidney infections and to boost energy. Elephant apple also shows promise for skin health, potentially improving elasticity and reducing signs of aging. While its name might be misleading, it bears no resemblance to an elephant, rather it is a juicy green fruit that has a delightful source of numerous health benefits. This talk will discuss the nutritional profile and pharmacological potential of elephant apple, offering practical advice on incorporating this versatile fruit into daily diets. Let us uncover the hidden benefits of this extraordinary elephant apple and discuss its impact on both nutrition and health.

PL11

From Foam to Function: Revolutionary Advances in Saponin Science

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The Agavaceae family comprises more than 200 species, and plants belonging to this family possess significant economic and ecological value. A wide range of biological activities and applications has been described since ancient times, including the production of fibre, beverages, and anti-inflammatory and anticancer properties. This family is recognised as the main source of steroidal saponins, natural products with a remarkable structure. They possess a hydrophobic core named aglycone linked to a hydrophilic sugar chain, and their unique structure confers a valuable range of biological properties. Nevertheless, their isolation remains a challenge; numerous purification steps that combine different solvents and stationary phases with various techniques are typically needed to isolate these compounds, which are often found in higher amounts or sometimes in mixtures [1]. For these reasons, the use of saponin-rich fractions has gained the attention of researchers.
Most extraction procedures used to obtain saponins are conventional methods, such as Soxhlet, maceration, or reflux. The work described herein provides an overview of advances made in the investigation of saponins, highlighting progress in structural elucidation of these particular natural products [2], isolation [3], and new approaches in extraction techniques aimed at greener and more sustainable technologies [4].

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PL12

Aminated polyphenols from Strecker degradation of amino acids in food processing: A class of novel bioactive components

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People always thought that the bioavailability of flavonoids is less than 10% due to poor absorption and rapid metabolism. In fact, dietary flavonoids entering the circulatory system are rapidly metabolized by the liver and excreted, leaving little opportunity for them to exert their effects. However, the majority of flavonoids remain in the digestive tract, where highly bioactive flavonoids with pyrogallol group, form quinone derivatives that directly degrade excess amino acids. The evidence demonstrated that high protein or amino acids consumption generated further dangerous metabolic disorders and liver injury. It helps alleviate the metabolic burden on the liver and kidneys, promoting digestion and reducing greasiness. Furthermore, flavonoids can interact with gut microbiota in intestine. These two points precisely explain the benefits of drinking tea. The Strecker degradation of flavonoid quinones with amino acids demonstrates how tea, through its unique chemical composition and interaction with the digestive system, provides tangible health benefits, particularly in aiding digestion and reducing the metabolic load from high-protein diets. The aminated polyphenols are formed through natural processes in the body and do not pose the same risks as synthetic compounds. This safety profile, combined with their degradation of amino acids, makes aminated polyphenols a unique and beneficial component. There are no data on the functions of NH2-flavonoids in vivo. NH2-flavonoids are much more stable than their precursor flavonoids, which provide them longer interaction time with the body. NH2-flavonoids maybe the key pathway through which flavonoids exert their biological functions.

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PL13

The potential of natural products in combating coronavirus infections

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Coronaviruses cause respiratory illnesses of varying severity in humans. The COVID-19 pandemic, resulting from the emergence of SARS-CoV-2, has led to profound global health and economic repercussions due to the virus's ability to cause severe respiratory complications in a significant proportion of patients. Substantial progress has been observed in a short time in understanding the pathology and clinical management of coronaviruses, as well as in the development of effective vaccines. Nevertheless, these viral infections continue to pose a public health challenge, due to the risk of recurrent epidemics linked to the emergence of new variants, suboptimal vaccination coverage among high-risk populations, and the lack of available specific antiviral agents targeting coronaviruses. Our overall strategy focuses on identifying pan-coronavirus antiviral agents by exploiting the structural diversity of natural products. This presentation will highlight collaborative research that has revealed the anti-coronavirus potential of selected plant extracts and specialized metabolites [1-5].

Acknowledgements
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PL14

In search of novel antibiotics from nature: the case of simamycin and the role of oxyprenylated phenylpropanoids

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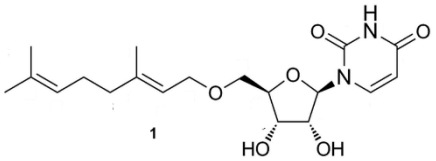
tRNA-2-selenouridine synthase (SelU) is a tRNA-modifying enzyme that is instrumental to bacterial translation by exploiting chalcogens. Specifically, this enzyme catalyzes the geranylation of a 2-thiouridine at the wobble position of three bacterial tRNAs to enhance the recognition of codons ending in guanine over adenosine using geranyl pyrophosphate as the cofactor. In addition, SelU is also the working enzyme for a selenation process at the same tRNA position in the presence of selenophosphate. It is a fundamentally interesting question of how this enzyme conducts two mechanistically different reactions. To gain more details about the substrate recognition of SelU, we synthesized and identified a small natural compound simamycin (5'-*O*-geranyluridine) **1**, isolated from the culture broth of a soil-derived Streptomyces spp., that has strong interaction with this enzyme. Through biophysical and NMR structural studies, we postulated an allosteric mechanism of SelU catalysis involving cooperativity among each domain and a conformational rearrangement around the active site of its *N*-terminal domain, which is supported by the bimolecular quenching constants, number of binding sites, and thermodynamic parameters calculated for this compound complexed with the *N*-terminal domain of SelU.



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Furthermore, we selected a small library of natural products and semisynthetic oxyprenylated phenylpropanoids based on their compositional resemblance to the purported SelU ligands. Specifically, these compounds contained one or more geranyl groups branching from aromatic frameworks, all of which were believed to heighten affinity to SelU. Meticulous screening of each compound against an *N*-terminal SelU construct via fluorescence quenching of W83 further revealed details on the enzyme-substrate binding mode. Conformational flexibility of residues around W83 was suggested by the slow bimolecular quenching constants calculated for each compound. This was consistent with the single binding site and the blend of interaction-types calculated at the active site. Lastly, we established this general oxyprenylated phenylpropanoid framework as a pharmacologic scaffold that can be further optimized into an antibiotic.

PL15

Investigating saponin biosynthesis and function: implications for natural product research

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As agriculture faces the urgent challenge of reducing reliance on synthetic pesticides, plants provide powerful inspiration through millions of years of evolutionary innovation. Among their diverse chemical defenses, saponins - amphiphilic triterpenoids - exemplify highly effective natural toxins that selectively disrupt pest membranes while sparing the plant's own tissues.

Our research investigates the biosynthesis, compartmentalization, and mode of action of saponins, combining molecular biology, analytical chemistry, and membrane biophysics. We study how plants produce and safely store these compounds in inactive forms, and how activation is triggered upon herbivore or pathogen attack. Biophysical assays and microscopy reveal how specific structural features - particularly glycosylation patterns - govern solubility, micelle formation, and membrane interaction under varying physiological conditions.

By uncovering the molecular logic behind saponin selectivity and self-protection, we contribute to the rational design of bioinspired pest management strategies. This work also sheds light on broader principles of membrane-active natural products and their evolutionary adaptation.

Our interdisciplinary approach integrates phytochemistry, cell biology, and evolutionary ecology to understand how plants achieve precise chemical defense. In doing so, we aim to inform the development of next-generation biopesticides that are both effective and environmentally benign - advancing natural product science and supporting more sustainable agricultural practices.

List of Short Lectures

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SL5 **Magdalena Kulinowska**
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SL6 **Martin Helcman, Juraj Markuš**
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SL11 **Yuxuan Zhao**
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SL12 **Ceren Öztürk**
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SL13 **Daniel Petrič**
Effectiveness of plant bioactive substances on the rumen environment in lambs with gastrointestinal parasites

SL14 **Milan** Malarz
Isolation of a unique monoterpene diperoxy dimer from Ziziphora clinopodioides subsp. bungeana together with triterpenes with antidiabetic properties

SL15 **Matej Leško**
Spent coffee grounds from filtered specialty coffee, their bioactive compounds and potential uses in animals

SL16 **Jakub Treml**
Identification of plant phenolics as novel PPARγ agonists and hypoglycemic agents

SL17 **Pola Sidoruk**
The role of fresh and ensiled Rugosa rose (Rosa rugosa Thunb.) pulp in in vitro modulation of basic ruminal parameters in dairy cows

SL18 **Jovana Lisičić**
Anti-inflammatory potential of sesquiterpene lactones from Schkuhria pinnata in an in vitro intestinal inflammation model

SL19 **Odimegwu, Joy I.**
A natural weapon: Nchuanwu (African basil) lipids as a sustainable bio-pesticide

SL20 **Kwan-Woo Kim**
Analysis of the active ingredients and pharmacological activity of Peucedanum japonicum cultivated in Korea

SL21 **Attila Ványolós**
Gymnopeptides: an emerging group of fungal cyclic peptides

SL22 **Yannik Karl-Heinz Schneider**
Screening the plant-like animals, 20 years of early drug discovery from Arctic marine invertebrates

SL23 **Polina Shpet**
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SL24 **Calisto Moreno Cardenas**
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SL25 **Anna Szakiel**
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SL26 **Paweł Kozaczuk**
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SL27 **Teresa Kaźmierczak**
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SL28 **Karolina Szyrner**
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SL30 **Anna Stasiłowicz-Krzemień**
Optimizing green extraction of cannabinoids using natural deep eutectic solvents

SL31 **Wenqi Huang**
Intergenerational inheritance of quercetin-induced abnormal immunity in mice

SL32 **Shiye Lin**
Transgenerational immunosuppression induced by sodium glutamate in Caenorhabditis elegans

SL33 **Wanning Ma**
Novel insights into the anti-obesity potential of myricetin using a drosophila model

SL34 **Katarzyna Gaweł-Bęben**
Cosmeceutical potential of Aronia melanocarpa (Michx.) Elliott fruits – preliminary identification of active fractions

SL35 **Quanyong Wu**
Effects of resveratrol on Drosophila lifespan: influence of gender and developmental stage

SL36 **Paulina Lechwar**
Coast plant extracts as a rich source of skin protecting ingredients

SL37 **Boya Ouyang**
Grape seed polyphenol extract enhances antioxidant stress response and prolongs life in Caenorhabditis elegans

SL38 **Zeliha Parlar**
Research on the effects of Myrtus communis L. used in Traditional Anatolian Medicine on Parkinson's pathology

SL39 **Xincheng Wu**

Effects of the aspartame on growth, development, immunity and neurological function of Caenorhabditis elegans

SL40 **Saba Shahrivari-Baviloliaei**

Nutritional, Chemical, Antioxidant and Antibacterial Screening of two Astragalus Species

SL1

New neuroprotective natural substances for the treatment of Parkinson's disease

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Parkinson's disease (PD) is the second most common motor-related neurodegenerative disease and numbers of globally diagnosed cases are expected to rise from 6 million in 2015 to more than 12 million by 2040. It is characterized by motor symptoms linked with specific degeneration and loss of approximately 30-70% of dopaminergic (DA) neurons in the substantia nigra pars compacta and their projections to the striatum. Some, of many, known molecular hallmarks of PD include enhanced oxidative and nitrosative stress, mitochondrial dysfunction, excitotoxicity [8], ubiquitin/proteasomal system dysfunction and neuroinflammation. Current treatments have various adverse side-effects and only offer symptomatic relief, so there are intense efforts to develop drugs with efficient curative effects on degenerating DA neurons. To extend knowledge of the protection (protective activity), we investigated activities of natural cytokinins (plant hormones) against salsolinol (SAL)-induced toxicity (a Parkinson's disease model) and glutamate (Glu)-induced death of neuron-like dopaminergic SH-SY5Y cells. We found that kinetin-3-glucoside, *cis*-zeatin riboside and N^6-isopentenyladenosine were active in the SAL-induced PD model. In addition, *trans*-, *cis*-zeatin and kinetin along with the iron chelator deferoxamine (DFO) and the necroptosis inhibitor necrostatin 1 (NEC-1) significantly reduced cell death rates in the Glu-induced model. Lactate dehydrogenase assays revealed that the cytokinins provided lower neuroprotective activity than DFO and NEC-1. Moreover, they reduced apoptotic caspase-3/7 activities less strongly than DFO. However, the cytokinins had very similar effects to DFO and NEC-1 on superoxide radical production. Overall, they showed protective activity in the SAL-induced model of parkinsonian neuronal cell death and Glu-induced model of oxidative damage mainly by reduction of oxidative stress. Development of a new generation of adenine-based antiparkinsonics will be also discussed here.

SL 2

In vitro plant cultures as a potential tool for the production of bioactive compounds - the example of *Achillea filipendulina*

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In vitro plant culture is a promising tool in biotechnology for the production of bioactive compounds for both pharmaceutical and cosmetic purposes. It allows the production of plant biomass under controlled conditions, independently from environmental factors. This method is especially valuable for cultivation of plant species that are rare, endangered, or have limited natural habitats. [1] *Achillea filipendulina* (fernleaf yarrow) is less known member of the *Asteraceae* family, with rich phytochemical profile. It is also a source of various secondary metabolites, including flavonoids, phenolic acids, and essential oils, with antioxidant, anti-inflammatory, and antimicrobial properties. [2]

The aim of this study was to compare the phytochemical composition and selected cosmetic properties of *A. filipendulina* extracts obtained from*: in vitro* microshoot cultures (AF *in vitro*), bioreactor-based cultivation (AF bio), and plants collected from the field (AF). AF *in vitro* were established from seeds and maintained on Murashige and Skoog (MS) agar medium supplemented with growth regulators. After 3 weeks of growth, biomass was harvested and some cultures were transferred to platform bioreactors for 21-day growth under controlled conditions.

Aqueous, hydro-ethanolic (50% EtOH, *v/v*), and ethanolic (96% EtOH, *v/v*) extracts were prepared using ultrasound-assisted extraction and compared for the total content of polyphenols, the antioxidant activity (DPPH scavenging assay), murine and mushroom tyrosinase inhibition, and *in vitro* cytotoxicity again human keratinocytes (HaCaT) and murine melanoma cells (B16-F10). The presence of bioactive compounds was compared using HPLC-ESI-QTOF-MS/MS.

The highest polyphenol content and antioxidant potential was observed for AF extracts (1.315 ± 0.043 mg GAE/g DW and 38.080 ± 2.156 µg/mL respectively). The 50% EtOH extracts exhibited the highest tyrosinase inhibitory activity, with the AF *in vitro* extract being the most active (63.84% inhibition at the 0.50 mg/ml). AF extracts showed significant cytotoxicity toward HaCaT cells, suggesting irritant potential of this ingredient. In contrast, AF *in vitro* extracts were not toxic to HaCaT cells and mildly cytotoxic to B16F10 cells, indicating selective activity and better skin compatibility. AF *in vitro* extracts (50% EtOH) contained the greatest diversity of active compounds, which may explain its tyrosinase inhibitory activity. Our presented results show that *in vitro* cultures of *A. filipendulina* can produce biologically active secondary metabolites with potential application in skin-lightening cosmetics.

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SL 3

Hybrid natural products in the hybrid *Leontodon × grassiorum* detected by HPLC-MS

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Leontodon x grassiorum Zidorn is a hybrid between the Azorean *Leontodon hochstetteri* M. Moura & L. Silva and the mainland European *Leontodon hispidus* L. Morphologically, *Leontodon* × *grassiorum* is intermediate between both parental species. Leaf size and shape resemble *L. hochstetteri* although they are narrower, softer and more petiolate. The flowering heads however resemble *L. hispidus* in size and flower colour. Phytochemically, the genus *Leontodon* s. str. is known to contain unique hypocretenolide type sesquiterpene lactones. Previous research showed that there is a distinct difference between the hypocretenolides from both parental species. *L. hispidus* contains 14-hydroxyhypocretenolide and its derivates, whereas *L. hochstetteri* contains 14-hydroxy-1,10-epoxyhypocretenolides. Furthermore, the 14-hydroxy-1,10-epoxyhypocretenolide-glycosides from *L. hochstetteri* are known to be substituted by *p*-hydroxyphenylacetic acid moieties. This substitution pattern is not known from *L. hispidus*. The hybrid *Leontodon* × *grassiorum* exhibits an additive inheritance pattern, meaning that phytochemicals from both maternal and paternal parents are found in the hybrid.

For a preliminary chemophentic study we prepared an acetone extract of the root of the hybrid species. With chemophenetic considerations supported by UHPLC-MS/MS we were able to detect several compounds that are potentially new sesquiterpenoid derivatives. Based on their losses in the MS/MS experiment those derivatives might be hybrid sesquiterpene lactones with 14-hydroxyhypocretenolide as the core moiety. One compound detected in the MS/MS experiment shows a molecular ion peak $[M-H]^-$ of 815 *m/z*. A subsequent loss of 260 *m/z* could be assigned to a loss of a 14-hydroxyhypocretenolide moiety. A further loss of 134 *m/z* could be assigned to a loss of *p*-hydroxyphenylacetic acid and lastly a loss of 162 *m/z* could be assigned to a loss of glucose, leaving a 259 *m/z* moiety that could be assigned to another 14-hydroxyhypocretenolide moiety $[M-H]^-$. These observations could lead to the hypothesis of a dimeric sesquiterpene lactone with a glucose as a linking molecule that is substituted with a *p*-hydroxyphenylacetc acid. The dimeric sesquiterpene lactone is already known from *L. hispidus* but has never been reported from *L. hochstetteri*. The substitution with a *p*-hydroxyphenylacetic acid moiety is known from compounds from L. *hochstetteri*, but has never been reported from L. *hispidus*. Therefore, this preliminary study indicates that the hybridization event in *Leontodon* × *grassiorum* may not only have led to an intermediate position of the hybrid in morphology and phytochemical patterns. It may also have led to the biosynthesis of new chemical compounds that are of hybrid origin.

SL 4

Metabolomics-guided discovery and characterization of dihydro-*β*-agarofuran sesquiterpenoids from *Maytenus senegalensis*

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Dihydro-*β*-agarofuran sesquiterpenoids are characterized by a polyoxygenated tricyclic skeleton core, bearing from as few as two to as many as nine ester groups [1,2]. The family Celastraceae is the major source of these sesquiterpenoids [2]. Previously reported dihydro-*β*-agarofurans have shown a great potential as anticancer compounds [1].

In this study, a metabolomics-based approach was combined with the ethnobotanical tradition, in the search for anti-leukemia terpenoids from the root bark of a plant belonging to the Celastraceae family commonly used in traditional medicine in Botswana, namely *Maytenus senegalensis.*

The root bark of this plant was extracted and analysed by NMR-based metabolomics. Preliminary tests showed that this extract was active against the human U937 leukemia cell line. The extract was then partitioned using several chromatographic steps to afford pure compounds. The structural elucidation of the pure compounds was carried out by extensive 1D and 2D NMR analyses.

The main compound, shown in the figure below, was reported for the first time to the best of our knowledge. Most of the isolated compounds were characterized by the same macrolide structure. However, NMR data suggested that different organic acids were involved both in the formation of this structural unit and in the esterification at the other hydroxyl groups.

Further studies are currently dedicated to the complete structural elucidation of the isolated compounds and to the evaluation of their bioactivity against leukemia cell lines.

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SL 5

A practical approach to Natural Deep Eutectic Solvents-Based extraction

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Green chemistry has gained increasing attention within the scientific community. Natural Deep Eutectic Solvents (NADES) are considered to fully align with the principles of it, particularly due to their low toxicity and minimal environmental impact. These next-generation solvents are composed of naturally derived compounds, including organic acids, amino acids, choline salts, and sugars, as well as secondary metabolites such as 1,8-cineole, menthol, camphor, and thymol.
Since their initial characterization in 2011, NADES have demonstrated favorable safety profile and considerable efficacy in extraction processes. Their application has been validated in the extraction of a wide array of bioactive compounds, including phenolic acid, flavonoids, chalcones, and others.
We aim to present our experience with these solvents, encompassing their preparation methods across the broadest feasible range of molar ratios (n:n) (1:9-9:1), utilization in extraction processes, and subsequent chromatographic analyses.
Stable mixtures were obtained for systems: menthol:camphor (9:1-6:4), camphor:thymol (2:8-6:4), thymol:benzyl alcohol (1:9-7:3), menthol:thymol (9:1-4:6), and thymol:cineole (1:9-9:1). Our research includes NADES-Based extraction of bioactive compounds in two representative case studies: usnic acid from *Cladonia uncialis* and shikonin derivatives from *Echium vulgare*.
Usnic acid, a yellow cortical pigment, is recognized as the one of the most extensively studied lichen-derived secondary metabolite, owing to its broad spectrum of biological activities, including analgesic, wound healing, antimicrobial, antiviral, UV-protective, and antitumor effects (Kulinowska et al., 2023).

SL 6

Natural phenolic compounds as novel quorum sensing inhibitors

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Quorum sensing (QS) is a communication mechanism used by unicellular organisms. Many Gram-positive and Gram-negative bacteria utilize the boron-containing molecule autoinducer 2 (AI-2) for QS, alongside species-specific signaling systems.

We isolated bioactive substances from *Cannabis sativa* L. (Cannabaceae), including neutral cannabinoids (cannabigerol, cannabinol), cannabinoid acids (tetrahydrocannabinolic acid, cannabigerolic acid, cannabidiolic acid, cannabinolic acid), and the prenylated flavonoid cannflavin B. The biological activity of these compounds, together with other plant-derived substances such as flavonoids (including semi-synthetic imins and oxims), arylbenzofurans, furocoumarins, and xanthones, was assessed for effects on QS-regulated bioluminescence in *Vibrio harveyi* MM30. This mutant strain, unable to produce but still sensitive to AI-2, enabled investigation into both AI-2-dependent and independent pathways. Significant anti-QS activity was observed, notably among arylbenzofurans and semi-synthetic flavonoid derivatives, marking their first reported activity. Newly identified QS-inhibitory compounds included amorfrutin 1 from *Glycyrrhiza yunnanensis*, moracin M (non-prenylated arylbenzofuran), and moracin C (prenylated arylbenzofuran) from *Morus alba* L. Moreover, hesperetin, naringenin, and adenosine displayed strong activity for the first time in *V. harveyi* MM30 assays, supporting their use as affordable positive controls. Comparative analysis suggested that prenylation consistently decreased anti-QS activity across all tested classes.

The effect of selected compounds on AI-2 production by methicillin-resistant *Staphylococcus aureus* (MRSA 7112) was evaluated using *V. harveyi* MM30 as a biosensor. Moracin M and cannabigerolic acid exhibited significant inhibition, highlighting their potential against MRSA. Although other compounds did not show QS-specific inhibition, amorfrutin 1, amorfrutin 2, and xanthones (toxyloxanthone C and gerontoxanthone C) demonstrated strong antibacterial properties.

This study offers new perspectives on the anti-QS potential of plant-derived compounds and their relevance as bacterial communication modulators.

SL 7

A TLC-based method for the quantification of quillaic acid saponins

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Saponins serve plant families such as *Caryophyllaceae*, *Fabaceae*, and *Araliaceae* as a chemical defense against pathogens and herbivores. They display medicinal properties like enhancing the drug delivery of biologicals and boosting immune responses within vaccinations.
As plant derived molecules, their phytochemical analysis is key to ensure pharmaceutical quality. The quantification of saponins is typically carried out by LC/MS. These complex systems are expensive and have high maintenance demands, so a simpler and quicker method is needed. Aim of this study was the development of a validated thin-layer chromatography (TLC) method for the quantification of the sapogenin quillaic acid (QA), the triterpenoid backbone of numerous saponins with pharmaceutical value.
The sapogenin was derivatized with 4-Hydrazino-7-nitro-2,1,3-benzoxadiazole hydrazine (NBD-H) to form a fluorescent hydrazone. Quantification was subsequently performed by TLC-densitometry. This study presents a new cost-effective method to quantify QA and possibly other carbonyl containing saponins, enabling their monitoring within plant cultivation and phytopharmaceutical quality control. All findings were validated via LC/MS.

SL 8

Cytotoxic activity of *Verbascum aydogdui* and isolation of secondary metabolites

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The genus *Verbascum* (Scrophulariaceae) consists of 466 accepted species distributed worldwide [1]. Some *Verbascum* L. (mullein) species such as *V. thapsus*, *V. densiflorum* and *V. phlomoides* have long been used as a folk remedy to relieve respiratory disorders, inflammations, as an expectorant and against skin diseases. Previous phytochemical studies reported the occurrence of iridoids, triterpene saponins, phenylethanoid glycosides and flavonoids as the main secondary metabolites of mullein species. *Verbascum* species exhibited remarkable bioactivities such as wound healing, antimicrobial, neuroprotective and anti-inflammatory [2]. *V. aydogdui* Karavel., Vural, B. Şahin & Aslan is a halophyte plant which is endemic to Türkiye [3]. This species has never been investigated previously in terms of its pharmacological activity and phytochemicals. In this study, aerial parts of *V. aydogdui* was extracted with EtOH. The crude extract was then dispersed in H_2O and partitioned against *n*-hexane, EtOAc and *n*-BuOH, respectively. The crude EtOH extract and the solvent fractions were tested for their *in vitro* cytotoxic activities against PC3, HEPG2, HGC27, A375, MCF7, SW480 cancer cell lines as well as a healthy one, L929. *n*-BuOH (IC_{50} 19.5-46.2 µg/mL) and EtOAc (IC_{50} 38.1-76.5 µg/mL) fractions exhibited significant cytotoxicity against all tested cancer cells being SW480 and HEPG2 are the most sensitive ones. Chromatographic separations on the *n*-BuOH and EtOAc fractions led to the isolation of eight triterpene saponins, craniosaponin A (1), mulleinsaponins III (2) and IV, buddlejasaponins I and Ia, ilwensisaponins A, C and D, six iridoid glycosides, aucubine, geniposidic acid, 6-*O*-β-D-glucopyranosylaucubine, eurostoside, 6-*O*-[3-*O*-(*trans*-feruloyl)-α-L-rhamnopyranosyl]-aucubine, nigroside III and two phenylethanoid glycosides, verbascoside and alyssonoside. This is the first bioactivity and phytochemical study on *V. aydogdui*. Evaluation of cytotoxic activities of the isolates are underway.

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3.

SL 9

Natural products of *Galium odoratum* (Rubiaceae) and the coumarin formation during drying

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Changes in the contents of coumarin and its precursors were monitored during the post-harvest drying process of *Galium odoratum* (L.) Scop. (Rubiaceae) to get scientific support for the traditional preparation of "Maibowle", an alcoholic beverage made of white wine and/or sparkling wine, and *G. odoratum* shoots and sometimes additional ingredients, which involves pre-drying of the freshly harvested herb for two to 24 hours [1]. Using quantitative UHPLC-DAD analysis, the contents of coumarin, *cis*-melilotoside, and *trans*-melilotoside in *G. odoratum* were determined over a period of 72 hours after harvesting. The drying process was finished after 48 hours. Afterwards no further weight loss of the plant material was detectable. During this 48-hour-period, *trans*-melilotoside seemed gradually to isomerize to *cis*-melilotoside. Furthermore, we demonstrated that coumarin decreases during drying due to sublimation, which supports the use of fresh or minimally air-dried material in traditional recipes with specific drying times.
In addition to coumarin and its precursors, the flavonoid glycosides, quercetin 3-*O*-[α-L-rhamnosyl-(1→6)-*β*-D-glucosyl]-7-*O*-β-D-glucoside and kaempferol 3-*O*-[*α*-L-rhamnosyl-(1→6)-*β*-D-glucosyl]-7-*O*-β-D-glucoside, the iridoids monotropein, geniposidic acid, scandoside, asperulosidic acid, deacetylasperuloside, and asperuloside, and the phenylpropanoids 3-*O*-caffeoylquinic acid (neochlorogenic acid) and 5-*O*-caffeoylquinic acid (chlorogenic acid) were isolated from the methanolic extract of *G. odoratum*.

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SL 10

Cytotoxic activities of *Achillea biserrata* extracts and isolation of their secondary metabolites

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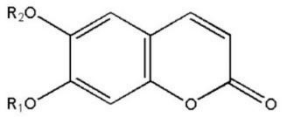
The genus *Achillea* (Asteraceae) comprises 139 species worldwide [1]. Essential oil and extracts obtained from various members of this genus exhibit a wide range of biological activities, including anti-inflammatory, antibacterial, antioxidant, cytotoxic, and hypoglycemic effects. Phytochemical studies on *Achillea* species revealed a diverse array of secondary metabolites, such as flavonoids, coumarins, sesquiterpene lactones, and lignans, as well as volatile components [2]. Previous bioactivity studies only investigated the antimicrobial potential of the essential oil of *A. biserrata* [3]. However, the bioactive properties of its extracts and isolated compounds remain undocumented. As part of our ongoing research on Türkiye's native flora, this study focused on isolating cytotoxic metabolites from *A. biserrata*, using bioassay-guided fractionation. The aerial parts were extracted with EtOH and DCM, EtOAc, *n*-BuOH, and H_2O subextracts were obtained through solvent-solvent extraction. The *in vitro* cytotoxic activity of EtOH extract and subextracts was evaluated against Colo 205, Colo 320, HeLa, MDA-MB-231, and KCR cells by MTT assay. Amongst, DCM exhibited moderate cytotoxicity against all tested cancer cell lines (IC_{50} = 26.45 ± 1.61 µg/mL - 61.81 ± 1.27 µg/mL), while EtOAc subextract showed weak cytotoxicity only on the MDA-MB-231 cell line (IC_{50} = 93.49 ± 2.97 µg/mL). Chromatographic separations on DCM and EtOAc subextracts led to the isolation and identification of scopoletin (1), scoparone (2), scopolin (3), isovitexin, isoorientin, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and chlorogenic acid based on extensive NMR and MS analyses. Evaluation of *in vitro* cytotoxic effects of the isolates is underway in our laboratory.



	R_1	R_2
1	H	CH_3
2	CH_3	CH_3
3	Glc	CH_3

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SL 11

LC-MS and GC-MS analyses reveal that amino acid-induced ammoniation of EGCG in tea enhances its structural stability

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Epigallocatechin gallate (EGCG) is one of the most abundant polyphenols in tea. Its transformation mechanism during the fermentation process has an important influence on the quality of tea and the formation of functional components. First, based on LC-MS full scan analysis, it was determined that the content of EGCG in the aqueous extracts of green tea, white tea, oolong tea and black tea decreased with the deepening of fermentation. GC-MS full spectrum scanning identification found EGCG and its derivative N-EGCG in fermented tea samples. The characteristic ions and retention times of N-EGCG were confirmed by LC-MS and GC-MS dual platforms, and the SIM mode was used to achieve its specific quantification in different tea samples. It was found that N-EGCG was significantly negatively correlated with EGCG in content. Further, the change trend of N-EGCG in methanol extract was consistent with the water extraction data, excluding the possibility of its thermal induction, and verifying that its generation was mainly driven by the fermentation process. Correlation analysis with free amino acids in tea leaves showed that L-Serine and L-Threonine were positively correlated with N-EGCG content and negatively correlated with EGCG, suggesting that they may participate in the C-N bond formation reaction of N-EGCG as nitrogen donors. This study combined multi-platform mass spectrometry to reveal the EGCG metabolic pathway in tea leaves, verified the formation of the stable product N-EGCG, and proposed a "polyphenol-amino acid" synergistic mechanism induced by fermentation, providing a theoretical basis for the study of the transformation mechanism of functional tea polyphenols and the development of metabolic markers for the degree of tea fermentation.

SL 12

Anti-inflammatory activity of *Cynanchica bornmuelleri* and bioassay-guided isolation of its secondary metabolites

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The genus *Cynanchica* (Rubiaceae), previously classified as the section *Cynanchicae* under the genus *Asperula*, has been reclassified as a distinct genus based on phylogenetic studies [1]. It is represented by 73 species distributed in the Mediterranean region [2]. To date, the phytochemical composition and bioactivities of the genus *Cynanchica* have not been explored. As a part of our ongoing search for new bioactive compounds from Rubiaceae species native to Türkiye, we aimed to isolate anti-inflammatory secondary metabolites from *C. bornmuelleri* through *in vitro* activity-guided fractionation. The whole plant of *C. bornmuelleri* was extracted with EtOH. The crude EtOH extract was dispersed in H_2O and then partitioned against *n*-hexane, EtOAc, and *n*-BuOH, respectively. The crude extract, and solvent fractions were evaluated for their NF-κB inhibitory activities in LPS-stimulated THP-1 cells. Amongst, *n*-BuOH fraction showed remarkable inhibitory effect on NF-κB (61%) which is comparable to positive control, prednisolone at 2 µM. Sequential chromatographic separations on the *n*-BuOH fraction using Polyamide CC, Sephadex LH-20 CC, MPLC (C_{18} and SiO_2), and semi-preparative HPLC yielded a new iridoid glycoside, bornmuellerioside (**1**), along with 11 known secondary metabolites namely, asperuloside, asperulosidic acid, asperulosidic acid methyl ester, deacetylasperulosidic acid, scandoside, geniposidic acid, 3',5'-di-*C*-β-glucopyranosylphloretin, vicenin 2, rutin, dehydroconiferyl alcohol 4-*O*-β-D-glucopyranoside, and chlorogenic acid. Additionally, ursolic acid was purified from the EtOAc fraction. The structures of the isolated compounds were elucidated by 1D and 2D NMR as well as HRMS. This is the first phytochemical and bioactivity study on *C. bornmuelleri*, an endemic species to Türkiye.

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SL 13

Effectiveness of plant bioactive substances on the rumen environment in lambs with gastrointestinal parasites

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This experiment is part of a larger study on natural plant chemotherapeutic alternatives for endoparasite control in lambs. We investigated the effect of grazing on a natural meadow enriched with experimentally sown chicory on parasitological status, larval contamination of the pasture, serum antioxidant parameters, histopathology, as well as on growth and rumen parameters. Two plots of mixed plant species diversity were selected and fenced with electric fencing, with the first plot consisting entirely of meadow grassland and 25% of the second plot used for chicory reclamation. Sixteen lambs infected with *Haemonchus contortus* endoparasite larvae were divided into two groups: lambs grazing on meadow grassland (Control) and lambs grazing on the experimental plot enriched with chicory (*Cichorium intybus*) (Experimental). The experimental period was 144 days and the lambs were euthanized following the rules of the European Commission. Qualitative analyses using ultra-high-resolution mass spectrometry identified phytochemical compounds (e.g., flavonoids, phenolic acids, etc.) in both pastures. Body weight differed on the day 89 ($P = 0.029$), day 131 ($P = 0.050$) and day 144 ($P = 0.027$) and reached higher values in the Experimental group (28.2, 33.0, and 38.2 kg) than in the Control group (24.5, 29.1, and 32.7 kg). The egg shedding reductions were highest from day 103 onwards in both groups. The mean number of abomasal worms recorded per lamb after the necropsy was lower in the Control than the Experimental group. Total bacterial populations and relative abundances of *S. bovis* were significantly higher ($P < 0.05$) in the Experimental group than in the Control group. It seems that the self-medication of grazing lambs improved the nutrition and well-being of the animals, but the meadow pastures with experimentally sown chicory increased the efficiency of nutrient use and improved the production indicators of the infected lambs. Infected grazing lambs could choose the medicinal plant themselves while having a variety of nutritional alternatives available to them. The availability of plants containing bioactive compounds in meadow grasslands may have a high potential to reduce parasite burdens in lambs and pasture contamination with infective nematode larvae which gives a future perspective in the control of parasitism in livestock.

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SL 14

Isolation of a unique monoterpene diperoxy dimer from *Ziziphora clinopodioides* subsp. *bungeana* together with triterpenes with antidiabetic properties

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Ziziphora clinopodioides subsp. *bungeana* (Juz.) Rech.f. is used in traditional medicine for various purposes. Previous phytochemical studies focused on phenolic compounds, but triterpenoids were almost overlooked. Therefore, this phytochemical study was focused on obtaining natural compounds lacking chromophores from the aerial parts of *Z. clinopodioides* subsp. *bungeana* with emphasis to find those displaying dual antidiabetic properties.

Extensive chromatographic separation of a chloroform-soluble fraction led to the isolation of twenty compounds, including a unique monoterpene diperoxy dimer (1). The structures were elucidated by 1D and 2D NMR experiments along with HRMS. Compound 1 was additionally identified by the single crystal X-ray diffraction method. α-Glucosidase inhibitory assay and GLUT4 expression and translocation in C2C12 myotubes were conducted to evaluate antidiabetic potential of selected compounds (1–11). Compounds 7 and 9–11 displayed more potent α-glucosidase inhibitory activity (IC_{50} 45.3–135.3 μM) than acarbose used as a positive control (IC_{50} 264.7 μM), while only pomolic acid (5) increased GLUT4 translocation in C2C12 myotubes in a significant manner. Although none of the isolated compounds demonstrated dual antidiabetic activity, selected triterpenes proved to be potent antidiabetic agents *in vitro*.

Acknowledgements
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SL 15

Spent coffee grounds from filtered specialty coffee, their bioactive compounds and potential uses in animals

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Specialty coffee is made from the highest quality green coffee beans, has a known geographical origin, and is produced using the best postharvest processing methods. It has a standardized production process from the selection of criteria for coffee plantations to coffee drinks. Spent coffee grounds (SCGs) are a major by-product of the preparation of coffee beverages produced through various brewing methods such as hot water extraction (e.g. aeropress) or steam (espresso). We investigated the effect of coffee by-products on fermentation and methane emissions in rumen fluid *in vitro*. SCGs from filtered specialty coffee (*Coffea arabica*) have been tested as a feed supplement. Meadow hay (MH) and barley grain (BG) (MH+BG, 500/500, w/w) were used as the substrates in rumen fluid fermentation, where SCG replaced an equal amount of MH (MH+SCG) or BG (BG+SCG). The experiment used an *in vitro* gas production technique with 35 ml of buffered rumen fluid inoculum and 250 mg of substrate incubated for 24 hours at 39 °C under anaerobic conditions. Three replicates (three incubation bottles) were prepared for each feed fermentation (i.e., MH+BG, MH+SCG, and BG+SCG) and the experiment was repeated three times within three consecutive days. Quantitative analyses of bioactive compounds by ultra-high resolution mass spectrometry in SCG indicated the presence of phenolic acids in the three main groups of chlorogenic acid (i.e., caffeoylquinic acids, feruloylquinic acids, dicaffeoylquinic acids). The total content of bioactive compounds was highest in SCG-from Ethiopian coffee, followed by SCG-from mixed coffees and Ethiopian coffee (35.2, 31.2, and 20.9 mg/g dry matter, respectively). Total gas and methane production values were significantly reduced in MH+SCG and BG+SCG. Values of total short-chain fatty acid concentration were correlated with methane and total gas production of incubated substrates. Replacing MH or BG with the same amount of SCG resulted in increased digestibility *in vitro* (20-40%). Due to bioactive compounds and relatively low cost, spent coffee grounds from filtered coffees could be used as alternative sources of feed with the potential to modulate ruminal fermentation, reduce methane emissions, and increase the digestibility of dietary substrates.

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SL 16

Identification of plant phenolics as novel PPARγ agonists and hypoglycemic agents

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There is a great need to find 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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SL 17

The role of fresh and ensiled Rugosa rose (*Rosa rugosa* Thunb.) pulp in *in vitro* modulation of basic ruminal parameters in dairy cows

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There is a growing interest in sustainable agriculture, particularly in reducing methane emissions from ruminants. A promising strategy involves dietary supplementation with industrial by-products rich in bioactive natural components (BAC), which can enhance ruminal fermentation, shift fatty acid profiles toward more beneficial compositions, and decrease greenhouse gas emissions. Bioactive compounds play a critical role in modulating ruminal microbiota and fermentation patterns. This study aimed to assess the impact of fresh (RFPF) and ensiled (RFPS) Rugosa rose pulp, rich in BAC, on basic ruminal parameters and methane production *in vitro*. Grass silage, whose chemical profile closely matched RFPS, was used as the control (CON). RFPF and RFPS chemical compositions (n=4) were determined using AOAC methods (2007), while fatty acid profiles were analyzed *via* gas chromatography. Neutral detergent fiber (NDF) was measured according to Van Soest et al. (1991). Phytochemical metabolome quantification was performed by the Folin–Ciocalteu method, and qualitative profiling was achieved using liquid chromatography (LC) coupled with mass spectrometry (ESI-QTOF-MS). Fermentation parameters, gas production, and methane output were assessed using the Hohenheim gas test. Both RFPF and RFPS were confirmed as valuable sources of nutrients (crude protein: 158 and 161 g/kg DM, respectively) and unsaturated fatty acids (UFA; C18:3 n-3: 29.13 and 29.56 g/100g FAME, respectively). A high polyphenol concentrations were observed (465 and 509 mg gallic acid equivalent/100 g DM, respectively), underscoring their potential functional benefits. Rugosa rose pulp supplementation significantly influenced ruminal parameters by modifying pH, ammonia, and VFA profiles, and notably reduced methane production compared to CON. Although digestibility was slightly lower in RFPF and RFPS groups after 24-hour incubation, likely due to seed content, both treatments demonstrated a marked decrease in methane yield (CH_4 mmol/g DM and CH_4/IVDMD). Additionally, a favorable shift in the acetic to propionic acid ratio was observed, suggesting improved fermentation efficiency. The altered FA profiles indicated a possible pro-health modulation, with an increase in beneficial UFA content. These preliminary findings highlight the value of BAC-rich Rugosa rose pulp in mitigating methane emissions while supporting ruminal health, offering a promising avenue for more sustainable ruminant production systems.

SL 18

Anti-inflammatory potential of sesquiterpene lactones from *Schkuhria pinnata* in an *in vitro* intestinal inflammation model

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Five sesquiterpene lactones were isolated from the above-ground parts of *Schkuhria pinnata* (Lam.) Kuntze ex Thell., Asteraceae, at the Department of Natural Drugs, Faculty of Pharmacy, Masaryk University. Among these, three compounds were isolated and characterized for the first time. Their potential anti-inflammatory effects were assessed *in vitro*. Initial screening using the human leukemia monocytic cell line THP1-Blue™ NF-κB, stimulated with lipopolysaccharide (LPS), revealed that two compounds exhibited a greater inhibitory effect on the pro-inflammatory transcription factor NF-κB signaling pathway than the standard drug prednisolone. The reduction in NF-κB activity was further confirmed by decreased mRNA levels of the pro-inflammatory cytokines TNFα and IL-1β in macrophages derived from THP1-Blue™ NF-κB cells. Both compounds were subsequently evaluated in a 3D *in vitro* model of intestinal inflammation, consisting of a co-culture of differentiated enterocyte-like Caco-2 cells and THP-1-derived macrophages in a Transwell® system stimulated with LPS. Within 24 hours, these compounds significantly reduced TNFα mRNA levels in Caco-2 cells. This inhibitory effect was further confirmed via ELISA, which quantified reduced TNFα levels in the basolateral media. These findings highlight the potential of these sesquiterpene lactones as promising anti-inflammatory agents.

SL 19

A natural weapon: Nchuanwu (African basil) lipids as a sustainable bio-pesticide

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Synthetic chemical pesticides, while effective, pose significant environmental and health risks. This growing concern has ignited a global search for sustainable, eco-friendly alternatives. Natural, plant-based pesticides offer a promising solution to reduce agricultural losses, food insecurity, and malnutrition caused by insect infestations. African basil (*Ocimum gratissimum*) locally called Nchuanwu meaning remover of insects in Eastern Nigeria has emerged as a potential source of natural pesticides. Its leaves contain bioactive lipids with potent insecticidal properties. To harness this potential, oils from the African basil plant were extracted using hydro-distillation and solvent extraction with n-hexane. Through Gas Chromatography-Mass Spectroscopy (GC-MS) analysis, several key bioactive compounds in the oils were identified and quantified, including thymol, eugenol, and ursolic aldehyde. These compounds belong to the terpenoid and phenylpropanoid groups, known for their diverse biological activities. *In vivo* experiments on maize grain weevils *Sitophilus zeamais,* a dangerous pest, demonstrated the remarkable insecticidal efficacy of the extracted oils. We observed significant mortality rates, particularly at higher concentrations (2% and 10%). After 24 hours, all insects exposed to these concentrations perished, while the control group (treated with Tween 20) remained unaffected. The underlying mechanism of action likely involves the disruption of insect neurotransmitter function, deterring feeding and ultimately leading to mortality. These findings highlight the potential of African basil-derived oils as a sustainable and effective bio-pesticide, offering a promising solution to address the global challenge of insect pest control.

SL 20

Analysis of the active ingredients and pharmacological activity of *Peucedanum japonicum* cultivated in Korea

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Peucedanum japonicum (*P. japonicum*) is a perennial herb native to Japan and parts of East Asia, traditionally used in Asian medicine for its diuretic, antipyretic, and analgesic properties. Recent studies indicate that its extracts have therapeutic potential against inflammation, viral infections, rheumatism, and urinary tract infections. This study evaluated the potential *of P. japonicum* as a functional food ingredient by analyzing its bioactive components and investigating effects on memory and gut health. Using UPLC-QTOF/MS analysis, 29 secondary metabolites were identified, with praeruptorin A–D and visnadine as major constituents. The ethanol extract (PJE) significantly inhibited pro-inflammatory mediators—nitric oxide (NO), prostaglandin E2 (PGE_2), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)—in BV2 microglial cells. This activity was associated with suppression of signaling pathways including NF-κB, ERK, JNK MAPK, Akt/GSK3β, and JAK2/STAT3 via modulation of the TLR4/MyD88 axis. PJE also promoted the growth of six beneficial bacterial strains during co-incubation with 12 probiotic strains in the absence of additional culture media. Notably, *Lactobacillus plantarum* and *L. reuteri* showed remarkable proliferation—approximately 1,000,000-fold and 150,000-fold, respectively. Fermented products of these strains exhibited reduced cytotoxicity and enhanced NO inhibition compared to PJE alone, suggesting improved anti-inflammatory potential. These findings indicate beneficial effects on gut and cognitive health through attenuation of neuroinflammation. Overall, the results support the development of *P. japonicum* as a promising natural source for health functional foods targeting gut health and cognitive function via modulation of neuroinflammatory responses and gut microbiota.

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SL 21

Gymnopeptides: an emerging group of fungal cyclic peptides

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The recently discovered gymnopeptides represent a distinct group of ribosomally synthesized and post-translationally modified peptides (RiPPs) of fungal origin.

RiPPs constitute a vast group of natural products distinguished by remarkable structural diversity. This diversity, stemming from various structural components, leads to a broad spectrum of biological functions, including favorable pharmacological properties. The biological activities of these compounds rely on structural features introduced through post-translational modifications of ribosomally produced precursor peptides. RiPPs are essential to several biological processes and are considered as potential lead compounds for drug development. The fungal RiPPs have been classified into four groups: the amatoxins/phallotoxins, dikaritins, epichloecyclins, and borosins.

Borosins are characterized by *N*-methylations on the peptide backbone's amide bonds. The first borosins were represented by the nematocidal omphalotins from the jack-o'-lantern mushroom (*Omphalotus olearius*). Besides the omphalotin-type borosins, gymnopeptides A and B have been identified in 2016 from the spindle shank mushroom (*Gymnopus fusipes*). *Gymnopus fusipes* is a parasitic fungus that can severely damage the roots of various oak species. Its edibility is disputed, however in some regions in Europe it is still consumed by locals. Recently, new members of this family of highly *N*-methylated fungal cyclopeptides, gymnopeptides C and D have been isolated from the same fungal species.

Gymnoppetides exerted remarkable antiproliferative properties on several human cancer cell lines. These peptides tested by the MTT method exhibited dose-dependent inhibition of cell proliferation with IC_{50} values in the nanomolar range. Further experiments revealed that all four peptides induced apoptosis by upregulating caspase-3 activity. Given the critical role of cell migration in tumorigenesis, the impact of gymnopeptides on the migratory potential of cancer cells was also investigated. The classical scratch assay demonstrated that gymnopeptides acted as suppressors of cell migration.

While studies have expanded the chemical understanding of gymnopeptides, their role in the parasitic lifestyle of *Gymnopus fusipes* and the absorption and metabolism of these compounds following consumption remain poorly understood. Further biological studies are needed to elucidate these aspects, thereby enhancing our understanding and exploration of the bioactive potential of gymnopeptides.

SL 22

Screening the plant-like animals, 20 years of early drug discovery from Arctic marine invertebrates

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Marbio was established as a bioprospecting platform for marine natural products at the University of Tromsø in 2005. For almost 20 years, Marbio has been screening extracts and extract-fractions from marine animals, in particular invertebrates, which has led to the discovery of several bioactive compounds. Throughout the 20 years of bioactivity screening, the extract-fractions have been prepared using minimally altered protocols and bioactivity assays, mainly anti-cancer, anti-microbial and anti-inflammatory. This provides a rare opportunity for bioactivity-profiling of marine-invertebrates.

The main focus of the screening effort has been anti-cancer, anti-microbial and anti-inflammatory bio-activity. The invertebrates were sampled predominantly in arctic waters in collaboration with the Norwegian Marine Biobank, Marbank. Invertebrates have provided compounds with anti-cancer and anti-microbial bioactivity. Among the compounds isolated are Securamine H-J (cytotoxic), Ponasterone A & F, Purpuroine (cytotoxic), Dendrobeaniamine A, Securidine A, Ianthelline (anti-fouling), Synoxazolidinones A & B (anti-bacterial & anti-fungal), and the Breitfussins (anti-cancer). A common challenge for the bioassay-guided profiling is unspecific bioactivity of the extracts/fractions, or the identification of previously described compounds. Our dataset indicates that the major source of bioactive compounds throughout the bioprospecting pipeline at Marbio have been sessile animals, which are in their morphology somewhat similar to plants. While bioactivity was observed in other invertebrates from crustaceans to mollusks, the sessile invertebrates have provided the largest share of bioactive isolated compounds.

SL 23

Prenylated phenolics as modulators of intercellular communication

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Prenylated phenolics are secondary plant metabolites known for a range of bioactive properties, including strong antioxidative and anti-inflammatory effects. Moreover, certain plant-derived phenolic compounds have demonstrated anti-proliferative activity. One possible mechanism of action of these phytochemicals is the restoration of impaired gap-junction intercellular communication (GJIC). Properly regulated GJIC is essential for maintaining tissue homeostasis. Dysregulation of GJIC has been linked to various pathophysiological conditions and diseases, including carcinogenesis. Recently, a variety of structurally different prenylated phenolics from *Paulownia tomentosa*, *Ficus cyathistipula*, and *Broussonetia papyrifera* were successfully isolated by our team, to investigate their ability to restore GJIC in tumorigenic cells.

To evaluate the effects on GJIC, an *in vitro* method called multiparametric scalpel loading-dye transfer assay (mSLDT) was used, followed by fluorescence microscopy with automated image analysis. The cell-based screening was performed using 2D (monolayer) *in vitro* model utilizing the tumorigenic rat liver cell line WB-*ras*, which exhibits downregulated GJIC due to *ras*-oncogene expression. Preliminary data obtained from the previous stages of research and our recent experiments on this 2D cell model showed that certain compounds have potent bioactivity to restore GJIC in WB-*ras* cells. The most active compounds are being investigated *in vitro* using secondary assays on 3D (tumor spheroids) cell models targeting other cancer hallmarks, such as cell proliferation and migration. The use of 3D cell models offers a significant advantage, as spheroids better mimic tumor-like microenvironment, reflecting several important aspects of tumor tissue, such as oxygen and nutrient gradients, multilayer cellular architecture, and exhibit barriers for drug penetration.

The research aims to (1) expand the current knowledge about the effects of prenylated phenolics on GJIC and (2) investigate in more detail their ability to attenuate cell proliferation or tumor formation. In this context, *in vitro* assessment of GJIC may (3) serve as a valuable tool in drug discovery and (4) support the identification of plant-derived compounds with therapeutic potential for individuals with a high risk of cancer.

SL 24

Sesquiterpene lactones from Arnica da Serra (*Lychnophora ericoides*) and their *in vitro* effects on multiple myeloma and acute myeloid leukemia

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Multiple myeloma (MM) and acute myeloid leukemia (AML) are very heterogeneous forms of bone marrow cancer, which account for more than 10% of all blood cancers [1]. Both types are associated with a wide variety of genetic abnormalities, making treatment extremely challenging. Therefore, novel therapies are urgently needed, and natural products represent a potential source of new therapeutics or lead compounds. Especially in cancer therapy, they still play an important role in the development of new therapeutics. In the present study, we focused on the isolation of potential lead compounds from arnica da serra (*Lychnophora ericoides* Mart, Asteraceae, Cichorioideae), which is traditionally used in Brazil for the treatment of pain, rheumatism and inflammation. Our study resulted in the isolation of four methylated flavonoids and three sesquiterpene lactones, which were tested against two AML (HL-60 and Molm-13) and two myeloma (AMO-1 and KMS-12-PE) cell lines. Testing of the antiproliferative activity of the compounds and their effects on cell viability revealed that all tested compounds showed dose-dependent activity with, however, much higher effects for the isolated sesquiterpenoids goyazensolide (1), centratherin (2), and lychnopholide (3). Compound 1 showed IC_{50} values ranging from 1.0 to 2.0 µM in both the proliferation and viability assays, while compound 2 showed similar effects with IC_{50} values from 1.1 to 2.7 µM. Compound 3 displayed somewhat higher IC_{50} values ranging from 1.3 to 2.6 µM in the proliferation assays and 1.8 to 4.0 in the viability assays. However, evaluation of selectivity indices (SI) of compound 3 using the non-malignant cell line HS-5, revealed SI values above 3 against both AML cell lines in proliferation testing. Likewise, compound 2 displayed an SI above 3 against Molm-13 in the viability testing. The results of our work indicate that the sesquiterpenoids contained in *Lychnophora ericoides* may be interesting starting points for the development of new therapeutics against multiple myeloma and acute myeloid leukemia.

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SL 25

Phytosterols and triterpenoids as functional compounds in edible and medicinal plants

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The phytochemical characteristics of edible and medicinal plants, as well as their derived foods, food preparations, and dietary supplements, often focus on bioactive polyphenols. However, data on the content of two specific groups of isoprenoid compounds - steroids (including phytosterols) and triterpenoids - are generally lacking. Phytosterols help lower lipid and cholesterol plasma levels; they are also valued for their antioxidant, anti-inflammatory and hypoglycemic properties, making them useful in the prevention of cardiovascular diseases, as well as fatty liver, rheumatoid arthritis and obesity-related diseases. Triterpenoids, known for their structural diversity and wide range of bioactivities, including anti-inflammatory, antimicrobial, antiviral, hepatoprotective, and anticarcinogenic properties, have significant pharmaceutical and industrial applications.
The aim of the ongoing studies conducted in the Department of Plant Biochemistry is to analyze the content of these two important groups of phytochemicals in various plants, foods and food-derived products. Gas chromatography-mass spectrometry (GC-MS) analyses were performed on lipophilic extracts obtained from different plant parts (leaves, flowers, fruits, seeds, sprouts) and plant-derived products, such as jams, juices, syrups, wines. In the investigated plants and plant-derived foods, the presence of phytosterols, primarily sitosterol, stigmasterol and campesterol, was often demonstrated, typically alonside stanols and steroid ketones. However, the content of this group of compounds was usually much lower than the therapeutic doses, which are estimated to be around 2 g per day. The amounts of bioactive triterpenoids, particularly triterpenoid acids (mainly oleanolic and ursolic acids), can be much higher than those of phytosterols, especially in plants from families known to be rich in triterpenoids, e.g., Rosaceae or Ericaceae. Despite this, the strong hydrophobicity and low absorption of triterpenoids result in low oral availability, reducing their physiological activity and hindering their therapeutic efficacy. A potential solution to this problem is the development of novel functional delivery systems for triterpenoids, such as encapsulated nanoparticles with improved stability, controlled release, and enhanced bioavailability. Although present in relatively low quantities, phytosterols and triterpenoids may significantly contribute to the health-supporting benefits and therapeutic properties of edible or medicinal plants by exerting synergistic effects with other phytoconstituents that act through different mechanisms. For example, the synergism between triterpenoids and phenolic compounds appears particularly promising in enhancing antioxidant and antimicrobial properties, due to increased efficacy and a broader spectrum of effects.

SL 26

The preparation of cannabis-based magistral formulations - personal experience

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This presentation distils six years of hands-on pharmacy experience in compounding prescription medicines from registered medical-cannabis raw materials available in Poland. It highlights three key domains: choosing the right chemotype for the individual patient, selecting the optimal pharmaceutical form, and demonstrating clinical value through illustrative cases.
Photographs of compounded preparations and short case descriptions illustrate outcomes: a psoriatic plaque almost cleared by a 2.5 % THC/CBD gel; a patient with refractory endometriosis gaining analgesia and functional recovery from combined sublingual drops and rectal suppositories.
These observations confirm the therapeutic potential of magistral cannabis preparations and underscore the pharmacist's pivotal position in personalised therapy.

SL 27

Comparative study of diosmin and its structural analogues with human red blood cells, hemoglobin and metal ions

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Oxidative stress, caused by free radicals and conditioned by prooxidative metals, is involved in atherosclerosis development. Structural analogues of diosmin (DMN) a flavonoid with anti-atherosclerotic activity *in vivo* - diosmetin (DMT) and diosmetin-7-*O*-glucoside (D7G) are less studied in the literature. The aim of this study is to determine the interaction of DMN, DMT and D7G with human red blood cells (RBCs) and hemoglobin (Hb). Furthermore, mechanistic studies investigating their chelation and reduction potential towards prooxidant metals (copper and iron) was performed.

The interaction of the flavonoids with RBCs: hemolytic activity, osmotic resistance changes, reduced glutathione (GSH) concentration in normal and AAPH-treated (2,2'-azobis(2-methylpropionamidine) dihydrochloride) cells were studied spectrophotometrically. The shapes of RBCs treated with compounds were observed under an optical microscope. The trans-membrane potential changes of RBCs and the interaction of the compounds with Hb were assessed using fluorimetry. Metals chelation and reduction were studied using spectrophotometry.

The results showed that none of the flavonoids possess hemolytic activity and do not change the biophysical parameters of RBCs: osmotic resistance and trans-membrane potential, confirming that they do not act destructively on erythrocyte membrane. Microscopic studies revealed that DMT led to stomatocyte formation and D7G to echinocyte formation, indicating different interactions with the cell membrane. The compounds did not alter GSH levels in RBCs; however, DMN and DMT protected GSH against AAPH-induced oxidation, better than the standard antioxidants: ascorbic acid and butylated hydroxyanisole (BHA). Static quenching of tryptophan fluorescence suggested that the compounds interact with Hb, forming stable complexes. DST chelated copper ions more effectively than iron ions, while DMN showed better iron-chelating activity. Furthermore, all compounds similarly reduced copper (II) ions.

All obtained results indicate that diosmin and its structural analogues possess antioxidant activity and therefore, may be effective in prevention of oxidative stress.

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SL 28

Between medicine, narcotic, food, and cosmetic: navigating cannabis analysis through analytical chemistry

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Cannabis-based formulations can fall under different regulatory categories, including food, cosmetics, medicines, or potentially narcotic substances, depending on their phytocannabinoid composition and intended use. Due to the complexity of the product matrices and the diversity of oils and cosmetic formulations containing cannabinoids, accurately measuring target analytes remains challenging. This highlights the importance of accurate and straightforward analytical methods to ensure consistent product quality, safety, and adherence to regulations.

A systematic literature review was conducted using the SPIDER framework to define search terms, applied to PubMed, Google Scholar, and Scopus, and followed PRISMA guidelines [1]. Inclusion criteria comprised open-access studies in English describing the development, validation, and application of analytical methods for quantifying cannabinoids using robust High-Performance Liquid Chromatography with ultraviolet detection (HPLC-UV). The review identified 55 relevant articles. Findings from the review informed method development, particularly in sample preparation, solvent selection, and chromatographic conditions.

This study aimed to develop and validate a practical HPLC-UV analytical method for quantifying cannabidiol (CBD), cannabinol (CBN), tetrahydrocannabinol (THC), and their acidic counterparts (CBDA, THCA) in medium-chain triglyceride (MCT) oil, hemp seed oil, olive oil, and cosmetic products.

The method was validated and applied to cannabis-based MCT oil. Further optimisation using hemp seed oil, olive oil, and cosmetic products was performed to expand applicability. Chromatographic separation was carried out using an Agilent 1260 Infinity system with a C18-AR column (250 mm × 4.6 mm; 5 µm), employing isocratic elution with acetonitrile and 0.5% acetic acid (75:25 v/v), a flow rate of 1.5 mL/min, column temperature of 30°C, detection at 220 nm, and injection volume of 10 µL. Validation followed ICH Q2(R2) guidelines [2].

Analysis of 11 commercial MCT oil products revealed label discrepancies, with 7 samples accurately reflecting the declared cannabinoid content. MCT oil validation showed accuracy (101%–114%), precision (RSD <15%), and linearity (R^2 = 0.99) over 0.03–0.5 mg/ml. The next phase will focus on method validation and application to hemp seed oil, olive oil, and cosmetic products to assess label accuracy and compliance. This validated HPLC-UV method has the potential to offer a practical, accessible analytical tool for cannabinoid quantification across complex matrices.

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SL 29

Lycium barbarum polyphenols improve NAFLD by regulating intestinal microbiota and one-carbon cycle

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Non-alcoholic fatty liver disease (NAFLD) has emerged as the most common chronic liver disorder worldwide, driven largely by modern lifestyle factors such as high-fat diets and sedentary behavior. Closely associated with obesity, insulin resistance, and dyslipidemia, NAFLD is increasingly recognized as a systemic metabolic disease involving both hepatic and extrahepatic dysfunctions. In this study, we explored the hepatoprotective potential of Lycium barbarum polyphenols (LB) in a murine model of diet-induced NAFLD, with a particular focus on their regulatory role in one-carbon metabolism and the gut-liver axis. C57BL/6J mice were fed a high-fat diet (HFD) with or without LBP supplementation. LBPs significantly improved glucose tolerance and insulin sensitivity, reduced hepatic lipid accumulation as evidenced by lower triglyceride, total cholesterol, and LDL-C levels and normalized HDL-C concentrations. Histological and biochemical assessments confirmed attenuation of liver injury. ELISA analysis demonstrated that LBPs effectively suppressed pro-inflammatory cytokines including TNF-α and IL-6 while restoring the anti-inflammatory cytokine IL-10 in both serum and liver tissue. 16S rRNA sequencing revealed that LB selectively modulated gut microbiota composition by increasing beneficial genera such as *Paramuribaculum*, Muribaculaceae, *Eisenbergiella*, *Lactobacillus*, and *Akkermansia*, thereby enhancing butyrate production and improving intestinal barrier integrity. Untargeted liver metabolomics further revealed that LBPs reshaped hepatic one-carbon metabolism by increasing levels of serine, methionine, and S-adenosylmethionine (SAM), while reducing levels of S-adenosylhomocysteine (SAH), cystathionine, and homocysteine. These coordinated shifts suggest enhanced methylation capacity and reduced oxidative stress burden. Collectively, our findings demonstrate that LBPs mitigate NAFLD progression through a multifaceted mechanism involving anti-inflammatory effects, antioxidant defense, gut microbiota restoration, and metabolic reprogramming centered on the one-carbon cycle. This study provides novel mechanistic insights into the diet-epigenetics interface in NAFLD and underscores the therapeutic potential of dietary polyphenols as safe, natural interventions for metabolic liver diseases.

SL 30

Optimizing green extraction of cannabinoids using natural deep eutectic solvents

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Cannabis sativa has garnered significant attention due to its great potential in various industries, including pharmaceuticals, cosmetics, and food. Efficient extraction methods are crucial for the later preparation of products. The use of eutectic solvents presents an innovative, eco-friendly approach to extraction, aligning with green chemistry principles. This study explores the optimization of cannabinoid extraction using eutectic solvents to maximize yield and efficiency while minimizing environmental impact and promoting sustainable practices.

The aim of the study was to optimize the extraction process using a eutectic solvent in order to obtain the highest possible cannabinoid content in the extracts.

The extraction process was optimized using the Design of Experiment (DoE) to enhance cannabinoid yield, which was quantified through High-Performance Liquid Chromatography (HPLC). The molarity and composition of the eutectic solvent (choline chloride and allantoin) were evaluated in preliminary studies. Three parameters were considered for optimization: extraction time, water content, and temperature. A Box-Behnken Design, a type of response surface methodology, was applied to reduce the number of trials while still identifying optimal conditions. The conditions for extraction time (15, 45, and 75 minutes), water content (5%, 10%, and 15%), and temperature (45°C, 60°C, and 85°C) were evaluated. The antioxidant properties of the optimized extract were evaluated using DPPH, CUPRAC, ABTS, and FRAP methods.

A eutectic mixture consisting of choline chloride and allantoin can serve as an effective cannabinoid extractant from *Cannabis sativa*, providing an extract with antioxidant properties. The Design of Experiments approach successfully determined the optimal extraction parameters, ensuring a green and sustainable process.

Acknowledgments

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SL 31

Intergenerational inheritance of quercetin-induced abnormal immunity in mice

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Quercetin, belonging to dietary flavonoids enrich in the human diet, has been reported to regulate immune-related models by epigenetic modifications in many organisms [1]. However, few studies were reported to explore its transmission of regulatory effects across generations to progeny. Here, we selected *Escherichia coli*, which is a conditional pathogen capable of causing gastrointestinal infections or various localized tissue and organ infections under specific conditions, as the pathogenic strain to infect mice [2]. We firstly provide evidence that Quercetin can not only induce responsiveness changes against systemic *Escherichia coli* infection in the directly exposed organisms, but rather in subsequent generations through the transgenerational inheritance of epigenetic traits. Both parental male mice and progeny exhibited cellular and phenotypic changes associated with metabolic alterations. Surprisingly, the male and female progeny of mice treated with Quercetin (200 mg/kg) for 6 weeks negatively enhanced the survival rate under systemic *E. coli* (1×10^8 CFUs/mL) infection, concurrent with an increase in bacterial loads of liver and spleen were also observed. Serum TNF-α, and IL-1β levels were significantly increased post-infection in the progeny. Our results provide the first evidence for the inheritance of immunity driven by Quercetin in mammals, and attenuating protection against bacterial infection.

Acknowledgments
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SL 32

Transgenerational immunosuppression induced by sodium glutamate in *Caenorhabditis elegans*

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Monosodium glutamate (MSG), a widely used flavor enhancer, represents one of the most common forms of glutamate found in foods. Despite its extensive application in the food industry to augment umami taste, accumulating evidence suggests that MSG exerts a range of adverse effects on human health, including metabolic syndrome, neurotoxicity, infertility, fetal developmental abnormalities, and immune dysfunction. However, whether MSG-induced immunosuppressive effects can be inherited across generations remains unclear. *Caenorhabditis elegans*, with its high degree of genetic conservation with humans and rapid reproductive cycle, has been demonstrated to be a powerful model to investigate innate immunity and transgenerational inheritance. In this study, we systematically explored the transgenerational immunosuppressive effects of MSG using *C. elegans* as a model organism. MSG was supplemented into the worms' standard diet, followed by infection with *Pseudomonas aeruginosa* PA14 to evaluate innate immune responses in both the parental generation (F0) and their offspring. Surprisingly, MSG significantly impaired host defense against pathogens, and this immunosuppressive effect continued to be appeared in the F2 generation. MSG exposure also compromised the worms' ability to avoid PA14 and disrupted pathogen-induced aversive learning behavior, suggesting potential neurotoxic effects. Moreover, developmental delays and reduced fecundity were observed in MSG-treated worms during early larval stages as well. Overall, our findings demonstrate that MSG exposure leads to heritable impairments in innate immunity, neural function, and development in *C. elegans*. These transgenerational effects, observed up to the F2 generation, may be mediated by epigenetic mechanisms. This study highlights the need for greater caution regarding the widespread use of MSG in food, given its potential long-term biological consequences.

SL33

Novel insights into the anti-obesity potential of myricetin using a drosophila model

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Obesity is a growing global health issue linked to metabolic syndromes, including type 2 diabetes and cardiovascular diseases. Excessive energy intake contributes significantly to weight gain and systemic metabolic disruption. Flavonoids exert anti-obesity effects by modulating lipid metabolism, reducing oxidative stress, and suppressing chronic inflammation [1]. Myricetin, a flavonoid found in fruits such as bayberry (Myrica rubra), has been shown to possess antioxidant, anti-inflammatory, and anti-obesity properties in both in vitro and mammalian models [2].
In this study, we established a high-sugar diet (HSD) induced obesity model in Drosophila melanogaster to further evaluate the anti-obesity potential of myricetin. Fed a standard diet, HSD, or HSD supplemented with myricetin, flies of separate genders exhibited differently. Behavior assays and expression of genes related to lipid metabolism are tested. Myricetin-treated flies showed significantly reduced fat accumulation, and improved physical activity compared to the HSD group. RT-qPCR results also suggested that myricetin may enhance lipolysis and inhibit lipogenesis by regulating genes.
Together, our data suggest that myricetin effectively alleviates diet-induced obesity in Drosophila, and this is consistent with its systemic metabolic benefits observed in mammals. These results highlight myricetin's potential as a natural therapeutic candidate for obesity management.

Acknowledgments
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SL34

Cosmeceutical potential of *Aronia melanocarpa* (Michx.) Elliott fruits – preliminary identification of active fractions

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Juice and extracts obtained from the fruits of black chokeberry (*Aronia melanocarpa*) are known as a rich source of bioactive compounds for food, pharmaceutical and cosmetic products, with various health-promoting properties. [1] Despite the abundance of scientific data confirming multi-directional action of *A. melanocarpa* extracts, there is still insufficient research connecting particular biological activity with isolated chokeberry compound and exploring the potential of these ingredients for developing cosmeceuticals. [2] In this study we aimed to implement Centrifugal Partition Chromatography (CPC) to fractionate a hydroethanolic extract from *A. melanocarpa* fruits and explore the antioxidant, anti-elastase and anti-tyrosinase potential of the obtained fractions. Twelve CPC fractions (FR1-FR12) were analysed using Thin Layer Chromatography (TLC) and compared for their DPPH scavenging activity, elastase inhibitory potential and inhibitor of tyrosinase. Cytotoxic potential was evaluated *in vitro* using HaCaT keratinocytes and Neutral Red Uptake Test. The study identified fractions FR8 and FR3 as the most active antioxidants (EC_{50} 0.22 and 8.15 µg/mL, respectively). Fractions FR3 and FR6 were the most potent inhibitors of elastase, decreasing its activity >20% at 0.1 mg/mL. The most effective inhibitors of tyrosinase were fractions FR5 and FR5, showing >50% inhibition at 0.1 mg/mL. In all performed assays the activity of the mentioned fractions was significantly higher than the activity of the whole extract. The extract and fractions were also not significantly cytotoxic to HaCaT cells. The obtained preliminary results confirm that the fractionation for *A. melanocarpa* fruit extract using CPC allows better exploration and utilization of the cosmetic potential of this valuable raw material.

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SL 35

Effects of resveratrol on *Drosophila* lifespan: influence of gender and developmental stage

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Resveratrol, a natural polyphenol with antioxidant and anti-inflammatory properties, has been linked to anti-aging and health benefits in various organisms, including *Caenorhabditis elegans* and *Drosophila* [1]. However, its effects across different life stages remain unclear. This study used *Drosophila* to investigate resveratrol's impact at various developmental stages and sexes. Resveratrol, dissolved in ethanol, was added to Drosophila culture medium at 10, 50, and 100 μg/mL. Growth, development, and lifespan were analyzed in eggs and adult flies (mated and unmated). Results showed that 10 and 50 μg/mL resveratrol accelerated egg development, pupation, and eclosion. However, flies developed from resveratrol-treated eggs had a shortened lifespan when later cultured in normal medium. Conversely, continuous exposure to 50 μg/mL resveratrol extended the lifespan of mated adult flies. When male and female flies were separately cultured without mating, resveratrol had no significant effect on male lifespan, while 10 and 50 μg/mL extended female lifespan. These findings suggest that resveratrol exposure during early development may negatively impact adult lifespan, potentially due to endocrine disruption, such as acting as an ecdysteroid antagonist [2]. However, appropriate concentrations can extend the lifespan of mated adult flies and unmated females treated from egg to adulthood, likely through complex interactions involving metabolism, hormones, gene expression, and signaling pathways [3].

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SL 36

Coast plant extracts as a rich source of skin protecting ingredients

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Coastal plants are a group of relict plants occurring in areas with a high content of easily soluble salts. Due to the environmental conditions in which they exist, these plants are exposed to abiotic stresses, including high soil salinity, strong wind, excessive sunlight. Therefore, they had to adjust to live in extreme environmental conditions by developing adaptive mechanisms. The most significant feature is increased biosynthesis of secondary metabolites such as polyphenols, flavonoids and carotenoids. The enhanced content of active components makes coastal plants a remarkable raw material as ingredient of nutraceuticals or cosmetic preparations [1].

The aim of the presented research was to compare skin protecting potential of aqueous and hydroethanolic (70%, *v/v*) extracts from the leaves and flowers of costal plants grown in Croatia: sea fennel (*Crithmum maritimum* L.) and sea lavender (*Limonium vulgare* Mill.).

All extracts have been analyzed for antioxidant properties using the DPPH radical scavenging method. Total polyphenols and flavonoids content was determined using spectrophotometric methods with Folin-Ciocalteu and sodium carbonate or aluminium nitrate and potassium acetate, respectively. The anti-pigmentation potential was determined using tyrosinase inhibitory assay and *in vitro* cytotoxicity was assessed using human epidermal keratinocytes HaCaT.

The extracts of sea lavender showed higher cosmetic potential than extracts of sea fennel. Hydroethanolic extracts from sea lavender flowers and leaves showed the greatest antioxidant capacity as they neutralize ca. 50% of DPPH radical at 0.008 mg/mL. These extracts contained also the highest levels of polyphenols (886.149 and 684.324 µg GAE/mL for flowers and leaves, respectively) and flavonoids (209.65 and 210.77 µg QE/mL for flowers and leaves, respectively). Hydroethanolic flower extract from sea lavender showed significant tyrosinase inhibitory activity (at 0.5 mg/mL reduced the activity of tyrosinase by 83.7%). The highest cytotoxicity was observed for flower extracts of sea fennel (cell viability reduced by ca. 34% at 0.2 mg/mL). Aqueous sea lavender extracts and hydroethanolic flower extract showed similar cytotoxicity (cell viability reduced approximately by ca. 30% at 0.2 mg/mL).

Based on obtained results it can be concluded that extracts from sea lavender have a high active potential on the skin and can be used as active ingredients in care and protective cosmetics.

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SL 37

Grape seed polyphenol extract enhances antioxidant stress response and prolongs life in *Caenorhabditis elegans*

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Grape seed extract (GSE), rich in polyphenolic compounds such as catechins and proanthocyanidins, exhibits strong antioxidant potential. This study explored the protective effects of GSE on oxidative stress resistance and longevity in *Caenorhabditis elegans*. Worms were treated with 100 and 500 µg/mL GSE from L1 to adulthood. *In vitro* assays confirmed enhanced DPPH and ABTS radical scavenging. *In vivo*, 500 µg/mL GSE significantly increased median lifespan by 16.67%, improved thermotolerance by 17.65%, and promoted body growth and motor activity. Fertility remained unaffected, suggesting non-toxic longevity benefits. Biochemical assays showed elevated SOD, CAT, and GSH-Px activities, with reduced MDA levels, indicating enhanced antioxidant defenses. RT-qPCR revealed upregulation of *skn-1*, *daf-16*, *sod-3*, *gst-4*, and *hsp-16.2*, pointing to activation of stress response pathways. Notably, GSE also increased expression of *set-2*, suggesting epigenetic regulation via H3K4 methylation. GC-MS-based metabolomics revealed GSE-treated worms exhibited altered amino acid metabolism, including elevated levels of glutamine, glutamate, and glycine—precursors for glutathione synthesis—alongside reduced pyruvate and succinate, indicating improved mitochondrial function and reduced metabolic stress. Increased norvaline and phenylalanine levels further suggested modulation of nitrogen metabolism and antioxidant pathways.

Collectively, these findings demonstrate that GSE confers significant anti-aging effects in *C. elegans* through coordinated activation of antioxidant enzyme systems, transcriptional upregulation of stress-responsive genes, epigenetic modulation, and metabolic reprogramming. This study provides mechanistic insights into GSE's bioactivity and supports its potential as a dietary polyphenol for managing oxidative stress-related aging and metabolic decline.

SL 38

Research on the effects of *Myrtus communis* L. used in Traditional Anatolian Medicine on Parkinson's pathology

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Myrtus communis L. is used in Traditional Anatolian Medicine for diabetes, hypercholesterolemia, and wound healing. The effects of *M. communis* leaf extracts and fractions on oxidative stress, inflammation, and *α*-synuclein fibrils associated with Parkinson's pathology were investigated *in vitro*. *M. communis* was examined anatomically and morphologically for pharmacopoeial quality. Antioxidant activity tests, including ABTS, CUPRAC, DPPH, FRAP, and NO; enzyme inhibition tests, including elastase, collagenase, and tyrosinase, were applied to 80% EtOH and aqueous extracts and subfractions. Metabolomics analysis supported the selection of the most appropriate extracts and subfractions for chromatographic methods. Six pure compounds isolated from 80% EtOH extraction of the plant leaves by chromatographic methods were determined as myrcitrin, quercitrin, ursolic acid, corosolic acid, asiatic acid, and gallomirtukommulon C by 1D-, 2D NMR and HR-MS methods. The effects of 80% EtOH and aqueous extracts, subfractions, and pure compounds on cell viability in the SH-SY5Y cell line were investigated. The effects of extracts and subfractions on low and high-molecular-weight *α*-synuclein fibril formation were tested by the Western blot method. Finally, the effects of isolated compounds on *α*-synuclein related to Parkinson's pathology were analyzed by the *in silico* molecular docking method.

All samples showed antioxidant properties; however, the extracts are much more active than the subfractions. None of the samples inhibited the elastase enzyme, while the collagenase enzyme inhibition test results showed that the highest activity was in the aqueous extract. In the results of the tyrosinase enzyme inhibition test, the highest activity was seen in the 80% EtOH extract. It was determined by Western blot method that MCE-Fr. E, which is rich in terpenic compounds, was the most effective sample in reducing *α*-synuclein fibrils. In computational investigation the best score was provided by corosolic acid (-10.04 kcal/mol), a triterpenoid.

Neuroprotective activities of triterpenoids have been observed in different studies. In the light of these data, it has been predicted that it would be appropriate to develop formulations that will have specific effects on the target protein during the product development process using the mentioned fractions and pure compounds.

SL 39

Effects of the aspartame on growth, development, immunity and neurological function of *Caenorhabditis elegans*

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The rising global obesity rates and increasing prevalence of diabetes have led to the widespread use of artificial sweeteners as sugar substitutes. Artificial sweeteners provide sweetness with minimal or no caloric intake, making them popular in sugar-free beverages, low-calorie foods, and dietary management for individuals with diabetes. However, concerns regarding their potential impacts on metabolic health, gastrointestinal disorders, and carcinogenesis have sparked extensive discussion. In this study, we investigated the effects of aspartame (ASP), one of the most commonly used artificial sweeteners, on the growth, immune function, and neurological responses of *Caenorhabditis elegans*. Our findings revealed that wild-type *C. elegans* exhibited a significant preference for Escherichia coli OP50 supplemented with high doses of ASP compared to OP50 alone. However, when given OP50 containing ASP and OP50 containing glucose, respectively, worms showed a strong preference for glucose. Such differences in food choice preferences are closely related to the sensory neurons of worms, and this may directly affect their food intake and growth. Morphological analysis indicated that ASP exposure significantly increased both body length and body width most of the time from the L4 stage to adulthood. In addition, the results of oil red O staining showed no significant difference in lipid accumulation between ASP and CON worms on the first day of adulthood. Therefore, the results suggested that ASP, as a non-caloric sweetener, has less risk of causing obesity after ingestion, but the increase in body length and width may be related to promoting the growth and development of worms. Additionally, ASP intake during the larval stage significantly reduced the survival rate of *C. elegans* when exposed to *Pseudomonas aeruginosa* (PA14) and exacerbated intestinal damage, as evidenced by intense blue staining of the intestinal tract. These results suggest that ASP consumption impairs the resistance of worms to pathogenic bacteria, likely due to impaired intestinal barrier. In addition, ASP exposure significantly impaired both the ability to avoid PA14 and the learned pathogenic avoidance of PA14, suggesting a potential neurotoxic effect.

Overall, we are further investigating the underlying mechanisms of these effects, particularly ASP's impact on worm immune function. Our preliminary findings suggest that these effects may be transmitted to offspring through epigenetic mechanisms, which will make people more cautious about the addition of artificial sweeteners in food.

SL 40

Nutritional, chemical, antioxidant and antibacterial screening of two *Astragalus* species

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The chemical composition and biological activity of *Astragalus* glycyphylos and *A. cicer* are scarcely investigated. In this study, the nutritional and chemical profiles of *A. cicer* and *A. glycyphyllos*, considering their different morphological parts (leaves, fruits and roots), were assessed together with their antioxidant and antibacterial potential. Our results showed that carbohydrates are the major macronutrients in both *Astragalus* species (above 62 g/100 g dry weight—DW). High amounts of ash (above 4.6 g/100 g DW) and protein (above 13.0 g/100 g DW) were also identified, particularly in leaves and fruits of *A. cicer* and *A. glycyphyllos*. Moreover, A. cicer was richer in sugars than *A. glycyphyllos*, while roots of both *Astragalus* species were the richest of fatty acids. Ten phenolic compounds were identified, with gallic acid and quercetin being predominant, above 49.84 and 37.27 μg/g DW, respectively. The mineral analysis revealed zinc and iron as the major constituents. Regarding the plants' antioxidant and antibacterial activity, both *Astragalus* species had antioxidant potential, and their water extracts showed antibacterial activity against *S. aureus* and *E. coli*. Altogether, these results provide insight into the potential of *A. glycyphyllos* and *A. cicer* as a source of nutritional benefits and active phytochemicals for many people, and they can be applied in the food sector as foods and as promising sources of natural ingredients.

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P3 **Daria Berezovska**
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P4 **Marcel Białas**
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P6 **Monika Bielecka**
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P1

Assessment of Volatile Organic Constituents of *Salvia* species by HS-SPME/GC-MS

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Salvia, the largest genus of Lamiaceae, includes about 900 species widespread throughout the world. In Flora Europea 36 taxa are described. The Lamiaceae family is rich in therapeutic and flavoring plant species with characteristics aroma. The aim of the study was to perform a comparative analysis of selected Salvia species' chemical profiles using Head Space Solid-Phase Microextraction (HS-SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS). Stems and leaves of *S. verticillata*, *S. glutinosa* and *S. nemorosa* subsp. *nemorosa* were used for the analyses. A total of 114 compounds were found in analysed plant material. Some of the commonly observed compounds were hex-(2e)-enal, *α*-thujene, *α*-pinene, benzaldehyde, sabinene, *β*-pinene, amylvinyl carbinol, hexanoic acid, purolan, *p*-cymene, limonene, *β*-phellandrene, phenylacetaldehyde, *t*-linalool oxide, linalyl anthranilate, phenethyl alcohol, *β*-copaene, *β*-*t*-bergamotene, *γ*-cadinene and allohimachalol. The results showed that stems and leaves of the selected *Salvia* species were rich in volatile organic compounds. Further analyses are necessary for the compounds isolation and the assessment of their bioactivities.

P2

Cytotoxic and Protective Activities of *A. atemoya* Crude Extracts

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Primary liver cancer ranks as the sixth most commonly diagnosed cancer worldwide and is the third leading cause of cancer-related deaths. Hepatocellular carcinoma (HCC) is the most prevalent type, accounting for 75 to 85% of liver cancer cases, and is the second leading cause of cancer-related deaths. The primary risk factors for developing HCC include chronic hepatitis B or C infections, obesity, excessive alcohol consumption, smoking, exposure to aflatoxin-contaminated foods, and type 2 diabetes. Systemic therapies for advanced HCC are required and natural products, such as herbal extracts or pure compounds have garnered attention from scientists as potential alternative anti-cancer agents. In recent years the Annona species have been the focus of much interest for their anticancer activities. *Annona atemoya* also known as the custard apple is a hybrid between two Annonaceae species: Cherimoya (*Annona cherimola*) and the sugar apple (*Annona squamosa*). It is widely cultivated in tropical and subtropical continents including north and south America, Asia, Africa and Australia. The biological activity of *A. atemoya* crude extracts were examined using Huh-7 cell line. Results from this study indicate that only two crude extracts, LH and SEY, displayed anti-cancer activity in Huh-7 cells, with IC50 values of 67.21 and 22.55, respectively. Both extracts exhibited a dose-dependent inhibition of cell viability within 24 hours, as assessed by the MTS assay. For the remaining crude extracts that did not demonstrate cytotoxic effects, their protective effects were studied against PA. Most of these extracts were found to block PA by increasing the levels of anti-apoptotic proteins like Bax and Bim, while reducing the levels of pro-apoptotic proteins such as Bcl-XL and Mcl-1.

P3

The role of *Cornus mas*, *Hippophae rhamnoides*, *Chaenomeles japonica* fruit extracts in modulation of CD73 and adenosine deaminase

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Ecto-5'-nucleotidase (CD73, EC 3.1.3.5) is a key enzyme in the extracellular adenosine pathway that catalyzes the hydrolysis of AMP to adenosine, whereas adenosine deaminase (ADA, EC 3.5.4.4) catalyzes the hydrolysis of adenosine to inosine, and overexpression of these enzymes correlates with tumor progression [1].

This study evaluates the inhibitory effects of 70% aqueous-ethanolic extract from fruits of *Cornus mas* L. (CM), and aqueous extracts from fruits *Hippophae rhamnoides* L. (HR) and *Chaenomeles japonica* (Thunb.) Lindl. ex Spach. (CJ) extracts on CD73 and ADA activity *in vitro*. The enzymatic activity was determined spectrophotometrically. CD73 activity was assayed with 5'AMP as substrate and Taussky-Shorr reagent for phosphate detection (λ =660 nm). ADA activity was assessed by the Blum and Shwed method with adenosine as substrate (λ = 625 nm).

The study showed that all three plant extracts of CM, HR, and CJ, were able to inhibit ADA and CD73 activity in vitro with a dose-dependent effect. The extract of CJ and HR had a significant inhibitory effect (>90%) on ADA, reaching at 7 mg/mL. CM fruit extract showed moderate activity, with a maximum inhibition of 94% of ADA at a significantly higher concentration (35 mg/mL), indicating its lower efficacy compared to other extracts. Moreover, at low concentrations (≤1mg/mL), CJ and HR extracts retain a noticeable inhibitory effect of ADA, while CM extract at the same concentration has almost no effect on enzyme activity. Similarly, CM showed weaker inhibitory properties towards CD73 compared to CJ and HR.

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P4

Anti-inflammatory and cytotoxic properties of *Glaucium flavum* extracts

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Glaucium flavum Crantz, commonly known as yellow horn poppy (Papaveraceae) is a coastal plant native to regions including Europe and Northern Africa (Macaronesia). Owing to its intriguing metabolic composition, extracts of G. flavum derived from *in vitro* organ cultures were evaluated for their potential anti-inflammatory and cytotoxic properties. G. flavum shoots were cultured on MH3 solid media supplemented with kinetin (KIN) at concentrations of 2.5, 4.5, and 7 µM, alongside 0.5 µM indole-3-acetic acid. Methanolic extracts from these cultures were tested for cytotoxicity and their impact on the secretion of cytokines IL-1, IL-8, and TNF-α using a human neutrophil model. For comparison, methanolic and ethanolic extracts were also obtained from the roots, stems with leaves, and flowers of plants grown in two botanical gardens (Kraków and Lublin). None of the tested extracts exhibited cytotoxic effects on neutrophil cells. The ability of the extracts to inhibit cytokine secretion was concentration-dependent and varied according to the plant part and specific cytokine examined. The stem extract from Lublin showed the most pronounced inhibition of TNF-α secretion at 1 µg/mL. In the case of IL-1 and IL-8, the most effective extracts were derived from in vitro shoots cultivated on MH3 medium with 7 µM KIN, and from shoots grown on hormone-free ½ MH3 medium, respectively, each at a concentration of 1 µg/mL. Further studies are warranted to characterize the phytochemical composition of these extracts and to identify the specific compound groups responsible for the observed biological activities.

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P5

Expression Analysis of Ribosome-Inactivating Proteins 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, the type I RIP, and seven additional RIP transcripts were identified in the transcriptome of the corn cockle[2]. However, the tissue-specific biosynthesis of RIPs has never been studied in detail in *A. githago*. To get insight into the mechanisms regulating the TTS at the transcription level, we performed expression analysis of its component, the RIPs, in various organs during plant's ontogenetic development. The plant material grown in the glasshouse of the Botanical Garden of Medicinal Plants (BGMP) at the Wroclaw Medical University (Poland) was used. Nucleotide sequences of genes encoding for RIPs 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 nine RIPs was studied.

The analysis contributed to an elucidation of the unexplored toxic synergism within plants of the carnation family. In a long run, our work paves the way for metabolic engineering of *A. githago* RIPs in heterologous systems, opening up the potential for their large-scale production and enabling this reservoir of toxic proteins to be harnessed and engineered for therapeutic applications.

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P6

Can Endophytic Fungi from *Salvia* Produce Bioactive Plant Metabolites?

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Endophytic fungi develop within tissues and organs of healthy plants without causing apparent disease symptoms. They are reported to improve plant fitness by supporting plant nutrition and participating in defensive mutualism. These fungi may also participate in metabolic pathways and boost their own natural biosynthetic activity, or may acquire some genetic information to synthesize biologically active compounds closely related to those produced by their host. With reference to the pharmaceutical importance of natural products from *Salvia*[1], we investigated the capacity by endophytic fungi recovered from *S. abrotanoides* and *S. yangii* to directly produce these compounds via *in vitro* cultures. 230 isolates of endophytic fungi were recovered from leaves, roots and stems of both *Salvia* species. Representative strains have been identified and characterized, using morphological characters and DNA barcoding. The isolates were *in vitro* cultured in the PDB. The species *Alternaria alternata*, *Boeremia exigua*, *Colletotrichum godetiae*, *Diaporthe eres*, *Diaporthe novem*, *Epicoccum nigrum*, *Fusarium acuminatum* and *Fusarium avenaceum* represent new records as endophytes of *Salvia* spp[2]. After incubation, the mycelium was separated from the culturing medium via filtering. The biomass and filtrate were analyzed alongside plant extracts using UHPLC-QTOF-MS for metabolic profiling. MetaboScape® (Bruker) software was used to verify whether the isolated endophytic fungi are able to produce plant-like metabolites, especially in the class of diterpenoids and phenylpropanoids.

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P7

Antioxidant potential of *Iris domestica* Pulse Electric Field (PEF) treatment as innovative approach for plant roots extraction

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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 model plants *Iris domestica* (syn. Belamcanda chinensis L. DC. Iridaceae), as well as parallel antioxidant potential of extracted substances. 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. We successfully extracted metabolites, plants survived this process and it could be then repeated on them. The most promising variants were treated with electric current in 3 repetitions, of which first had 75 pulses, second - 150 pulses and the third 225 electrical pulses, voltage was set to 9kV and the pulses lasted for 0.05ms. For extraction following solvents were used: methanol 50% diluted 1:10. For antioxidant potential we developed DPPH model. 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 compounds were identified in qualitative and quantitative manner. The main component – irigenin, was identified in all samples wit concentration range from 0.0088mg/mL to 0.032 mg/mL.

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P8

Amino acid based derivatives of Chrysin and evaluation of antifungal activity

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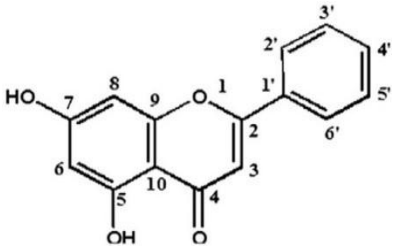

Propolis is a plant-based honey bee-derived resinous product with numerous health benefits, which is the primary effect of its complex chemical constitution. Propolis is rich in polyphenols and flavonoids which are secondary plant metabolites. Chrysin is one of the abundant flavonoids in poplar propolis with known pharmacological activities. However, its antimicrobial activity is seldom studied due to its poor solubility and low bioavailability. Derivatisation of the molecule is a potential solution to overcome these properties. This study focuses on the development of amino acid derivatives of chrysin and the assessment of its antifungal activity. Amino acid derivatives are known for their biological activities and improved water solubility. Selected amino acid derivatives were synthesised through selective esterification on C7 hydroxyl groups of chrysin. The antifungal activity of the obtained compounds was evaluated against *Candida albicans* and *Candida glabrata* keeping activity against chrysin as the reference.

P9

Impact of edible fruit extracts on bacterial nucleases *in silico* and gut microbiota *in vitro*

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The extracts from fruits of *Hippophaë rhamnoides* L. (HRE), *Chaenomeles japonica* (Thunb.) Lindl. ex Spach (CJE), and *Cornus mas* L. (CME) are inhibitors of hydrolases, such as pancreatic lipase and *α*-amylase [1]. In this study, we raise the question of whether the constituents of these plant materials influence i) bacterial hydrolases participating in the replication of nucleic acids, ii) the metabolic activity of bacterial biofilm of selected microbial strains; iii) the gut microbiota composition.
The study aimed to evaluate the binding mode of the most abundant constituents of HRE, CJE, and CME with the bacterial nucleases in silico. The molecular docking study of isorhamnetin glycosides, procyanidins B1 and B2, catechin/epicatechin [1], loganic acid, and cornuside [2] with bacterial enzymes was performed. Endonuclease 1 (EC 3.1.21.1), colicin E9, and ribonuclease H (EC 3.1.26.4) from *Escherichia coli* were considered. The second aim was the in vitro evaluation of the influence of the CJE, HRE, and CME on the metabolic activity of bacterial biofilm and suspensions of selected microbial strains (*E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Lactobacillus reuteri*) and gut microbiota composition with next-generation sequencing of bacterial 16S rRNA.
Most virtual hits were predicted to inhibit the proteins under investigation, except for procyanidin C1. The most relevant flexibility of binding mode with *E. coli* endonuclease 1 was observed in the case of procyanidin B1. The docking score values obtained for the same protein varied significantly depending on the compound. This suggests that the antimicrobial activity exhibited by the plant extracts results from the additive action of multiple components. This preliminary research did not reveal significant changes in gut microbial populations between extracts. The impact of CME and CJE on gut microbiota was tested for the first time, and further research is still required.

Acknowledgements
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P10

Biologically active compounds of parasitic plants of the Orobanchaceae family

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The presented work is focused on botanical and phytochemical characteristics of parasitic plants of the Orobanchaceae family to enlarge a knowledge of the chemical composition of parasitic plants and try to identify their constituents that have not been determined yet.
Five parasitic plants of the mentioned family were chosen for the phytochemical analysis. The main subject of investigation was holoparasitic species *Lathraea squamaria* L., including both of its subspecies. Four hemiparasitic plant species, *Euphrasia rostkoviana* Hayne, *Melampyrum nemorosum* L., *Melampyrum sylvaticum* L. and *Rhinanthus minor* L. were chosen for comparison of their chemical composition. We focused especially on the compounds of iridoid and phenolic structure. Prepared extracts of individual plant parts were analyzed by means of HPLC. The determination of content substances was carried out using available spectral methods (UV, IR MS, NMR). Isolation of chosen compounds was performed using preparative liquid chromatography.
The presence of the iridoid glycoside aucubin, that has chemotaxonomical significance for these types of plants, was confirmed in all investigated parasitic species. Free benzoic acid was identified in all plant parts of *L. squamaria* for the first time, in smaller amount it was found out in extracts of three poloparasitic plants, except for eyebright (*Euphrasia*). Another new finding is the presence of phenylethanoid glycoside acteoside and its isomer isoacteoside in both subspecies of holoparasitic toothwort. These compounds were determined also in two hemiparasitic species *E. rostkoviana* and *R. minor*. In addition to the mentioned phenylethanoids we isolated and identified the non-glycosidic iridoid compound scyphiphin B_1 and other iridoid aglycones from the underground part of *Lathraea squamaria* subsp. *squamaria* L. None of these substances have been determined in this species to date.

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P11

Impact of altered ethylene production on phytohormonal profiles and stress resilience in *Arabidopsis* mutants

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Ethylene is a potent phytohormone that plays a pivotal role in regulating essential plant processes, including fruit ripening and senescence. In the realm of plant biology, ethylene significantly influences growth, development, and stress responses. By modulating gene expression and signaling pathways, ethylene enhances plant resilience and adaptability, thereby affecting overall plant fitness (Lin et al., 2009).

This study investigates the impact of altered ethylene production in *Arabidopsis* mutants (ethylene overproducer *eto2* and ethylene limited biosynthesis *aco2aco3* mutants) on the profiles of various phytohormones under abiotic stress conditions, specifically heat shock, salinity stress, and their combination. We employed a validated method to accurately measure levels of 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor, along with other phytohormones. This approach allowed us to assess the interactions between ethylene and other hormonal signals under stress conditions.

Our study demonstrates significant interactions among the ACC, abscisic acid (ABA), salicylic acid (SA), cytokinin, and other phytohormones. Stress-induced changes in ACC levels resulted in increased response to heat shock in Col-0 and *aco2aco3*, but not for the *eto2* mutant, and showed similar increases under salinity stress and combined salinity and heat shock conditions across Col-0, *aco2aco3,* and *eto2* seedlings. SA levels exhibited a complex pattern: during heat shock stress, SA levels rose in 12-day-old plants but decreased in 14-day-old plants. In contrast, the *eto2* mutant displayed a decrease in SA at 12 days and an increase at 14 days. Under salinity stress and combined stress conditions, SA levels consistently decreased across Col-0, *aco2aco3,* and *eto2*, which presents intriguing insights into their stress adaptation mechanisms.

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P12

Comparative antimicrobial evaluation of *Origanum vulgare* leaf and rhizome extracts via isothermal microcalorimetry

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Origanum vulgare L. (oregano) is traditionally valued for its aromatic and therapeutic properties, yet its underground parts remain underexplored. This study presents a comparative analysis of water extracts prepared from oregano leaves (OVL) and rhizomes (OVR), focusing on their chemical composition, cytotoxicity, and antimicrobial properties. High-performance liquid chromatography coupled with mass spectrometry (LC-DAD-MS/MS) identified key phenolic compounds. Quantitative analysis confirmed significant differences in the phytochemical profiles between leaves and rhizomes. Cytotoxicity was assessed using the MTT assay on human keratinocyte HaCaT cells, revealing that both extracts reduced cell viability in a dose-dependent manner. Antimicrobial activity was initially determined by broth microdilution, indicating potent effects against methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* strains with minimal inhibitory (MIC) and minimal bactericidal (MBC) concentrations equal to 2.5 mg/mL for both bacterial strains and both extracts. Isothermal microcalorimetry (IMC) was employed to monitor bacterial metabolic activity in real time. IMC data demonstrated a marked inhibition of MRSA and MSSA growth by OVR at significantly lower concentrations compared to OVL. Results were expressed as the time to peak and total heat values. These results highlight oregano rhizomes as a promising and sustainable source of bioactive compounds and validate isothermal microcalorimetry as an advanced, sensitive method for real-time assessment of antimicrobial efficacy.

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P13

GreenPharming: CO_2-neutral biomanufacturing of therapeutic proteins using tobacco plants

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Research and society are confronted with the challenge of finding resource-efficient solutions for the production of biological pharmaceuticals. Currently most recombinant therapeutic proteins are produced in mammalian cells. This expression system harbors the risk of contamination with human pathogens and is cost-intensive. Plants offer a more efficient protein production process with lower cultivation needs, easier scalability, and an enhanced flexibility for tailoring processes for the production of various therapeutic proteins.
The project "GreenPharming" refers to the plant-based production of active ingredients for drug manufacturing. The project aims to enhance the resource efficiency of therapeutic protein production. By integrating this process into a circular economy, its sustainability will be promoted and its environmental impact will be reduced.
In the current project, various pharmaceutical proteins, e. g. interleukins and target modules for adapter CAR-T-cells, will be produced in tobacco plants. The data presented here focus on vector construction, plant cultivation and optimization of the infiltration procedure.
Different strains of *Agrobacterium tumefaciens* were used to establish transient expression in tobacco (*Nicotiana benthamiana*) for the production of the respective recombinant proteins. The cloning process was carried out using the GreenGate system [1]. Our constructs contain reporter (Myc-tag) and purification tags (His-tag), which can be cleaved off without leaving any residue. Vacuum infiltration was performed on 6-7-week-old tobacco plants, and various infiltration buffers and conditions were tested to optimize the process. Additionally, different mechanical extraction methods and buffer systems were evaluated.
The biomass production of the plants at 14 h versus 16 h illumination at approx. 6000 to 8000 lux and 24°C was examined. Tween 20 turned out to be an unsuitable surfactant for the infiltration buffer. However, 0.01% Silwet-L77 led to improved infiltration. The absolute infiltration pressure (100 to 800 mbar) strongly influenced the infiltration success, but could also be overdosed and thus lead to massive plant tissue damage.
The results presented here serve as a basis for defining cultivation conditions in an S1 greenhouse, where the trial concept will later be implemented in a CO_2-neutral circular economy process. Future experiments will focus on the purification of the therapeutical proteins and the analysis of their biological activity.

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P14

Chlorinated and brominated flavone derivatives: dual action as anticancer agents and membrane modulators

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Halogenated flavone derivatives are emerging as promising anticancer candidates due to their ability to interfere with cellular signaling and membrane integrity. Flavones, naturally occurring compounds with diverse biological activities, can be structurally modified to enhance pharmacological properties. Introducing halogen atoms such as chlorine or bromine increases lipophilicity, improves membrane permeability, and strengthens interactions with biological targets. Halogenation often enhances metabolic stability and binding affinity, as seen in many clinically used drugs. In this study, we investigated two derivatives, 6,8-dichloroflavone and 8-bromo-6-chloroflavone [1], for their anticancer potential against canine cancer cell lines, a model for translational oncology. Cytotoxicity assays showed IC_{50} values of 38–78 µM after 72 hours of incubation in CLBL-1 (B-cell lymphoma), GL-1 (B-cell leukemia), and CLB70 (chronic B-cell leukemia) cell lines. Flow cytometry demonstrated early apoptosis induction in CLBL-1 and CLB70 cells. In the presence of the compounds, increased expression of H2AX, a histone variant and marker of DNA double-strand breaks was observed. Additionally, analysis of apoptotic markers revealed decreased expression of the anti-apoptotic proteins Bcl-2 and Bcl-XL, supporting apoptosis induction. Biophysical studies using model lipid bilayers mimicking cancer cell membranes confirmed, via ATR-FTIR spectroscopy, that the compounds interact both at the membrane surface and within the hydrophobic core. Our findings highlight the potential of halogenated flavone derivatives as dual-function anticancer agents, targeting both intracellular signalling and membrane structures, thus offering new avenues for anticancer drug development.

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P15

Xanthones from *Maclura pomifera* and their electron absorption spectra

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Xanthones, as secondary metabolites with significant biological activity, are present in only a few plant families. *Maclura pomifera* (Moraceae) appears to be a relatively rich source of these compounds. In the course of our work to date, twelve xanthones, mostly prenylated, have been isolated from stem and root bark. The isolation of these compounds was based on extraction and chromatographic methods such as ultrasonic extraction, liquid/liquid extraction as well as column chromatography, preparative TLC and semi-preparative HPLC. Subsequently, the substances were identified using TLC, analytical HPLC, MS and 1D and 2D NMR experiments.
The major compound in the root cortex appears to be macluraxanthone, whereas in the stem bark it is mainly toxyloxanthone C.
Structurally, the isolated xanthones can be divided into several groups according to the presence and mode of attachment of the prenyl substituent, the position of the hydroxyl groups or the relative position of the rings in the xanthone skeleton.
In this context, UV electron absorption spectra were evaluated, not only for future identification of the compounds in the extracts but also trying to find a possible relationship between the structure and a particular modification of the spectrum. Already with this set of substances, it appears that it is possible to observe certain dependencies and changes according to the structure visible at the first sight on the shape of the given spectrum.
The results of this analysis are useful for future faster identification of other isolated xanthones or for selection of compounds suitable for separation, especially together with data on various biological activity of these substances.

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P16

UNCHARTED TERRITORY
Biologically active compounds among the bryophytes

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Bryophytes, a diverse group of non-vascular plants, comprise three major clades: approximately 14,000 species of mosses (*Bryophyta*), 6,000 species of liverworts (*Marchantiophyta*), and around 300 species of hornworts (*Anthocerotophyta*). Despite lacking sophisticated physical defense structures, bryophytes are rarely targeted by herbivores or pathogens. Instead, they rely on a wide array of secondary metabolites that perform various ecological and protective roles, including phytotoxic, antibacterial, antifungal, insect antifeedant, and molluscicidal activities. In addition to defense, these secondary metabolites also enhance the plants' ability to withstand environmental stressors, offering protection against ultraviolet radiation, drought, and freezing temperatures. Historically, bryophytes have played a role in traditional medicine across many cultures. Indian and Chinese systems of medicine, in particular, have used them to treat conditions ranging from fevers and skin infections to pain relief.

While modern scientific analysis has revealed that some species once considered medicinal may be ineffective or even toxic, numerous others have demonstrated significant therapeutic potential. Confirmed bioactivities include antiseptic, anti-cancer, enzyme-inhibitory, antioxidant, anti-HIV-1, neurotrophic, cardiotonic, and muscle-relaxing effects. Some compounds found in bryophytes have also shown promise as plant protection agents due to their selective toxicity against pests.

As global concerns about antibiotic resistance and the need for new therapeutics grow, bryophytes are attracting increased scientific attention. Researchers continue to discover novel and unique bioactive compounds within this plant group, highlighting their untapped potential in medicine, agriculture, and biotechnology.

P17

Yeast extract and mannitol elicitation effect on the *Agrostemma githago in vitro* cultures

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Corncockle (*Agrostemma githago*) is an annual flowering plant native to Europe. Once a widespread weed in grain fields, particularly wheat, it is easily recognized by its tall, slender stems and vibrant pink to purple flowers. Despite its ornamental appeal, corncockle is toxic if ingested, as all parts of the plant contain poisonous compounds. Modern agricultural practices, especially seed cleaning and crop management, have led to a significant decline in its occurrence in the wild.
The objective of this experiment was to examine the effects of different elicitors on the morphological development of *A. githago*. Two-node seedlings served as the initial plant material and were transferred to Murashige and Skoog (MS) medium adjusted to pH 5.8. The medium was supplemented with either yeast extract or mannitol as elicitors. Yeast extract was applied at concentrations of 125, 250, 500, and 1000 mg/L, while mannitol was used at 50, 100, 200, 400, and 800 mM. The MS medium without additives functioned as the control.
All cultures were maintained in a phytotron chamber under controlled conditions: a temperature of 25 ± 2 °C, an 18h light/6h dark photoperiod, and illumination provided by white LED lighting. Samples were collected at four time points: 2, 7, 14, and 28 days after treatment. Morphological parameters assessed included stem and root length, fresh weight, and the number of stems, nodes, leaves, and roots. The presence of flowers and callus formation was also recorded. After collection, plant material was stored at -80 °C for subsequent phytochemical and molecular analyses.

P18

Biological activities and NMR structure elucidation of the Ent-labdane diterpenoids from leaves of *Andrographis paniculata*

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Antimicrobial resistance (AMR) is a major threat to global health. The World Health Organization stated multi-drug resistant (MDR) bacteria as one of the top three major public health threat [1]. It was projected that by 2050, about 1.91 million deaths as a result of AMR will be recorded [2]. This necessitates the search for and assessment of new antimicrobial agents effective against MDR bacterial strains. Plants being an excellent source of antimicrobials have been used for the management of bacterial diseases for several years. Developing natural antibacterial agents from plants requires the identification and characterization of these chemicals. In view of this, this study aims to evaluate the antimicrobial activity of *Andrographis paniculata*, a member of the Acanthaceae family, traditionally used in the treatment of dysentery, skin infections, respiratory tract infections etc. [3].

Methanol crude extracts of the leaves of *A. paniculata* collected in Nigeria was tested against different microbial strains. The methanol crude extract that exhibited 50% growth inhibition against only *Candida albicans*, out of the microbial strains tested, was split by liquid-liquid separation in an apolar (n-hexane) and a medium-polar (dichloromethane) fractions. Following a bio-guided approach the dichloromethane fraction has been selected based on their highest activity against *C. albicans*.

In order to isolate and identify the compounds likely responsible for the observed activity, the dichloromethane extract was subjected first to SiO_2 column chromatography, followed by preparative thin-layer chromatography (TLC) which led to the isolation of four ent-labdane diterpenes. The chemical structures of these compounds were established by spectroscopic analyses, including ^{1}H, ^{13}C, and 2D NMR. The isolated pure compounds were tested for their antimicrobial activity against different microbial strains.

In conclusion, the fight against antimicrobial resistance is an ongoing battle that can only be won by continuous search and development of novel drug candidates from natural sources.

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P19

Methyl jasmonate effect on rosmarinic acid production in *Salvia atropatana* shoot culture

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Salvia atropatana is a medicinal plant from the Lamiaceae family, native to Middle Eastern countries. It exhibits antioxidant, antimicrobial, and anticancer properties and is traditionally used to treat inflammation, infections, wounds, and diabetes. *S. atropatana* contains a wide range of polyphenolic compounds, with rosmarinic acid (RA) being the principal bioactive metabolite.
In our experiment, to stimulate RA production, *S. atropatana* cultures were treated with methyl jasmonate (MJ) at two concentrations (50 and 100 µM). The elicitor was added on day 30 of culture to shoots cultivated in liquid Murashige and Skoog (MS) medium supplemented with 0.1 mg/L indole-3-acetic acid (IAA) and 1 mg/L 6-benzylaminopurine (BAP). Control cultures consisted of explants maintained under identical conditions with the addition of ethanol, the MJ solvent. Plant material was harvested at 1, 3, 5, and 7 days post-elicitation. Shoot biomass (fresh and dry weight) and RA content, determined *via* high-performance liquid chromatography (HPLC), were evaluated.
The elicitor's impact on shoot growth and rosmarinic acid (RA) accumulation was influenced by both its concentration and the duration of exposure. A higher methyl jasmonate (MJ) concentration (100 µM) suppressed shoot growth, with the most significant inhibition observed on day 3 post-treatment. Maximum shoot biomass was recorded across all treatments on day 5, with fresh weights ranging from 4.8 to 4.9 g and dry weights between 0.38 and 0.40 g per explant. Following this peak, biomass declined significantly in MJ-treated cultures, a trend not observed in the control group. RA levels in MJ-treated shoots began to rise significantly from day 3 post-treatment, peaking at 52.6 mg/g dry weight with 50 µM MJ on day 5, and at 53.5 mg/g dry weight with 100 µM MJ on day 7. Considering both biomass accumulation and RA content, the highest productivity was achieved with 50 µM MJ on day 5, yielding 805.3 mg RA per liter of culture medium - approximately 20% higher than the control. These findings suggest that elicitation with MJ can enhance RA production in *S. atropatana* shoot cultures, with optimal results achieved by balancing concentration and exposure time.

P20

The production of phenolic diterpenes and phenolic acids in hairy root culture of *Salvia atropatana*

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Salvia atropatana L. (Lamiaceae) is widely used in Turkish and Iranian folk medicine for the treatment of inflammation, infectious diseases, digestive system disorders, spastic conditions, and diabetes. The plant's biological activity has been attributed to polyphenolic acids, diterpenes, and essential oil. Plant biotechnology can be used to increase the production of bioactive compounds, with transformed roots being a particularly promising material. They offer high production of secondary metabolites, fast growth, genetic stability and the ability of growth without the addition of growth regulators, which reduces the costs of the culture.

The aim of the study was to determine the phytochemical profile of hydromethanolic extract of *S. atropatana* hairy roots and to select root clone exhibiting the highest content of bioactive metabolites.

Following infection *of S. atropatana* explants with the bacterial strain *Rhizobium rhizogenes* A4, twelve hairy root clones (designated C1–C12) were obtained, and their transformed nature was confirmed by PCR (polymerase chain reaction). Qualitative and subsequent quantitative analyses of hydromethanolic extracts from these clones were performed using high-performance liquid chromatography.

The qualitative analysis of extracts by UPLC-PDA-ESI-MS enabled the identification of 23 compounds, including 11 polyphenolic acid derivatives and 12 phenolic diterpenes. Among the polyphenolic acids, rosmarinic acid (RA) was the predominant compound. The RA content in the analyzed clones ranged from 14.9 to 35.4 mg/g dry weight (DW), with the highest concentration observed in C2 and the lowest in C10. The total polyphenol content in the roots of clone C2 exceeded 40 mg/g DW, whereas in clone C10 it was more than twofold lower. Additionally, eleven of the detected phenolic diterpenes were identified as isomers of carnosic acid and carnosol. Quantitative analysis of these metabolites in the hairy root clones is currently in progress.

The profile of secondary metabolites identified in the hairy roots of *S. atropatana*, as well as their content, suggest that these cultures possess promising pharmacological potential.

P21

Polygoni cuspidati rhizoma et radix decoction as a potential chemopreventive agent

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Colorectal cancer (CRC) poses a significant global health burden, with increasing incidence and growing resistance to standard chemotherapeutics such as doxorubicin. This study evaluated the chemopreventive potential of water-based decoctions prepared from Polygoni cuspidati rhizoma et radix (PC) a traditional medicinal plant widely used in East Asian medicine on normal colon epithelial cells (CCD 841) and two colorectal cancer cell lines: LoVo and the doxorubicin-resistant LoVo/Dx. Two types of decoctions were analyzed: one derived from pharmacopoeial-grade material (PH-PC) and another from self-collected raw material (SC-PC), along with their respective polysaccharide fractions. Cytotoxicity was assessed using MTT and SRB assays. The self-collected *Polygonium cuspidatum* (SC-PC) decoction demonstrated the most favorable cytotoxic profile, showing low toxicity towards normal cells (IC_{50} = 3564.4 µg/mL) and strong activity against LoVo (IC_{50} = 40.6 µg/mL) and LoVo/Dx cells (IC_{50} = 154.0 µg/mL). Among reference compounds, resveratrol and polydatin were effective against cancer cells but also moderately toxic to normal colon cells. The SC-PC decoction exhibited a high therapeutic index (TI = 87.8 for CCD 841/LoVo), indicating promising selectivity. Pro-apoptotic effects were confirmed by fluorescence microscopy, with SC-PC fractions inducing apoptosis in both drug-sensitive and resistant cancer lines.
Our study showed that a water decoction prepared from self-collected rhizomes of *Polygoni cuspidati* rhizoma et radix exhibited selective cytotoxicity toward colorectal cancer cells, while showing low toxicity to normal colon epithelial cells. These findings suggest that plant-derived compounds may offer a favorable safety profile while maintaining anticancer activity. This makes them promising candidates for colorectal cancer treatment. Further research is needed to evaluate in vivo efficacy and bioavailability.

P22

Polygoni cuspidati rhizoma et radix decoction as a regenerative agent in gingival fibroblast models

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The aim of this study was to obtain polysaccharide-rich decoctions from Polygoni cuspidati rhizoma et radix, isolate their polysaccharide fractions, and evaluate their phytochemical composition and regenerative potential. Despite the known anti-inflammatory and antioxidant properties of this pharmacopoeial herbal raw material, little is known about the biological effects of its polysaccharides. Therefore, we investigated their impact on gingival wound healing, with the goal of assessing potential applications in regenerative dentistry.

Decoctions were prepared from two sources: pharmacopoeial-grade material (PH) and self-collected material (SC). Polysaccharide fractions (POS) were isolated using graded ethanol precipitation and characterized using HPLC-MS and FT-IR. The decoctions and fractions were tested in vitro on human gingival fibroblasts (HGF-1). Viability (MTT assay), proliferation marker expression (Ki-67), cell cycle distribution (Muse® Cell Cycle Kit), and apoptotic activity (Muse® Annexin V & Dead Cell Kit) were assessed. Genotoxicity and antioxidant properties were also evaluated under oxidative stress (H_2O_2) using flow cytometry.

The results showed that decoctions and fractions increased HGF-1 viability and Ki-67 expression in a concentration-dependent manner. Notably, the POS1 fraction, rich in high-molecular-weight polysaccharides, showed the most significant enhancement of cell proliferation and S-phase cell cycle entry. Cytoprotective effects were confirmed by reduced apoptosis and DNA damage under oxidative stress, particularly in samples SC POS3 and PH POS2. Spectroscopic analyses confirmed the presence of pectins, xylans, glucomannans, and xyloglucans. These findings suggest that decoctions and polysaccharide fractions from P. cuspidatum possess wound-healing and antioxidant potential, which may be relevant for future applications in regenerative therapies. Further investigation is needed to elucidate the precise mechanisms and therapeutic relevance of these plant-derived polysaccharides.

P23

Structural elucidation of phenolic compounds from edible Apiaceae plants: NMR Challenges and Insights

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In this study, we investigated aqueous and methanolic extracts of three edible Apiaceae species – *Anthriscus cerefolium* (L.) Hoffm., *Anthriscus sylvestris* (L.) Hoffm., and *Chaerophyllum bulbosum* L. – to isolate and characterize bioactive phenolic metabolites. We identified four flavonoid glycosides (including one novel compound) and five previously undescribed caffeoylquinic acid derivatives.

Structural elucidation relied on advanced NMR spectroscopy but faced challenges such as: rapid hydrogen–deuterium exchange at malonyl methylene sites, reduced resolution caused by conformational flexibility, and subsequent inaccuracies in coupling constants. Additionally, solvent-induced changes in conformation altered splitting patterns and coupling values, making signal interpretation difficult.

To address these challenges, we employed a combination of 1D and 2D NMR techniques. Particular attention was paid to the splitting patterns of H-3, H-4, and H-5 protons in the quinic acid core, which were essential for determining relative configuration. These NMR strategies enabled confident differentiation of isomers and substitution motifs, including malonyl and caffeoyl groups.

Biological activity was assessed using DPPH radical scavenging and cytotoxicity assays on Vero E6 cells. While most compounds exhibited antioxidant activity, two dicaffeoylquinic acids showed notable cytotoxicity (IC_{50} < 10 µM), indicating a structure–activity relationship that may limit their dietary or therapeutic use.

Overall, this study highlights the role of NMR spectroscopy in elucidating structurally complex natural products and demonstrates how precise NMR analysis can uncover subtle features critical for understanding biological activity.

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P24

Optimalization and evaluation of semi-solid magistral formulations based on *Cannabis* extract for use in individualizing therapy in particular for oncology patients

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The skin is promising application place for the delivery of cannabinoids from semi-solid drug forms due to the reduction of psychoactive effects, adverse effects, and interactions with concomitant chemotherapy compared to other routes of administration [1]. Additionally, cannabinoids interact with CB1, CB2, and TRP receptors of the endocannabinoid system, modulating cell proliferation, differentiation, apoptosis, and the expression of pro-inflammatory cytokines in the skin. Extensive research has shown the potential benefits of topical formulations containing [2]. *Cannabis* extracts in promoting wound healing (including post-radiotherapy wounds), managing psoriasis, and reducing cutaneous inflammation. Additionally, these formulations exhibit analgesic properties in neuropathic pain and in cancer patients undergoing chemotherapy, radiotherapy, or cancer treatment itself [3]. In Poland, physicians prescribe compounded dermatological formulations containing oil-based extracts of *Cannabis flos*. However, there are no official guidelines regarding their composition and use. Pharmacists have pointed out the lack of scientific data on the compounding technology of semi-solid dosage forms containing herbal drug preparations of *Cannabis sativa* L. Therefore, the study aimed to develop an optimized technology for preparing semi-solid formulations of prescription drugs containing *Cannabis* oil extract. For this purpose, the oil extract of *Cannabis flos* developed based on the technology proposed in the Department of Drug Form Technology was introduced into modern substrates of different nature, viz: Celugel® (hydrophilic), Oleogel® (lipophilic), Pentravan® (multiphase, liposomal) and cholesterol ointment (absorbent). A technology for the preparation of semi-solid forms[4] was proposed, together with a qualitative and quantitative assessment of the cannabinoids contained in the designed formulations, i.e., THCA, CBDA, THC-8, THC-9, CBN, and CBD, using the HPLC method.

The study evaluated the conditions for the preparation of the proposed semi-solid drug forms, i.e. the speed and time of homogenization in the unguator, the selection of the type of packaging, the storage conditions in the using pack, its stability with the determination of cannabinoid content over time and microbiological evaluation of formulation sample. For the optimal semi-solid dosage forms, an evaluation of their pharmaceutical properties was carried out, including viscosity, consistency and pharmaceutical availability of cannabinoids using Franz diffusion chambers through a lipophilic membrane, among others. A comprehensive approach including formulation technology and formulation composition in addition to assessing the pharmaceutical compatibility of the oil extract with four different substrates will allow the selection of formulations to meet clinical needs and indicate how to handle the formulation of semi-solid drug forms with *Cannabis* raw material.

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P25

Effect of physicochemical parameters on the solubility of prepared *Cannabis flos* soft extract in pharmacy compounding practice

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Herbal preparations of *Cannabis sativa L.* are increasingly used in pharmacy compounding practice, particularly in the preparation of semi-solid and solid dosage forms. Among various dosage forms, oil *Cannabis sativa L.* extracts offer a convenient method of cannabinoids administration, providing improved bioavailability and prolonged therapeutic effect[1]. The aim of this study was to evaluate the influence of selected physicochemical parameters, such as the amount of solvent and dissolution time, on the solubility and stability of *Cannabis flos* soft extract in selected pharmaceutical oils. The oil extracts were obtained using a previously optimized three-step method: 1/ dynamic double maceration with solvent following prior decarboxylation of the raw material; 2/ the resulting ethanol extract was subjected to freezing and solvent evaporation to obtain a soft extract; 3/ in the final step, the soft extract was dissolved in selected oils, including rapeseed oil, under controlled temperature and stirring conditions, at various extract-to-oil m/m ratios[2]. Solubility was evaluated under different solvent proportions. The cannabinoids content i.e. THC, THCA, CBD, CBN, CBG was determined by HPLC according to the guidelines of the German Pharmacopoeia monograph (DAB 2021[3]).
The stability of the oil solutions was analyzed after 30, 60, and 90 days of storage at 2–8°C and in time points cannabinoids content by HPLC was determined. The results of the study may serve as a basis for developing standardized procedures for processing *Cannabis sativa flos* under pharmacy compounding conditions, especially given the absence of a *Cannabis* extractum normatum monograph in the European and Polish Pharmacopoeias[4]. This study supports the development of practical guidelines for pharmacists preparing personalized cannabinoid therapies with using oily extracts as herbal components into solid and semi-solid magistral formulations.
Keywords: *Cannabis sativa flos*, solubility, cannabinoids, oil extract, pharmaceutical compounding technology, magistral formulations, personalized therapy.

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P26

Quantification of polyphenols and antioxidant activity of extracts from different plant parts of *Glechoma* species

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Members of the genus *Glechoma* are perennial plants belonging to the *Lamiaceae* family, widely distributed across Europe and Asia. The *Glechoma* genus comprises 7 species, two of which are native to Poland [1]. These include: *Glechoma hederacea* L. and *G. hirsuta* Waldst. & Kit. Several studies indicate that *G. hederacea* health-promoting properties are associated with the presence of polyphenolic compounds. The main objective of the study was to evaluate and compare the total phenolic content (TPC) as well as quantity and distribution of dominant polyphenols in different morphological parts of the two *Glechoma* species, native to Poland collected in the flowering phase as well as after this stage. Thus, the contents of rosmarinic acid (RA), chlorogenic acid (ChA), caffeic acid (CA), protocatechuic acid (PCaA), rutin (Ru) and isoquercetin (IsoQ) were determined in the roots, stems, leaves, and flowers using HPLC method [2]. Furthermore, the antioxidant potential of hot water extracts from investigated plant parts was assessed using DPPH, ATBS and CUPRAC methods [3].
The phenolics content varied depending on the morphological part and the harvest period. Among the phenolic acids, the highest levels were noted for RA and were found in the flowers of both species. Approximately twice lower content of this phenolic acid was found in the leaves collected during the flowering phase and in the stems of *G. hederacea,* regardless of the time of harvest. The analysis also showed that phenolic acids such as ChA and CA dominate in the leaves of the studied species, particularly those collected during the flowering phase. The highest content of ChA was found in the leaves of *G. hirsuta*, while CA in the plant material obtained from *G. hederacea.* Among plant parts analyzed, the flowers of both *Glechoma* species had the highest flavonoid content, with IsoQ being the major compound. The highest antioxidant activity was found in extracts from flowers, followed by leaves (collected during the flowering period) of the species studied.

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P27

Multivariate metabolomic profiling of polyphenols in five *Artemisia* L. species for revealing pathway-specific signatures and chemotaxonomic markers

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The genus *Artemisia* L. includes several hundred species of aromatic plants (Trifan et al., 2022). Several species of *Artemisia* are of noticeable scientific interest due to their widespread use in traditional and conventional medicine across many countries (Sharifi-Rad et al., 2022). Understanding the diversity of secondary metabolites in the *Artemisia* genus is critical for exploiting their ecological and pharmacological potential.

In this study, we applied correlation analysis and Principal Component Analysis (PCA) to liquid chromatography mass spectrometry (LC/MS) profiles of positive and negative ionization datasets from five *Artemisia* species: *A. annua* L., *A. argyi* H. Lév. & Vaniot, *A. austriaca* Jacq., *A. japonica* Thunb. and *A.ludoviciana* Nutt. The raw plant materials were harvested in 2023 from the M. Gryshko National Botanical Garden in Kyiv, Ukraine. The dried aerial parts of the studied plants were used for the analysis.

The PCA revealed clear metabolic differentiation between groups, with the positive ionization dataset explaining over 70% of variance in the first two components. Notable group-specific metabolites included *prunetin*, *biochanin*, and *kaempferol neohesperidoside* in positively clustered accessions, while negatively grouped samples were distinguished by *brazilin*, *chrysoeriol*, and *quercetin derivatives*.

The correlation heatmaps highlighted tightly co-expressed clusters of free flavonoids and their glycosides, suggesting conserved biosynthetic regulation within groups. Through pathway mapping, several metabolites were linked to the Kyoto Encyclopedia of Genes and Genomes **(**KEGG) flavonoid (ko00941), isoflavonoid (ko00943), and phenylpropanoid (ko00940) biosynthesis pathways. These findings indicate the presence of robust chemotaxonomic markers and pathway-specific metabolic strategies across the studied *Artemisia* species. This study provides a framework for integrating metabolomic data into plant systematics, trait selection, and functional genomics.

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P28

Utilizing Pulsed Electric Field in a novel approach to *Cicerbita alpina* L. Wallr. compound extraction

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Cicerbita alpina L. Wallr., also known as alpine sow-thistle, is a perennial herb found in mountainous regions of Europe. The plant is valued for its mildly bitter taste and nutritional properties, including vitamins and minerals. Though not widely cultivated, it remains an important wild edible in some European regions [1]. However its underground parts, containing various active compounds from the sesquiterpene group, are severely understudied and its potential use in pharmacy is yet to be determined.In our study, a series of extraction experiments were performed at Wroclaw Medical University's Botanical Garden of Medicinal Plants. Underground organs of 17-month-old C. alpina cultivated in hydroponics were harvested and used for the experiments. The above-ground parts were discarded, and the underground material – roots and rhizomes – were separated and cut into slices approx. 0.5 cm in length. Methanolic extraction was conducted using several techniques: ultrasound, Soxhlet (thermal) and maceration. Additionally, an extraction using Pulsed Electric Field (PEF) was performed, during which, an eutectic solvent – a mixture of choline chloride : xylose (1:2 + 30% deionised water) – was used as an extraction medium. This solvent was then removed via Solid Phase Extraction (SPE), and the rest of the liquid was evaporated. The residue was then dissolved again using 80% methanol. All extracts were then purified and sterilized using 0.45 µm filters. Qualitative and quantitative analyses of the acquired extracts were conducted using HPLS-MS. The results were compared using statistical tests (t-test, ANOVA) and explorative data analysis (PCA, OPLS-DA), which showed significant differences between extraction methods used. The most promising results stemmed from the use of PEF, which resulted in high extraction efficiency and slightly altered phytochemical content of the extracts. Additionally, significant differences in metabolic makeup of the root and rhizome extracts were noted. This is a first comparative study of underground organ extracts derived from this plant, and it also highlights the use of PEF as a reliable method in facilitating extraction of secondary metabolites from plants, as was noted by other authors [2].

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P29

Antibacterial effectiveness of selected herbs used in polish traditional medicine

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For centuries, medicinal plants have played a fundamental role in traditional healing systems, offering a rich reservoir of biologically active substances. These natural compounds—produced by plants as secondary metabolites—are increasingly recognized for their potential to combat modern health challenges, particularly due to their antioxidant, antimicrobial, and anticancer properties. In recent years, the rise of multidrug-resistant pathogens has intensified the global search for alternative antimicrobial agents, especially those derived from natural sources. Among the promising candidates further down the road are herbs traditionally used in Polish folk medicine, which remain under-researched despite their historical importance and therapeutic reputation.

This study focuses on selected Polish medicinal plants traditionally applied for treating infections and inflammation. Although the exact mechanisms of their action are still being elucidated, many of these plants are known to contain phytochemicals capable of inhibiting microbial growth. This study aimed to evaluate the antibacterial and antifungal properties of ethanol and aqueous extracts from 15 herbs commonly used in Polish traditional medicine: *Tanacetum vulgare, Matricaria chamomilla, Allium ursinum, Mentha × piperita, Urtica dioica, Hypericum perforatum, Taraxacum officinale, Trifolium pratense, Artemisia absinthium, Nasturtium officinale, Glechoma hederacea, Achillea millefolium, Angelica archangelica, Inula helenium*, and *Valeriana officinalis*. The antimicrobial activity was tested in vitro against five pathogenic bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Micrococcus luteus*, and *Salmonella enterica*). Ethanol-based extracts, in particular, have shown notable promise, with several demonstrating broad-spectrum antibacterial activity. These findings suggest that certain native herbs may harbor compounds effective against both Gram-positive and Gram-negative bacteria, potentially offering new strategies for managing resistant infections. Conversely, water-based extracts tended to show weaker or more selective activity, highlighting the influence of extraction methods on the biological efficacy of plant materials.

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P30

Profile of secondary metabolites during the development of generative organs in *Nigella damascene*

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Nigella damascena, an important member of the buttercup family (Ranunculaceae), is an ornamental and medicinal plant native to the Mediterranean region. Known for its ability to produce a wide range of secondary metabolites, this species has considerable medicinal potential. The main constituents of the essential oil extracted from its seeds are *β*-elemene and damascenine. *β*-elemene has antibacterial, anticancer and anti-inflammatory properties, while damascenine has anti-inflammatory, antipyretic, analgesic and diuretic effects.
The aim of this study was to identify the stage of generative organ maturation characterized by the highest accumulation of damascenine and *β*-elemene. The profile of secondary metabolites during different stages of Nigella damascena generative organ development was analyzed using gas chromatography coupled with mass spectrometry (GC-MS).
Results revealed a substantial increase in fruit mass during development, with the mature fruit exhibiting a mass 10-fold greater than that of the unpollinated flower. At the same time, significant changes were observed in the secondary metabolite profile. A total of 16 metabolites were detected, including damascenine, *β*-elemene, fatty acids and their derivatives. Damascenine, *β*-elemene, linoleic acid and 2-amino-3-methoxy-benzoic acid methyl ester showed increased accumulation during fruit development, with linoleic acid exhibiting the highest increase, up to 6-fold. In contrast, palmitic acid *β*-monoglyceride, 6-octadecenoic acid methyl ester and two unidentified metabolites decreased over time.

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P31

A natural compound with anticancer promise: chetomin's effects on melanoma cells

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Melanoma is a highly aggressive form of skin cancer that originates from melanocytes. It has a strong tendency to metastasize, and in its advanced stages, it is associated with poor prognosis. Although excessive ultraviolet (UV) exposure is the predominant risk factor, other contributors include indoor tanning, genetic susceptibility, immunosuppression, congenital conditions, nevi, and obesity [1]. Despite significant progress in treatment strategies, long-term patient outcomes remain suboptimal, highlighting the urgent need for new therapeutic options [2].

Chetomin, a bioactive metabolite derived from *Chaetomium* fungi, exhibits antifungal, antibiotic, and anticancer properties. Previous research has shown that it can suppress HIF-1-regulated genes, suggesting potential anticancer activity in various tumor types [3]. However, its specific effects on human melanoma cells remain largely unexplored, necessitating further study.

This study aimed to investigate the antitumor effects of chetomin on A375 human melanoma cells, with a focus on its influence on cell viability and apoptosis induction.

Chetomin (NSC 289491, extracted from *Chaetomium cochliodes*, with a purity of ≥98% confirmed by HPLC) was dissolved in DMEM to prepare a stock solution at a final concentration of 100 nM. A375 melanoma cells were exposed to various concentrations of chetomin (5–100 μM) for 24, 48, and 72 hours. Cell viability was assessed using MTT and CellTiter-Blue® assays, while apoptotic cell death was evaluated using the RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay.

Chetomin exposure significantly reduced the viability of A375 melanoma cells in a dose- and time-dependent manner. Increased phosphatidylserine externalization in chetomin-treated cells confirmed apoptosis induction.

The findings suggest that chetomin has potent anticancer activity against melanoma cells, as it inhibits proliferation and induces apoptosis. These results underscore its potential as a promising therapeutic agent for melanoma. Further, *in vivo* studies are essential to evaluate its efficacy and safety profile in preclinical models.

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P32

Flavonolignans and their halogenated derivatives as possible inhibitors of *α*-glucosidases

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Milk thistle (*Silybum marianum* (L.) Gaernt.) from the Asteraceae family, with its flavonolignan content in the fruits is one of the most researched plants for the treatment of liver diseases [1]. Halogenated structural analogues of natural products often have a stronger effect than their parent substances [2]. The aim of the study was to analyze the inhibitory activity of pure silymarin flavonolignans and their two brominated derivatives against αglucosidases and to establish the structure-activity relationship. The inhibitory activity of the tested constituents of *Silybum marianum* against α-glucosidases was determined using enzymes of two origins: mammalian from rat intestine, and yeast from *Saccharomyces cerevisiae*. Acarbose, an approved αglucosidase inhibitor, was used as a positive control and *p*nitrophenyl-*α*-D-glucopyranoside as a substrate in a spectrophotometric method [3].
The silymarin complex had a higher inhibitory activity than acarbose with yeast enzyme, while 2,3-dehydrosilychristin was the most active substance. The two brominated derivatives of 2,3-dehydrosilybin were more active than acarbose. Using the mammalian enzyme, none of the substances tested, including the halogenated derivatives, was a more potent inhibitor of αglucosidase than the registered drug acarbose. Although flavonolignans were potent inhibitors of yeast *α*-glucosidases, their effect on the mammalian enzyme was weak.

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P33

Analysis of the active ingredients and pharmacological activity of *Peucedanum japonicum* cultivated in Korea

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Peucedanum japonicum (*P. japonicum*) is a perennial herb native to Japan and parts of East Asia, traditionally used in Asian medicine for its diuretic, antipyretic, and analgesic properties. Recent studies indicate that its extracts have therapeutic potential against inflammation, viral infections, rheumatism, and urinary tract infections. This study evaluated the potential *of P. japonicum* as a functional food ingredient by analyzing its bioactive components and investigating effects on memory and gut health. Using UPLC-QTOF/MS analysis, 29 secondary metabolites were identified, with praeruptorin A–D and visnadine as major constituents. The ethanol extract (PJE) significantly inhibited pro-inflammatory mediators—nitric oxide (NO), prostaglandin E2 (PGE_2), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)—in BV2 microglial cells. This activity was associated with suppression of signaling pathways including NF-κB, ERK, JNK MAPK, Akt/GSK3β, and JAK2/STAT3 via modulation of the TLR4/MyD88 axis. PJE also promoted the growth of six beneficial bacterial strains during co-incubation with 12 probiotic strains in the absence of additional culture media. Notably, *Lactobacillus plantarum* and *L. reuteri* showed remarkable proliferation—approximately 1,000,000-fold and 150,000-fold, respectively. Fermented products of these strains exhibited reduced cytotoxicity and enhanced NO inhibition compared to PJE alone, suggesting improved anti-inflammatory potential. These findings indicate beneficial effects on gut and cognitive health through attenuation of neuroinflammation. Overall, the results support the development of *P. japonicum* as a promising natural source for health functional foods targeting gut health and cognitive function via modulation of neuroinflammatory responses and gut microbiota.

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P34

Evaluation of anti-neuroinflammatory effects in LPS-induced BV2 microglial cells using ethanolic extract of native Korea plant species

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Neuroinflammation is a complex process that significantly impacts cognitive functions, particularly memory. It involves the activation of glial cells, such as microglia, and the release of inflammatory mediators within the brain, which can disrupt normal brain function. For instance, LPS-induced neuroinflammation has been shown to impair spatial memory and learning by activating microglia and increasing the levels of pro-inflammatory cytokines like IL-1β and TNF-α in the hippocampus, a region crucial for memory formation. This inflammation can lead to a reduction in the number of NMDA glutamate receptors, essential for synaptic plasticity and memory consolidation. Moreover, neuroinflammation is associated with cognitive deficits in various neurodegenerative diseases, including Alzheimer's disease, where it disrupts large-scale network connectivity and contributes to memory impairments. Understanding the mechanisms behind neuroinflammation-induced memory deficits is crucial for developing effective therapeutic strategies to mitigate these effects. In this study, we investigated the anti-inflammatory properties of native Korean plants, including *Dendrathema zawadskii* var. *Latilibum*, *Chrysanthemum indicum*, *Valeriana sambucifolia*, *Agastache rugosa*, and *Agrimonia pilosa* to assess their potential as ingredients for health functional foods aimed at enhancing memory.

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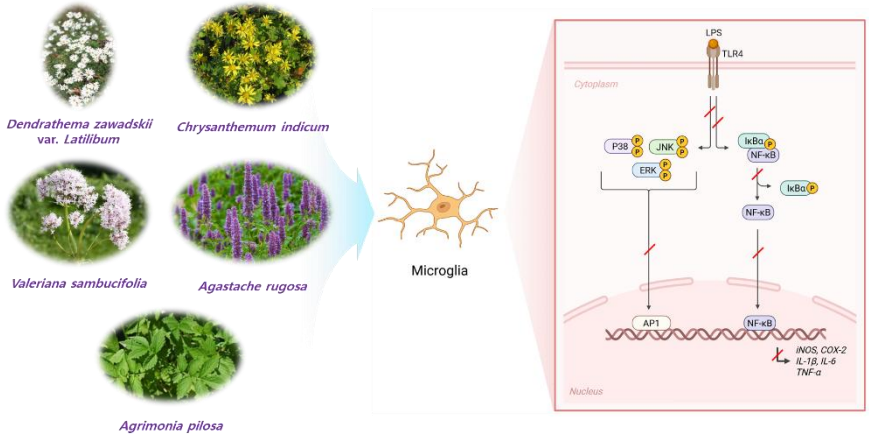

Figure. Anti-neuroinflammatory effects of Korean native plant species in LPS-induced BV2 microglial cells.

P35

Comparative antibacterial activity of aqueous, aqueous-methanolic, and methanolic extracts of *agrimonia eupatoria* against *P. aeruginosa*

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The growing problem of antibiotic resistance has prompted the search for new antimicrobial agents from natural sources. *Agrimonia eupatoria* (Rosaceae) is a perennial plant native to Europe, traditionally used in folk medicine and known for its diverse secondary metabolites. These include compounds with varying degrees of polarity and solubility such as tannins, flavonoids, triterpene aglycones, saponins, and polysaccharides. To assess the influence of solvent extraction on antibacterial efficacy, aqueous, aqueous-methanolic, and methanolic extracts of *A. eupatoria* were prepared and evaluated for their activity against a reference strain of *Pseudomonas aeruginosa*.

Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method. The methanolic and 50% aqueous-methanolic extracts demonstrated higher antibacterial activity (MIC = 2.5 mg/mL) compared to the aqueous extract (MIC = 5 mg/mL), indicating the significance of solvent selection in maximizing bioactive compound yield.

To further investigate the antibacterial effects, isothermal microcalorimetry (IMC) was employed using a calScreener™ instrument. This technique enables real-time monitoring of bacterial metabolic activity by detecting the heat produced during growth. Parameters such as time to peak, maximum metabolic rate, total heat, and time to activity were analyzed to provide a detailed comparison of extract efficacy.

In parallel, a phytochemical analysis of all three extracts was conducted using modified pharmacopoeial methods. The screening focused on quantifying total polyphenols, tannins, and hydroxycinnamic acid derivatives. This analysis confirmed the presence of key secondary metabolites and supported the observation that solvent polarity influences both chemical composition and biological activity.

The results of this study demonstrate a clear trend in antibacterial potency correlating extraction solvent, with methanol-based extracts being the most effective. These findings highlight the importance of selecting appropriate extraction techniques to optimize the recovery of active constituents. Overall, *A. eupatoria* represents a promising source of natural antibacterial agents, particularly against Gram-negative pathogens such as *Pseudomonas aeruginosa*.

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P36

Isoquinoline alkaloids: unlocking the potential of ultra-high performance supercritical fluid chromatography in the analysis of quaternary compounds

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Several isoquinoline alkaloids have been recognized for their broad-spectrum antiviral activity against both animal and human coronaviruses. Building on our previous research, a number of these compounds have shown promising efficacy against the SARS-CoV-2 spike protein in pseudovirus neutralization assays. In this study, we explore the potential of Ultra-High Performance Supercritical Fluid Chromatography (UHPSFC) for the analysis of isoquinoline alkaloids. Twenty-two isoquinoline alkaloids from quaternary and tertiary protoberberine, protopine, aporphine, acyclic and cyclic bisbenzylisoquinoline alkaloid's group were selected for primary screening. Unmodified stationary phases such as bare silica and BEH and stationary phases with various attached ligands such as PFP, CSH PFP, Biphenyl, Naphtyl, BEH Shield RP18, Diol, Amide, 2-EP, 2-PIC, 1-AA and DEA were evaluated with combination of different organic modifiers and additives. For initial screening gradient elution was programmed from 5% - 45% of organic in 3 minutes. Neat MeOH, 10 mM ammonium formate in MeOH, 10 mM ammonia in MeOH and 98% MeOH were selected as organic modifiers of first choice. Based on primary screening the stationary phases with Diol and 2-PIC chemistry were selected for further optimization. The effect of 25% and 50% MeOH in ACN was further tested (neat/10 mM ammonium formate/10 mM ammonia). Gradient elution was programmed from 5% - 45%, 5% - 50% and 5% - 55% of organic component, both in 3, 5 and 10 min. In the next step, we developed a solid-phase extraction (SPE) protocol for sample preparation using a mixed-mode polymeric sorbent that combines weak cation-exchange and reversed-phase properties. This purification strategy efficiently eliminates unwanted acidic and neutral secondary metabolites from the barberry crude extract, enhancing overall analytical performance. Our profiling approach was successfully employed for non-targeted UHPSFC-UV-MS profiling of various species from genus *Berberis* L. (Berberidaceae).

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In the shadow of the claw: unraveling bioactive alkaloids from *Erythrina variegata* L. via Medium-Pressure Liquid Chromatography

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The genus *Erythrina* L. (Fabaceae) includes approximately 115 species of trees and shrubs naturally distributed across tropical and subtropical regions worldwide. The stem bark has been widely used in traditional medicine systems across Asia and the Pacific due to diverse therapeutic properties. Common uses include decoctions for treatment of internal ailments and poultices for topical application, reflecting its traditional roles in relieving inflammation, infections, insomnia, and parasitic infestations. The stem bark presents an alluring phytochemical profile, abundantly enriched with bioactive constituents including alkaloids, flavonoids, terpenoids, saponins, tannins, phenolic compounds, and glycosides, highlighting its promising therapeutic versatility. Erythrinan alkaloids are a characteristic group of tetracyclic isoquinoline alkaloids with neuropharmacological effects, such as anticholinergic, sedative, anticonvulsant, and CNS depressant activity. Although a few animal studies have been conducted, erythrinan alkaloids still represent a largely uncharted territory in terms of their therapeutic potential in human medicine.

The crude ethanolic extract was obtained from ground and dried stem bark. The subsequent pH-dependent liquid-liquid extraction of crude extract facilitated the selective removal of acidic and neutral constituents, thereby yielding an alkaloidal fraction of enhanced purity.

The alkaloid fraction was subsequently separated using normal-phase Medium-Pressure Liquid Chromatography into more than 200 fractions, which were combined into 14 larger pooled fractions based on High-Performance Thin-Layer Chromatography profiling. To assess their cholinesterase-inhibitory capacity, the crude extract, alkaloid-enriched extract, and 14 systematically combined fractions were evaluated *in vitro* against recombinant human acetylcholinesterase and butyrylcholinesterase.

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Rhododendron ferrugineum L. tissue cultures *in vitro*

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Rhododendron ferrugineum L., commonly known as the Alpine rose, is a small evergreen shrub belonging to the Ericaceae family, predominantly found in the Alps and the Pyrenees, with a single documented natural site in Poland located in the Karkonosze Mountains. This study aimed to establish callus cultures from sterile seedlings of *R. ferrugineum*, by using explants derived from cotyledons, hypocotyls, and leaf blades. The callus cultures were maintained in a controlled conditions within a phytotron chamber at a temperature of 25°±2°C, under an 18/6 hour photoperiod (light/dark) with white LED lighting. The growth medium was enriched with plant growth regulators, specifically the cytokinin kinetin (KIN) and synthetic auxin-like 1-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D). This research contributes to the understanding of *in vitro* micropropagation techniques for *R. ferrugineum*, supporting both its *ex-situ* protection and future physiological and phytochemical studies on this distinctive alpine species.

P39

Optimization of supercritical CO_2 extraction of *Petasites hybridus* rootstocks

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Petasites hybridus (L.) G.Gaertn., B.Mey. & Scherb. (known as butterbur) of the Asteraceae family, possesses clinically proven anti-migraine properties. The primary bioactive constituents of its extract are sesquiterpenoids, such as petasin derivatives (petasin, neopetasin, and isopetasin). However, the individual activity profile of these compounds is poorly understood and the reference standards are not readily available. Furthermore, crude extracts of *P. hybridus* contain hepatotoxic and cancerogenic pyrrolizidine alkaloids (PAs) [1].
Consequently, several patented extraction techniques have been developed to eliminate PAs [2]. However, the previous methodologies often lack specific data targeting individual isomers.
In this work, we conducted a comprehensive evaluation of the extracting methods of butterbur. Utilizing Response Surface Methodology (RSM) with a Box-Behnken design, we optimized the extraction process to minimize toxic PAs concentration while maximizing the yield of bioactive sesquiterpenoids. Supercritical carbon dioxide ($scCO_2$) extraction was selected due to its many advantages: high efficacy, selectivity, flexibility, scalability, and low environmental impact [3]. The optimization of the methods included three parameters, i.e., temperature, pressure, and the CO_2-to-plant material ratio at three different levels (-1, 0, +1). The experimental ranges were as follows: temperature 40–60 °C, pressure 200–400 bar, and CO_2 50-100 kg CO_2/kg of plant material. A total of 15 extracts were prepared with the use of scCO2 with different parameter levels. A statistical model based on the yields of sesquiterpenoids enabled the identification of optimal extraction conditions for *P. hybridus* rhizomes: a temperature of 40 °C, pressure of 300 bar, and CO_2 of 100 kg/kg plant material. These optimal conditions were validated through additional $scCO_2$ extraction experiments. The proposed protocol can be feasibly scaled up for industrial applications to produce extracts enriched in therapeutically relevant sesquiterpenoids while minimizing toxic impurities.

Acknowledgments
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P40

Anti-migraine activity of petasin isomers isolated from *Petasites hybridus* in migraine-like pain model of mice

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Eremophilane-type sesquiterpenoids, specifically petasin derivatives (petasins), are considered the bioactive components of *Petasites hybridus* (L.) G.Gaertn., B.Mey. & Scherb. (butterbur) rootstocks [1]. Numerous clinical studies have proven the efficacy of butterbur in migraine [2]. However, the activity of pure petasins of *P. hybridus* in migraine has been never evaluated in preclinical and clinical studies. The objective of the present study was to assess the antimigraine activity of both crude extract of *P. hybridus* as well as petasin-rich fraction (PRF) (neopetasin, petasin, and isopetasin 1:3:2) in migraine-like model in mice. Fraction was isolated from crude methanolic extract of butterbur with the use of liquid-liquid chromatography technique, following the methodology established in our previous study [3]. Migraine-like symptoms were assessed in male C57BL/6J mice using nitroglycerin-induced migraine model. Systemic injection of nitroglycerin (10 mg/kg, i.p.) induced vasodilation and significant mechanical hypersensitivity (allodynia) in the paw and periorbital region of animals, as measured using von Frey filaments [4]. Based on preliminary data, doses of 25 and 50 mg/kg i.p. of butterbur extract, and 25 and 50 mg/kg i.p. of petasins fraction were administered 30 min after vehicle or nitroglycerin. Our findings demonstrate that the PRF (50 mg/kg) significantly attenuated mechanical hypersensitivity in periorbital region of the head after nitroglycerin administration and elicited a greater analgesic effect compared to the crude butterbur extract.
This study provides evidence supporting the enhanced efficacy of purified petasin derivatives compared to extract and offers insights into the molecular basis of butterbur's antimigraine activity.

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P41

High anticandidal activity of propolis extracts and its relation with flavonoid content

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Bee products, including propolis, are widely applied in natural medicine but their activity is variable and inconsistent which poses challenge. The ethanolic extracts of propolis from Poland were investigated for their potential against different *Candida* spp. strains (*C. albicans*, *C. glabrata*, *C. krusei*, and *C. parapsilosis*). Less than 25% of the investigated propolis extracts exhibited lower Minimum Inhibitory Concentration (MIC) ≤ 128 µg/mL against *Candida* spp. and only a few, particularly high anticandidial potential (MIC ≤ 32-64 µg/mL). The detailed phytochemical profiles were investigated by LC-MS, and evaluated using multivariate analysis techniques. The main components identified were phenolic acids and their esters as well as variety of flavonoids. Some of the latter were found more abundant in the most active propolis samples and were associated with elevated activity. In particular, chrysin (up to 36 mg/g), galangin (up to 29 mg/g), pinocembrin (up to 46 mg/g), pinobanksin-3-acetate (up to 38 mg/g), pinobanksin (up to 13 mg/g), pinostrobin (up to 31 mg/g), techtochrysin (up to 9 mg/g) and isosakuranetin (up to 27 mg/g). Although pure compounds were not particularly active and characterized by high MIC >256 µg/mL against *Candida* spp., their mixtures were much more efficient. The lowest values of MIC = 64 µg/mL and Minimum Fungicidal Concentration (MFC) = 128 µg/mL were observed for a mixture of chrysin, galangin, pinobanksin, pinobanksin-3-acetate and pinocembrin. This suggest that propolis activity is conditioned by composition of different compounds and their synergistic action and not some ingredients alone. This may allow elaboration of propolis standardization methods or development of effective therapeutic compositions for treatment of *Candida* spp., consisting of pure compounds.

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P42

Bioactive metabolites and phytotoxicity of *Cynara cardunculus* L.: a step toward green bioherbicides

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Cynara cardunculus L. has attracted increasing attention as a potential source of bioherbicides, owing to its pronounced phytotoxic effects, which are largely attributed to its rich content in specialized secondary metabolites [1]. This study investigates the correlation between the metabolite profile of *C. cardunculus* and its herbicidal activity.

A systematic review of phytochemical data related to the aerial parts of *C. cardunculus* L. reveals a broad spectrum of allelopathic compounds, notably flavonoids and their glycosides, triterpenes, polyphenols, and sesquiterpene lactones [2]. Among these, sesquiterpene lactones such as cynaropicrin and grosheimin, as well as flavonoids like luteolin and apigenin, are recurrently identified as major contributors to the species' phytotoxicity. Other compounds, including aguerin B, *p*-coumaric acid, and quercetin, may act synergistically to reinforce these effects [3, 4].

To complement the literature review, an experimental approach was conducted involving TLC and UHPLC-MS analyses of crude extracts from three distinct *C. cardunculus* genotypes. Extractions were carried out using conventional solvents, among which ethyl acetate proved most efficient in yielding bioactive fractions. This solvent enabled the recovery of a metabolite-rich extract displaying strong herbicidal activity, with key constituents including cynaropicrin, chlorogenic acid, luteolin, cynaroside, apigenin, aguerin B, cynarinin A/B, and *p*-coumaroylquinic acid. The wild genotype consistently exhibited the highest concentrations of these compounds.

The findings support a robust association between the phytotoxic potential of *C. cardunculus* and the abundance of specific secondary metabolites, particularly sesquiterpene lactones and flavonoids. These results underscore the relevance of chemotypic variability in determining bioactivity and offer a rational basis for targeted compound isolation.

Future research will focus on the optimization of extraction methods, the identification of additional active constituents, and the evaluation of compound synergies under pre-emergence conditions. This integrative approach is intended to support the development of *C. cardunculus*-based bioherbicides as sustainable and environmentally friendly alternatives to synthetic chemical herbicides.

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P43

Fatty acid profiles, nutritional quality, and antioxidant properties of cold-pressed oils and herbal macerates after prolonged storage

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According to recent studies, maceration with aromatic herbs is a promising method for improving the oxidative stability, shelf life, and sensory properties of edible oils. Despite the abundance of literature addressing the effects of storage on the fatty acid profiles of unconventional cold-pressed oils, little is known about how maceration affects these profiles, particularly in the context of long-term storage.
The study investigated the effects of herbal additives and prolonged storage affect the fatty acid profiles, nutritional quality, and antioxidant properties of cold-pressed oils from eight plant species (black cumin, safflower, borage, evening primrose, hazelnut, walnut, sea buckthorn, and oilseed rape). The oils were macerated with basil or sage leaves (in two doses, 50 g or 100 g) and stored under refrigeration for 10 months. The fatty acid composition was assessed by gas chromatography with flame ionisation detector, while the total low molecular weight antioxidant activity (TAA) was analysed by DPPH assay. Nutritional indices derived from the fatty acid profiles included the ratios of ω-6 to ω-3 fatty acids and polyunsaturated fatty acids (PUFAs) to saturated (SFAs) fatty acids, atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic/hypercholesterolemic ratio (HH). Principal component analysis (PCA) was applied for multivariate evaluation.
The PCA was performed separately for each oil, revealing significant species-specific differences in response to herbal additives and long-term storage in terms of fatty acid composition, percentage content specific to each oil, as well as nutritional indices (AI, TI, HH indices and the ratios of PUFAs/SFAs and ω-6/ω-3), and TAA. The most significant differences were observed between the black cumin seed oil and the rapeseed oil. The most statistically significant effects were observed in black cumin seed oils, with the effects of herbal additives, storage, and their interaction also being significant. In contrast, rapeseed oil exhibited no immediate alternations in these parameters upon the addition of herbs. However, significant effects became evident after a prolonged time period. The findings provide new insights into how both maceration and storage influence the nutritional quality of unconventional cold-pressed oils.

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P44

Comparative analysis of antioxidant, anti-pigmentation and cytotoxic properties of extracts from different species of the genus *Rosa*

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Extracts obtained from various *Rosa* species have been widely utilized for centuries in both traditional medicine and modern cosmetology. Their enduring popularity is primarily attributed to the richness in biologically active compounds, such as polyphenols and flavonoids, which exhibit a range of beneficial properties including antioxidant, anti-inflammatory, and skin-regenerating effects. Extracts obtained from rose buds are of particular interest, as their chemical composition and biological activity can vary significantly depending on the extraction solvent used.

This study presents a comparative evaluation of aqueous (**A**), hydroethanolic (70%, *v/v,* **EtOH**), hydroglycerinic (20%, *v/v*, **GLC**), and hydroglycolic (20%, *v/v***, GLL**) extracts derived from the buds of five rose species: *R. damascena*, *R. alba*, *R. damascena* 'Kazanlik', *R. platyacantha*, and *R. centifolia*. The extracts were assessed for their antioxidant, anti-pigmentation, and cytotoxic properties. Antioxidant activity was determined using the DPPH and ABTS radical scavenging assays. Tyrosinase inhibition was evaluated using both fungal tyrosinase and murine tyrosinase obtained from B16F10 melanoma cells. Cytotoxicity was assessed using the neutral red uptake assay on untreated and hydrogen peroxide-stressed HaCaT keratinocytes.

All tested extracts demonstrated significant antioxidant activity, regardless of the solvent used. The strongest anti-pigmentation effect against fungal tyrosinase was observed for EtOH extracts, with the extract from *R. platyacantha* showing the highest inhibition (29% at the concentration of 5%). In contrast, the GLL from *R. centifolia* exhibited the most potent inhibitory effect on murine tyrosinase (36% inhibition at the concentration of 5%). None of the extracts exhibited significant cytotoxicity towards HaCaT cells. Rose extracts were also protecting the HaCaT cells from H_2O_2-induced death, confirming their potent cytoprotective activity against oxidative stress.

P45

Immune enhancement effect of fermented products of the extract of *Acanthopanax sessiliflorus* fruits in RAW264.7 and BV2 cells

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This study evaluated the prebiotic potential of a 30% ethanol extract from *Acanthopanax sessiliflorus* fruit (ASE) and compared its immune-enhancing effects with those of fermented derivatives produced using three probiotic strains: *Lactobacillus plantarum* (ASE-LPF), *Streptococcus thermophilus* (ASE-STF), and *Lactobacillus helveticus* (ASE-LHF). ASE significantly promoted the growth of all three probiotic strains. Furthermore, immune-modulating effects were assessed in RAW264.7 macrophages and BV2 microglial cells treated with ASE and its fermented products. Among the treatments, ASE-LHF notably enhanced nitric oxide (NO) production and upregulated the expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-*α*), when compared to ASE alone. These effects were linked to the activation of the nuclear factor kappa B (NF-*κ*B) and extracellular signal-regulated kinase mitogen-activated protein kinase (ERK-MAPK) signaling pathways. Collectively, these findings indicate that *A. sessiliflorus* fruit, by fostering the growth of beneficial bacteria and boosting immune activity, may serve as a promising ingredient for health functional foods aimed at enhancing intestinal health and immunity.

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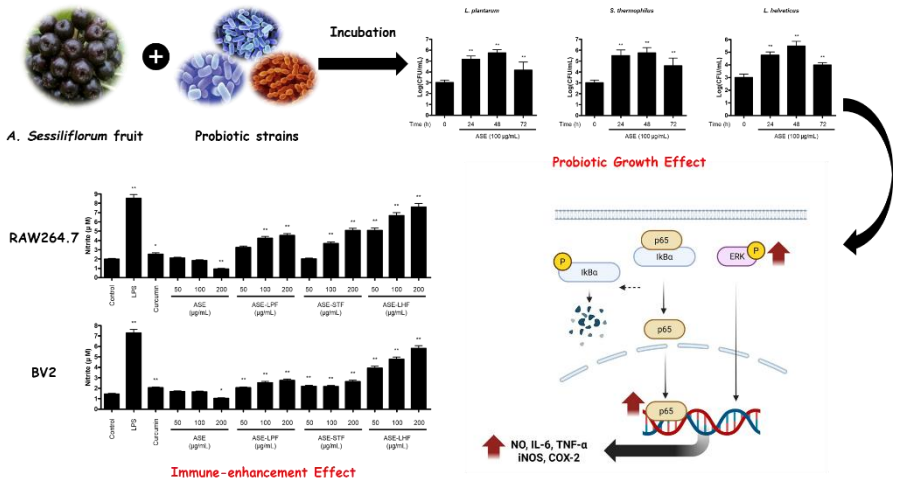

Figure Probiotic growth effect of *A. sessiliflorus* fruit extract and immune-enhancement effect of fermented products of *A. sessiliflorus* fruit extract

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Micropropagation of *Rhododendron ferrugineum* for biodiversity conservation

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High-mountain ecosystems are especially sensitive to the impacts of climate change, which significantly accelerates biodiversity loss. Among the species affected is Rhododendron ferrugineum, an alpine shrub native to the Alps and Pyrenees. A particularly isolated and vulnerable population has been identified in the Karkonosze Mountains, comprising just 68 individuals, an alarmingly low number that underscores the species' precarious conservation status.

In response to this decline, conservation efforts have focused on in vitro propagation as a means of safeguarding the species. Shoot cultures were initiated from sterile, germinated seeds and maintained under controlled phytotron conditions (25°C, 18/6-hour photoperiod, white LED lighting). *Anderson's Rhododendron Medium* was enriched with the cytokinin 2-isopentenyladenine (2iP) and the auxin indole-3-acetic acid (IAA) at varying concentrations to promote shoot induction and root development. During acclimatization, young plantlets were transferred to peat-based substrates, with gradual humidity reduction and supplementation of macro- and micronutrients to support transition to *ex vitro* conditions. Signs of successful adaptation included progressive lignification of seedlings, and the emergence of a flowering shoot. This study highlights the viability of *in vitro* culture as an effective strategy for the conservation of endangered alpine species. By combining biotechnology with ecological conservation, it offers a sustainable approach to preserving genetic diversity in the face of ongoing environmental change.

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Influence of simulated *in vitro* gastrointestinal digestion on the polyphenolic profile and biological activity of blackthorn fruit

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Continuous exposure to negative factors related to the changing lifestyle, such as an unbalanced diet low in fruit and vegetables and rich in highly processed food, causes prolonged oxidative and inflammatory stress in human organisms, leading to the development of many disorders. The number of cases of gastrointestinal inflammation, including its chronic forms, is increasing dramatically.
In the face of a growing problem, more and more attention is paid to the search for food products that could be used to prevent or treat this group of diseases. One example of such a product is the fruit of *Prunus spinosa* L. (blackthorn), used both as food and as a traditional herbal medicine, recommended by European phytotherapy in the treatment of intestinal inflammation. However, the existing data on the biological activity of sloe fruits are incomplete, and the lack of broader analyses and confirmation of activity in biological models that take into account the specific environment of the gastrointestinal tract limits the wider use of the fruits as health-promoting agents.
Given the above premises, the aim of the phytochemical part of the presented work was to perform qualitative and quantitative analysis using LC-MS^3, HPLC-PDA, and spectrophotometric methods of polyphenolic compounds of blackthorn fruit extracts: the native one and the one obtained after simulated *in vitro* digestion. In the biological part, the aim was to assess the effect of extracts on the secretion of pro- and anti-inflammatory mediators (TNF-α, IL-18, IL-10, IL-8, MMP-8) by immune system cells involved in intestinal inflammatory processes. In the biological part, the analyses were carried out using immunoenzymatic and spectrophotometric methods.
A polyphenolic composition different from the initial extract characterized the extract obtained in the process of simulated digestion. Qualitative and quantitative changes mainly concerned the fractions of anthocyanins, phenolic acids, and procyanidins. The extracts tested in the concentration range of 5-150 μg/mL, in particular, reduced the secretion of pro-inflammatory factors, including MMP-8, IL-18, and TNF-α (even by ~ 65%). The results might partly support the traditional use of the plant material in inflammatory intestinal diseases and encourage further *in vitro* and *in vivo* studies in that matter.

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P48

Chemical composition and evaluation of the effects of the *Prunus* genus plant substances on oxidative modifications of plasma components

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Peroxynitrite ($ONOO^-$) is one of the primary oxidants, and its biological effects include oxidation/nitration reactions that lead to modifications of protein and lipid components of cells and biological fluids. The stress conditions induced by $ONOO^-$ may influence functional molecules in the vascular endothelium and smooth muscle, eventually leading to cardiovascular diseases (CVDs). According to the accumulated research, plant extracts/natural compounds may successfully prevent or block key CVDs risk factors such as nitrative/oxidative stress.
Prunus is a genus of flowering trees or shrubs from the Rosaceae family, including plums, cherries, and apricots. Traditional medicine suggests that various herbal substances (flowers, branches, or leaves) from blackthorn (*P. spinosa* L.) and American black cherry (*P. serotina* L.) may have health benefits in the prevention or supportive treatment of oxidative-inflammatory diseases, i.a. CVDs.
The study focused on the chemical composition of blackthorn leaves and branches, as well as American black cherry flowers, and the impact of the dry hydroalcoholic extracts prepared thereof on the human plasma components under $ONOO^-$-induced oxidative stress. Phytochemical analyses were performed using LC-MS^3 and spectrophotometric methods. The *ex vivo* studies included measuring the levels of biomarkers, such as 3-nitrotyrosine (3-NT), thiobarbituric acid reactive substances (TBARS), and the total antioxidant capacity of plasma (NEAC). The ability of the extracts to scavenge oxidants was also tested in *in vitro* models. In activity studies, the immunoenzymatic and spectrophotometric methods were used.
The main compounds identified in *P. spinosa* leaves were flavonols (mono-, diglycosides of kaempferol and quercetin) and caffeoylquinic acids. In *P. spinosa* branches, flavan derivatives predominated, in particular di- and trimers of type A procyanidins, whereas in *P. serotina* flowers, mainly flavonols (mono- and diglycosides of quercetin, isorhamnetin and kaempferol) and caffeoyl-, feruloyl-, coumaroylquinic acids were identified. All tested extracts (1-50 µg/mL) significantly reduced 3-NT levels (up to ~ 72%) and increased NEAC (even by ~ 47%). Moreover, they neutralized various oxidants of physiological significance ($O_2^{\bullet-}$, $OH^{\bullet}$, and H_2O_2). The results might, in part, support the traditional use of plant materials and encourage further *in vitro* and *in vivo* studies on the topic.

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P49

Does microgravity affect chemopreventive activity and bioactive compounds content in *Brassica* sprouts? – preliminary *in vitro* studies in thyroid cancer cell lines

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Sprouts of edible plants have gained popularity in recent years. They can be a source of functional food, which, apart from nutritional properties, can reduce the risk of cancer [1]. Microgravity, as a state of near zero gravity, can cause stress in growing plants, which can affect the secondary metabolites biosynthesis and biological activity of sprouts, such as cytotoxic and anti-proliferative potential. Due to our previous promising results [2] and the research gap, our team decided to check if microgravity can improve *Brassica* sprouts chemopreventive potential.

Kale and broccoli sprouts were cultivated with the use of Random Positioning Machine, which simulates the microgravity conditions. Additionally, different lighting conditions (day/night and total darkness) and harvesting periods (5, 6, 7 days) were applied. After harvesting, the sprouts were extracted and examined for cytotoxic (MTT assay) and anti-proliferative (crystal violet assay) activity on different thyroid cancer (8505C, TPC-1, FTC-133) and normal (Nthy-ori 3-1) cell lines. Additionally, HPLC and UPLC-MS/MS methods were used to determine the content of polyphenolics and sulfur compounds.

Our preliminary results showed that microgravity caused an increase in the phenolic acids and sulfur compounds biosynthesis. As far as biological activity is concerned, sprouts exhibited cytotoxic activity against thyroid cancer cells (especially TPC-1 cells, which were the most sensitive) and the most active were sprouts grown in microgravity and/or darkness at the highest concentration used (300 µg/mL). What is worth adding, the sprouts did not show significant toxicity against normal thyroid cells. Broccoli and kale sprouts exhibited anti-proliferative activity against thyroid cancer cells, in a time-dependent manner. As the sprouts decreased also the proliferation of normal thyroid cells, this suggests that they could inhibit not only thyroid cancer progression, but also excessive hyperplasia of thyroid.

Microgravity-grown sprouts is a fascinating idea, which combines topics of plant biology, space technology and food studies. We believe that these insights could contribute to innovative approaches for enhancing the nutritional and chemopreventive potential of Brassica sprouts, unlocking their full potential as functional foods in diverse dietary applications.

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P50

Isolation of compounds with potential anti-inflammatory activity from *Glycyrrhiza uralensis* via bioassay-guided fractionation

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Glycyrrhiza species have been used worldwide in traditional medicine for their expectorant, anti-inflammatory, and antimicrobial activities. The anti-inflammatory effects are attributed primarily to prenylated phenolics, which exhibit unique interactions with biological membranes, making them promising candidates for drug development.

The main objective of this study was the isolation of prenylated phenolics with potential anti-inflammatory activity from *G. uralensis*. The chloroform fraction from *G. uralensis* was selected, as its extracts demonstrated the highest bioactivity in our previous study.

The plant material was obtained through cooperation with the Faculty of Horticulture, Mendel University. An ethanolic extract from freshly harvested roots was subjected to liquid-liquid partitioning with *n*-hexanes, chloroform, ethyl acetate, and water, respectively. The chloroform fraction was chromatographed on RP-C18, yielding fifteen unique fractions. These fractions were evaluated for cytotoxicity and subjected to biological testing for NF-κB inhibition using the Quanti-Blue™ assay on THP-1 cells. Compounds from fractions exhibiting the highest anti-inflammatory activity were isolated by semi-preparative HPLC. The structures of the isolated compounds were elucidated by 2D NMR spectroscopy.

In conclusion, we successfully isolated and identified a total of nine prenylated isoflavonoids from fractions exerting the most prominent bioactivities, which will undergo further biological testing.

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Chemical and genetic variability among accessions of *Cicerbita alpina* (L.) Wallr., an alpine plant with anthelmintic properties

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Cicerbita alpina (L.) Wallr., a perennial plant species belonging to the Asteraceae family, is typically found thriving in the challenging alpine environment, particularly at altitudes more than 1000 metres above sea level. While traditionally recognised and utilised as a local delicacy, *C. alpina* has recently attracted significant scientific attention due to the discovery of its potent antiparasitic activity. In Europe, the use of chemical treatments to combat nematodes has been met with challenges. Many of these treatments have either been prohibited due to their detrimental environmental impact or have become increasingly ineffective due to the rise of drug resistance in these parasites. Consequently, the agricultural sector faces a critical need for novel, alternative anthelmintic treatments that are both effective and environmentally sound. The specialised metabolites present in extracts derived from *C. alpina* offer a compelling prospect for a sustainable and biologically based approach to nematode control.

A comprehensive analysis of the chemical diversity present within a unique *C. alpina* germplasm collection, encompassing samples originating from eight distinct natural populations scattered across the Italian Alps was performed employing state-of-the-art targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS-MRM) to identify and quantify the key metabolites present. This analysis revealed a predominance of several distinct classes of metabolites, among which were caffeic acid derivatives, sesquiterpene lactones, along with the flavone glycosides, as well as their corresponding aglycones.

To complement the chemical analysis, the genetic diversity of eighty individual *C. alpina* plants within the germplasm collection was assessed. This was achieved through the utilization of ten DNA molecular markers (Simple Sequence Repeats; SSRs) which were selected based on their informative nature and were transferred from two closely related species, *Cichorium intybus* and *Tanacetum parthenium*. This genetic analysis revealed a substantial degree of genetic diversity among the examined *C. alpina* populations, enabling the differentiation and grouping of the individuals into three distinct genetic clusters.

The combined chemical and genetic insights provide a robust foundation for future efforts aimed at the domestication of *C. alpina* through targeted plant breeding programs. These programmes can be strategically designed to ensure the consistent production of high-quality products, as well as to facilitate the successful cultivation of this valuable species in more diverse geographic regions, expanding its potential applications and contributing to the development of sustainable agricultural practices.

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P52

Nanoencapsulation of bioactive compounds from plant by-products to produce sensitive skin cosmetics

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NanoCosmos (www.nanocosmos.eu) represents a collaborative, international initiative uniting researchers and industry partners to drive the transformation of the bioeconomy through the synergistic application of bioscience and nanotechnology. The central objective of **NanoCosmos** is the establishment of a robust multidisciplinary and intersectoral network. This network will focus on maximizing the recovery of valuable bioactive compounds from a range of post-harvest by-products, thereby contributing to a circular economy model. The specific by-products targeted for this recovery effort include Saffron petals, Chamomile and Lavender post-distillation biomass, and *Rhodiola rosea* leaves. The **NanoCosmos** project aims to achieve key innovative outcomes including the development and implementation of green extraction techniques for the recovery of bioactive compounds, and the subsequent utilization of these compounds in the production of innovative nanocosmetic products specifically designed for sensitive skin applications. These advancements will be guided by the principles of the circular economy, ensuring resource efficiency and minimizing waste. The project's activities will encompass several interconnected areas:

- Knowledge Exchange: Facilitating the exchange of cutting-edge knowledge regarding the bioactive properties of compounds derived from plant by-products.
- Green Extraction Development: Developing and optimizing environmentally friendly extraction methods for the efficient recovery of bioactive compounds from the biomass sources.
- Nanoencapsulation for Cosmetics: Creating innovative biocomponents suitable for application in the cosmetics industry through the implementation of nanoencapsulation technology.

To accomplish these ambitious objectives, **NanoCosmos** will undertake a multifaceted approach. This will include a thorough evaluation and exploration of plant biodiversity, cropping technologies, and the diverse sources of by-products. Advanced chemical analysis techniques and green extraction technology will be employed. The recovered bioactive compounds and extracts will be analyzed using state-of-the-art metabolomics tools which will be complemented by comprehensive bioactivity and efficiency testing to validate the efficacy of the recovered compounds. Furthermore, the project will investigate the impact of the drying process, both on the original plant material and the resulting by-products, on their chemical profiles. Finally, the bioactive extracts and compounds will be incorporated into novel formulations for skin cosmetics using nanotechnology.
Beyond the core scientific research, **NanoCosmos** is committed to maximizing the broader impact of its work by fostering long-term, sustainable network relationships between academic institutions and industry partners. This collaborative approach will ensure the effective translation of research findings into practical applications and contribute to the **growth of a sustainable bioeconomy.**

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P53

Tissue-specific expression of genes related to triterpenoid saponin biosynthesis in *Agrostemma githago* L.

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A two component-system (TTS) is responsible for the toxicity of corn cockle seeds (*Agrostemma githago L*). The TTS consists of type I ribosome-inactivating proteins (RIPs) and specific triterpene saponins [1]. *A. githago* has been found to produce triterpenoid saponins such as gypsogenin and quillaic acid glycosides. The biosynthesis of saponins has not been studied in *A. githago* to date. To get insight into the mechanisms regulating the biosynthesis of saponins at the transcription level, we performed expression analysis of genes encoding for respective enzymes in various organs during *A. githago* ontogenetic development. The plant material grown in the glasshouse of the Botanical Garden of Medicinal Plants (BGMP) at the Wroclaw Medical University (Poland) was used. Nucleotide sequences of genes putatively involved in the biosynthesis of triterpenes 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 *squalene synthase*, *squalene monooxygenase*, *β-amyrin synthase* as well as two cytochromes P450 homologous to *CYP716* and *CYP72*, was studied. Our work contributed to an elucidation of the triterpenoid saponin biosynthesis within plants of the carnation family. In the 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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P54

α-Glucosidase inhibitory activity of geranylated flavonoids from *Paulownia tomentosa* (Thunb.) Steud.

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Paulownia tomentosa (Thunb.) Steud. ("princess tree"), belonging to the Paulowniaceae family, is a traditional Chinese medicinal plant, which had been used for centuries as a component of remedies for various illnesses. It is a rich source of geranylated flavonoids and other secondary metabolites, that are currently being studied for their promising biological activities.

Diabetes mellitus (DM) is a serious chronic metabolic syndrome characterized by hyperglycemia, which is caused by insufficient insulin production or insulin resistance. DM affects millions of people worldwide and is currently a global public health problem. Although specific medication for the management of DM is used, new therapeutic approaches that minimize side effects are needed. A new potential strategy is the use of natural compounds, mainly due to their multiple targeting effects. One of the therapeutic approaches is to inhibit the enzyme α-glucosidase, which breaks down carbohydrates in the digestive tract.

Geranylated flavonoids were isolated from the chloroform portion of the ethanolic fruit extract of *P. tomentosa*. Compounds were tested for their inhibition of α-glucosidase, which was performed according to the standard method based on *p*-nitrophenyl-α-D-glucopyranoside as described previously [1]. The released *p*-nitrophenol was quantified spectrophotometrically at λ 405 nm.

Tomentone II, diplacol, and tomentone B exhibited the highest α-glucosidase inhibitory activity. Two of these compounds possess geranyl chain modified by cyclization forming a pyranoflavanone skeleton.

Geranylated flavonoids isolated from *P. tomentosa* have shown the ability to inhibit α-glucosidase, which is one of the possible targets for management of DM.

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P55

Evaluation of physicochemical properties of nanoemulsions containing Resveratrol

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Resveratrol (3,5,4'-trihydroxystilbene, RSV) is a natural polyphenolic stilbene compound. In recent years, it has attracted increasing scientific interest due to its broad therapeutic potential, particularly in the adjunctive treatment of diabetes, rheumatoid arthritis and various cancers [1]. Despite these promising effects, the clinical application of resveratrol is significantly limited, primarily due to its poor bioavailability. This is due to its poor pharmacokinetic profile, characterised by low aqueous solubility, limited photostability, rapid metabolism and a pronounced first-pass effect. Given these challenges, it is essential to protect such natural compounds and carefully design advanced pharmaceutical formulations that can enhance their stability and improve absorption in the body. One of the most promising approaches is the use of nanoscale drug delivery systems, which have the potential to overcome physicochemical and biological barriers by increasing solubility, protecting the active substance from degradation and enhancing its permeability across biological membranes. Nanoemulsions, in particular, offer advantages such as small droplet size, a high surface area, and the improved dispersion of lipophilic compounds such as RSV [2].

A total of 30 placebo formulations were prepared using an oil phase of Capryol 90, water and one of the selected surfactants (Tween 80, Kolliphor EL or Labrasol). A co-surfactant (propylene glycol, PEG 200 or triacetin) was then added at various concentrations. These formulations were then subjected to high-pressure homogenisation at 500 and 1000 bar. The resulting systems were then evaluated in terms of their stability, particle size, polydispersity index (PDI), osmotic pressure, refractive index, surface tension and viscosity. Based on these results, three placebo formulations were selected to prepare corresponding resveratrol-loaded nanoemulsions.

Particle size analysis revealed that the nanoemulsion comprising Capryol 90 (oil phase), Kolliphor EL (surfactant) and propylene glycol (co-surfactant) exhibited the smallest droplet size of all the tested variants. All nanoemulsions containing co-surfactants achieved resveratrol solubility of over 90%. The formulation incorporating triacetin exhibited the greatest solubility of the active compound, reaching 93%.

Resveratrol-loaded nanoemulsions obtained via high-pressure homogenisation are a promising drug delivery system for improving the bioavailability of this compound. Further optimisation of the formulation and physicochemical characteristics is required to develop an effective resveratrol dosage form.

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P56

The effect of aloe-emodin loaded liposome in photodynamic therapy

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The presenting study focused on investigating the enhanced photoactive properties of liposomal aloe-emodin for photodynamic treatment (PDT) on melanoma cell lines. Aloe-emodin is a natural anthraquinone found In the Aloe, Rheum and Rhamnus species. It displays a variety of biological activities, among which- a antineoplastic activity [1]. Aloe-emodin exhibits light-absorption capacity, thus it can be employed as a photosensitizer agent [2].
Photodynamic therapy (PDT) is a treatment that uses light-sensitive substances (photosensitizers, PS) in combination with light to destroy abnormal cells. Depending on the efficacy of PDT, apoptosis, necrosis or autophagy can cause cell death [3]. In this study, a unique aloe-emodin formulation has been used, in which aloe-emodin was encapsulated in liposomes, which exhibited high stability owing to the relatively rigid liposome bilayer, and therefore, low aloe-emodin diffusion properties. In this study, we examined the phototoxic and anticancer effects of liposome-encapsulated aloe-emodin on melanoma cell lines, including FM-6, Buf1286, and MUG-Mel2 cell lines. To assess the effectiveness of liposome aloe-emodin as a photosensitizer in PDT on melanoma cell lines, we performed MTT assay to measure the cytotoxicity of a natural compound, ROS analysis by confocal microscopy, and Real-Time PCR gene expression analysis. The melanoma cells showed increased phototoxicity after therapy compared to the normal cells in the MTT assay. Moreover, liposome aloe-emodin-based photodynamic therapy resulted in an increased ratio of apoptotic cells, increased ROS levels, and increased or decreased gene expression. In conclusion, the study concluded that aloe-emodin encapsulated in liposomes could be a promising and safe photosensitizer for melanoma photodynamic therapy, with potential for clinical development.

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P57

Natural substances in anticancer therapy

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Natural substances have the potential to prevent and treat many diseases and have been used for centuries, and thanks to their effects, they have attracted the attention of scientists. This is evidenced by the multitude of studies, both in vitro and in vivo, and the increasing number of clinical studies. Many studies show the anti-cancer effects of plants and the compounds or dyes found in them. These substances are a new therapeutic trend in the treatment of patients. They have anti-cancer, antiviral, antioxidant and anti-inflammatory properties. These compounds not only trigger apoptosis and inhibit cell development, but also demonstrate a reduced effect of general toxicity for the body, which results in fewer complications during therapy. Recently, we can observe a significant increase in publications in which the method of treating cancer is plant-based substances, such as: curcumin, hypercin, emodin, resveratrol, piperine or geraniol. Every day, researchers confirm the phenomenal effect of natural and plant substances on cancer cells.

One of the more popular substances of plant origin is curcumin. It comes from turmeric, commonly known as curcumin. Curcumin has a number of positive properties that affect the entire body. It is most often used in the case of digestive problems. It owes its positive effect to choleretic properties. It also has antioxidant, anti-inflammatory, antibacterial and anticancer properties. In the case of anticancer effects, studies confirm the health-promoting effects of curcumin: antioxidant (fights damage caused by free radicals), and is also associated with stimulating the death of cancer cells by reducing the level of Bcl-2 protein (which increases in the early stages of carcinogenesis). As described above using curcumin as an example, naturally derived compounds have a number of properties, including anti-cancer properties.

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Onion thiolanes: multifunctional modulators of oxidative stress, PPAR-γ2 signaling, and bacterial growth

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Onions (*Allium cepa* L.*)* are known for their sulfur-containing compounds with reported biological activities. In this study, 24 thiolane derivatives isolated from onions were evaluated for their potential effects on oxidative stress, metabolic regulation, NF-κB signaling, and antimicrobial properties. At a concentration of 10 μM, none of the compounds markedly impaired THP1-Blue™ NF-κB cell viability; however, several compounds demonstrated moderate cytotoxicity at higher concentrations (50 μM). Given the previously reported anti-inflammatory potential of sulfur-containing compounds, a reduction in NF-κB pathway activity was anticipated [1]. Most thiolanes showed minimal or no inhibition of LPS-induced NF-κB activation, indicating alternative anti-inflammatory mechanisms. Most reduced ROS levels under oxidative stress, though cepadithiolactones A1–A3 were mildly pro-oxidative. Selected thiolanes (A5–A7, B4, B5) activated the PPAR-γ2 pathway, achieving up to 26% of the activity induced by rosiglitazone. Thiolanes from onion exhibited limited antimicrobial activity against *E. coli* and *C. albicans* at the tested concentrations. However, certain thiolanes showed inhibitory effects against *S. aureus* subsp. *aureus*, with minimum inhibitory concentrations ranging from 3.125 to 6.25 mM. These findings suggest that onion-derived thiolanes possess moderate antioxidant capacity and metabolic modulatory properties, with selective antimicrobial effects and limited anti-inflammatory activity via NF-κB inhibition. Further studies are needed to elucidate alternative anti-inflammatory mechanisms and explore their potential applications in functional food development.

Acknowledgements
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P59

Metabolites from the liverwort-associated fungus *Penicillium concentricum*

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Natural products are recognized as the richest sources of novel drug molecules for combating diseases threatening humanity. Plants, especially, serve as valuable natural sources. Liverworts, belonging to lower plant groups, are among the oldest terrestrial plants descended from bryophytes. Numerous studies on liverworts aim to determine their phytochemical composition and biological activity. Consequently, many of their constituents have been reported and structurally identified. While research on the endophytes of higher plants has been promising, studies focusing on the bioactive metabolites of endophytic microorganisms associated with bryophytes have remained relatively limited.

The interactions between endophytic fungi and bryophytes are largely enigmatic. Although recent articles have begun to explore this area, research on the metabolites produced by endophytes is still insufficient. The mutualistic interactions between host plants and endophytes are diverse and complex. These interactions maintain a delicate balance that is crucial for the host plant's protection against external threats while enabling the production of bioactive natural products by the endophytes. These unique metabolites have demonstrated significant biological effects, making them promising candidates for future drug development.

Therefore, we emphasize the secondary metabolites obtained from *Penicillium concentricum*, isolated from the liverwort *Trichocolea tomentella*, based on HPLC dereplication and NMR-based metabolomics investigations, complemented by TOCSY studies. The current investigation provides an overview of the metabolites derived from the endophytic fungus *Penicillium concentricum* cultured in various media types, focusing on isolation and structural characterization regarding biosynthetic pathways. Future studies should aim to isolate different components using diverse media conditions and assess their potential biological activities.

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Isoflavones content and biological properties in different clover species: a comparative study

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In our previous study, we described the effects of red (*Trifolium pratense*), white (*Trifolium repens*), crimson (*Trifolium incarnatum*), and Persian (*Trifolium resupinatum*) clover sprouts on different breast and prostate cancer cell lines. We demonstrated that estrogen-dependent MCF-7 breast cancer cells and androgen-dependent LNCap prostate cancer cells were the most susceptible to the tested sprouts compared to hormone-independent cell lines. Notably, the observed cytotoxic effect was not related to the isoflavone content in the sprouts [1].

As a continuation, in the present study, we conducted a qualitative and quantitative analysis of isoflavone content in ten clover species. The plants were collected during the flowering period from natural habitats in the Małopolska region. Plant material was extracted for 60 minutes under a reflux condenser using 50% methanol, maintaining a raw material-to-solvent ratio of 1:125. The extraction conditions were optimized in a previous study using a fractional factorial design. The isoflavone content in the extracts was determined by the HPLC-UV-VIS method, while the antioxidant potential was assessed using the DPPH and FRAP assays. To investigate the effects of the extracts on the viability of estrogen-dependent MCF-7 and estrogen-insensitive MDA-MB-231 breast cancer cell lines the MTT assay was performed.

The highest total isoflavone content was found in extracts from *T. medium* (26.7 mg/g dry weight) and *T. pratense* (12.56 mg/g dry weight). The greatest diversity of isoflavones was observed in *T. medium* and *T. pratense*, with six different compounds identified. Interestingly, no isoflavones were detected in four of the tested species. The highest antioxidant potential in the DPPH and FRAP assays was exhibited by the extract of *T. arvense*. The examined clover extracts had a moderate but varied impact on cell viability, with *T. resupinatum* and *T. incarnatum* showing the highest effects.

Our results indicate that *T. medium* can be considered as a new, rich source of isoflavones. The observed antioxidant and cytotoxic activities of the ten clover species suggest their potential for chemoprevention of breast cancer.

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P61

Anticancer potential of herbs used in Polish traditional medicine

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Plants are a rich source of bioactive compounds with potential anticancer, antibacterial, antifungal, and antiviral properties, among others. Many species used in traditional Polish herbal medicine contain phytochemicals that may play a role in cancer prevention and therapy. Malignant tumors remain one of the leading causes of death worldwide, with lung and colorectal cancers being among the most prevalent. While chemotherapy remains the primary treatment for malignant tumors, its toxicity to healthy cells necessitates the search for alternative therapies. *In vitro* studies suggest that certain plant extracts can selectively induce cancer cell death, highlighting their potential as a source of safer cytostatic substances.

In this work, we studied the influence of plant aqueous and ethanolic extracts obtained from 15 herbs used in Polish traditional medicine (*Tanacetum vulgare, Matricaria chamomilla, Allium ursinum, Mentha × piperita, Urtica dioica, Hypericum perforatum, Taraxacum officinale, Trifolium pratense, Artemisia absinthium, Nasturtium officinale, Glechoma hederacea, Achillea millefolium, Angelica archangelica, Inula helenium, Valeriana officinalis*).

The anticancer activity of plant extracts was assessed using an *in vitro* model of colorectal cancer, employing Caco-2 human epithelial colon adenocarcinoma cells. Normal colon epithelial cells (CCD841 CoTr line) served as the control. Antitumor effects were evaluated via the XTT assay, which measures cellular metabolic activity and provides insight into the effects of the extracts on cell proliferation and viability. Extracts were tested at concentrations ranging from 25 to 200 µg/mL.

Aqueous extracts generally exhibited stronger and dose-dependent antiproliferative effects on cancer cells compared to alcoholic extracts. The latter mostly did not significantly inhibit cancer cell growth and often stimulated the proliferation of normal cells, particularly in the case of meadow clover and tansy at higher concentrations, suggesting regenerative potential. In contrast, many aqueous extracts suppressed cancer cell proliferation in a concentration-dependent manner, with the strongest effects observed for yarrow, peppermint, dandelion, elecampane, and watercress. While most aqueous extracts had limited impact on normal cells, watercress notably reduced their viability. Therefore, extracts from yarrow, peppermint, dandelion, and elecampane exhibited selective activity, supporting their potential as sources of anticancer agents.

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Pharmacy calls for aid. And history will answer!
Pharmaceutical-historical research as a source of new active compounds and synergies

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In the face of the rising antimicrobial resistance and stagnation in the discovery of new antibiotics, pharmaceutical sciences are in urgent need of unconventional solutions. One promising but underexplored path lies in the interdisciplinary cooperation between pharmaceutical research and the history of medicine. Historical sources, among others from the early modern period, offer a complex repository of therapeutic practices that differ significantly from current standards. This includes now-forgotten plant species, like for example *Valeriana celtica*, pre-modern processing techniques, like calcination or (dry) distillation, and alchemical preparations, like *Antimonium diaphoreticum* or *Cornu cervi philosophice praeparato*. All of them are ranging from metallic compounds to transformed organic substances—often combined in complex multi-ingredient recipes. While these remedies were shaped by humoral theory, they also reflect centuries of empirical refinement, environmental adaptation, and local (also indigenous) medicinal knowledge, vulnerable to erasure. Under the scrutiny of the interdisciplinary team, during meticulously designed historical reconstructions, the sole script of recipes becomes a tangible preparation, ready to be analysed for expected, and hoped for, new active compounds or synergies. Thus, historical reconstruction, far from being a purely academic exercise, is a valuable tool in the ongoing search for new therapeutic agents.

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Phytochemical profiling and *in vitro* propagation of *Buphthalmum speciosissimum* L. – a rare alpine endemic with significant medicinal potential

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Buphthalmum speciosissimum L. (syn. *Xerolekia speciosissima* (L.) Anderb., *Telekia speciosissima* (L.) Less.) is a rare endemic species from the southern Alps in northern Italy, representing the sole species of the genus *Buphthalmum* within the Asteraceae (tribe Inuleae, subtribe Inulinae) [1,2]. Despite its relation to medicinal taxa, the phytochemical composition and micropropagation potential of this species have only recently been studied. This study offers the first integrated analysis of its phytochemical and propagation characteristics.

HPLC-DAD-MS^n analysis of hydroalcoholic extracts from aerial parts identified a wide spectrum of caffeic acid derivatives, including mono-, di-, tri-, and tetracaffeoyl conjugates. Among the most abundant compounds were 3-*O*-, 4-*O*-, and 5-*O*-caffeoylquinic acids, 1,3-, 3,4-, 1,5-, 3,5-, and 4,5-di-*O*-caffeoylquinic acids, dicaffeoyl and tricaffeoyl hexaric acid isomers (I–IV), and a tetracaffeoyl hexaric acid. Uncommon acylated derivatives such as isobutyryl- and 2-methylbutyryl/isovaleryl-dicaffeoylquinic acids were also detected, highlighting the species' potential as a source of structurally diverse and bioactive phenolics [3]. HPLC-DAD analysis of the chloroform extract of *B. speciosissimum* identified three major constituents, characterized by ^{1}H NMR spectroscopy as innviscolide [4], 4,5-epoxy-4,5-*cis*-inunolide [5], and alantolactone [6]. Phenolic content was higher in soil-grown plants (61.7 ± 2.1 mg GAE/g in leaves, 80.1 ± 1.1 mg GAE/g in roots), emphasizing the influence of environmental conditions. Plants cultured on MS medium with 5.37 µM/L NAA and 13.32 µM/L BAP accumulated 38.0 ± 0.7 mg GAE/g, while those with 2.0 µM/L BA and 0.1 µM/L NAA showed slightly higher levels (42.2 ± 1.6 mg GAE/g). *In vitro* propagation was successfully established using nodal explants cultured on MS medium supplemented with 5.37 µM NAA and 13.32 µM BAP, yielding the highest shoot regeneration rate (8.39 ± 3.07 shoots/explant).

These results highlight both the phytochemical richness and the successful propagation potential of *B. speciosissimum*, supporting its conservation and future exploration as a source of bioactive natural products.

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Purification of hyperforin and adhyperforin by preparative HPLC from *Hypericum perforatum* L.

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The COVID-19 pandemic caused by the SARS-CoV-2 virus has highlighted the urgency of developing, alongside vaccines, effective antiviral compounds. In a previous study, we demonstrated the antiviral potential of hyperforin, one of the major metabolites of *Hypericum perforatum* L. (Hypericaceae). This compound showed pan-coronavirus activity with IC_{50} values ranging from 0.24 to 2.55 µM on four coronaviruses (SARS-Cov-2, SARS-CoV, MERS-CoV and HCoV-229E). Hyperforin was also active in primary human airway epithelial cells, a preclinical model [1]. However, the initially tested standard hyperforin also contained 20% adhyperforin. Therefore, the objective of the present study is to develop an HPLC separation method to test the activity of these two prenylated phloroglucinol derivatives and other subsequently purified analogues.

A solid/liquid extraction of *H. perforatum* aerial parts was first performed in methanol, followed in a second step by a liquid/liquid extraction with dichloromethane/water. The separation gradient optimization was first performed by analytical HPLC before transposition to preparative HPLC. Several columns and gradients were tested, and the Kinetex F5 column was chosen as the stationary phase. The developed mobile phase was a segmented gradient with water and acetonitrile. The purified products were then analysed by UHPLC-UV-MS and 1D and 2D NMR.

Fractionation of the DCM sub-extract allowed us to purify hyperforin with a purity of 83% and adhyperforin with a purity of 96%. We were also able to isolate an additional compound with a purity of 84%. This compound has a retention time close to hyperforin and an identical molecular mass ($[M+H]^+$ *m/z* 537 in positive mode). We now need to improve the purification of hyperforin from the DCM sub-extract and continue the elucidation of the purified compounds.

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Chemical investigation of bioactive specialized metabolites from *Trichoderma* spp.

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Trichoderma spp. are endophytic fungi broadly used in agriculture for the biocontrol of different pathogens affecting crops, such as fungi, nematodes, and insects [1]. *Trichoderma* strains can be biosynthesized metabolites that can inhibit plant pathogens in the soil through their highly potent antimicrobial activity [2]. One of the major challenges in agricultural crop production is the capability of parasitic nematodes, such as *Meloidogyne* spp., to cause considerable damage and yield losses in vegetable crops invading plant root tissues and determine the formation of galls or "knots" which disrupt the plant's ability to absorb water and nutrients [3].
In particular, *Meloidogyne incognita*, a globally distributed root-knot nematode, attacks more than 2,000 plant species including tomato, and Indian ginseng.[1]
The nematicidal activity of *Trichoderma* spp. against the plant-parasitic nematodes *Meloidogyne* spp. has been reported in guava, okra, mung bean [1]. To the best of our knowledge, there are no reports about the use of *Trichoderma parceramosum* and *T. citrovirinide* as biological control agents against *M. incognita.*
Based on this background this study aims to investigate crude extracts of both fungi strains to isolate compounds potentially responsible for the nematicidal properties. The hydroalcoholic extract of fungal cultures in Solid State Fermentation (SSF) was subjected to liquid-liquid repartition, first with n-hexane and then with dichloromethane. This latter fraction showed strong activity against *M. incognita*. The purification of most active fractions, through chromatographic techniques, led to the isolation of heterocyclic lactones as 5-(1-Hydroxyethyl)oxolan-2-one, 4-methyl-2,3-dihydropyran-6-one, 4-hydroxy-4-methyloxan-2-one. 1D-NMR experiments and exhaustive 2D-NMR investigations allowed us to determine the structure of the pure compounds. The nematicidal activity of pure compounds against *M. incognita* has been evaluated.

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P66

Identification of natural ligands from mediterranean plants for DNA secondary structure modulation

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Phytochemicals are widely used as drugs or as scaffolds for the design of new molecules for the treatment of various ailments, including cancer[1]. Metabolites characterized by aromatic moieties have been pinpointed as possible anticancer compounds, since they are able to interact with the regions of G-quadruplex DNA (G4) located in the promoters of many disease-relevant oncogenes, thus interfering with the expression of oncogenes[2].

In the context of the SMALL project, aimed at identifying bioactive small molecules from plants as ligands of DNA secondary structure, the results of the metabolite profiling of numerous plants from Mediterranea area is herewith reported. Mediterranean plants growing in Southern Italy have been selected. Different parts of plants, including roots, leaves, stems, seeds, fruits, etc., have studied by 1D and 2D-NMR using a metabolomic protocol.

The lyophilized plant samples have been extracted with a mixture of methanol:water (1:1). The obtained extracts were enriched in aromatic compounds thanks to a purification step carried out using SPE cartridges. The obtained fractions were analyzed by NMR.

Among the analysed plants, there were several belonging to the Fabaceae family. These samples were characterized by the presence of flavonoids and several other aromatic compounds. For what concerns flavonoids, they were mainly glycosides of quercetin and/or kaempferol. Plants belonging to the Asteraceae family were also interesting, since they were characterized by the presence of flavonoids and several aromatic compounds of terpenoid nature. Further extracts of interest were identified from other plant sources.

The fractions enriched in aromatic compounds will be screened for their capability of interacting with G4 regions and the purification of target compounds will be carried out using different and complementary chromatographic methods.

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P67

Effect of chicory (*Cichorium intybus* L.) sward on fatty acids profile in different lamb's tissues

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This study investigated the influence of a chicory (*Cichorium intybus*) sward, rich in bioactive compounds such as polyphenols and sesquiterpene lactones, on the fatty acid composition of rumen fluid, liver, muscle, serum, and subcutaneous fat tissues in lambs. Bioactive compounds are increasingly recognized for their capacity to modulate lipid metabolism, inhibit ruminal biohydrogenation, and enhance the deposition of biologically valuable fatty acids (FAs), such as n-3 polyunsaturated fatty acids (PUFAs), in animal tissues. We hypothesized that chicory supplementation could improve the fatty acid profile of lamb tissues by reducing ruminal biohydrogenation and promoting the accumulation of health-beneficial FA. Sixteen Tsigai lambs (male: female, 1:1; 2-3 months old; initial body weight 13.61 ± 2.85 kg) were randomly assigned to two grazing treatments: a control group grazing natural meadow pasture, and an experimental group grazing chicory-enhanced pasture (¼ chicory, ¾ natural meadow). All lambs additionally received a commercial concentrate (300 g DM/day) and ad libitum meadow hay. The experiment lasted 145 days (May to October 2023), after which animals were slaughtered and samples collected. Rumen fluid was sampled from five rumen locations, and tissues (*Longissimus dorsi* muscle, subcutaneous fat, liver, and blood) were collected. Fatty acid profiles were analyzed by gas chromatography, and statistical analyses were conducted using ANOVA and Student's t-test in RStudio (Version 2022.12.0+353). Chicory supplementation significantly decreased saturated fatty acid (SFA) concentrations in the rumen fluid, liver, and serum ($p<0.05$), confirming the modulating role of bioactive compounds on ruminal lipid metabolism. Although SFAs numerically increased in the *Longissimus dorsi* muscle, a significant enhancement of linolenic acid (LNA) content was observed. Furthermore, a notable reduction in the n-6 to n-3 FA ratio was detected in lambs grazing chicory sward, aligning meat quality with contemporary consumer demands for healthier, n-3-rich products. These results underscore the potential of BAC-rich forage such as chicory not only to improve the nutritional profile of meat but also to promote sustainable livestock production.

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Molecular mechanisms of cross-talk between light signaling and aquaporins in plant tolerance to abiotic stress

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In plant seeds, aquaporins control the specific transport of water and some other small molecules across membranes and are involved in various physiological processes. Aquaporins belong to a large family of channel proteins called major intrinsic proteins (MIPs). There are currently five major subfamilies recognised in plants based on sequence similarities. The plasma membrane intrinsic proteins (PIPs), the tonoplast intrinsic proteins (TIPs), the NOD26-like intrinsic proteins (NIPs), the small basic intrinsic proteins (SIPs), and the plant-specific subfamily of X-intrinsic protein (XIPs). In the tomato genome, 47 aquaporin genes are present; some of them might be pseudogenes or expressed in particular conditions. Above all, PIPs and TIPs have been proven to participate in responses to abiotic stresses, such as drought, salinity, or chilling. In addition, aquaporin gene expression can be regulated by these abiotic stresses. Interestingly, the expression of aquaporins can also be modulated by irradiance, with theirent expression patterns strongly dependent on the wavelength of visible light.

Recently, we have revealed a relatively new phenomenon of regulation of gene expression of aquaporins by light of different wavelengths during the germination of tomato seeds. Seed germination is a physiological process which is influenced by light. Although very little is known about the perception of blue light by seeds, it was shown that in tomatoes blue light mostly reduces seed germination. This finding prompted our further research, which was directed at analysing the expression of aquaporin genes to uncover the mechanisms of blue light-induced reduction of tomato seed germination. The second part of our research was focused on the regulation of aquaporin transcription under the treatment of AQP-blockers or during osmotic stress. For the analysis of aquaporin gene expression in tomatoes we chose RNA sequencing because it is a very robust method that provides information about the presence and absolute quantity of every transcript. In addition, for aquaporin genes showing significant changes during the experimental conditions, the expression was verified by qRT-PCR. The next objective of our project is to verify these findings at the protein level and consider the role of post-translational modifications of the proteins.

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Controlling Sugar Transport: Future of Agriculture?

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What if we could manipulate sugar flow? Sugars play an irreplaceable role in plant metabolism as an energy source and as signaling and building molecules essential for other biochemical processes. This makes sugar transport a fundamental process in plant physiology. Moving sugars from source tissues to sink tissues is a complicated and tightly regulated system. This makes it challenging to create biotechnological tools that could improve crop yields, plant resistance to stress, or increase the nutritional value of crops. To create tools to control sugar transport for agriculture, we need a deeper understanding of how it is controlled. We recently discovered CANAR, the first receptor identified that can regulate sugar transport from source to sink tissues in plants. CANAR offers a new level of control by adjusting the rate and destination of sugar transport. The ability to manipulate sugar transport in an organ-specific manner could lead to crops with boosted productivity and improved nutritional content in desired plant tissues. These unique properties make CANAR a highly promising target for future biotechnological applications. At this point, the exact molecular mechanism of the CANAR effect on sugar transport is still unknown. The precise knowledge of molecular components of the CANAR signalling pathway is an essential prerequisite for developing next-generation tools to boost crop yield.

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Cytotoxic effect of *Agrostemma githago* root and herb extracts against normal and cancerous cell lines

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Corncockle (*Agrostemma githago* L.) is a well-known weed. Contemporary, it has been eliminated from fields and has become more popular as a component of wildflower meadows. In the past, due to its various properties, it was used in traditional medicine for the treatment of ulcers, toothache, cough, and rashes.

It is known, that the toxic effects are caused by saponins and ribosome-inactivating proteins (RIPs), which are primarily found in the seeds of corncockle. However, the chemical compounds potentially responsible for the beneficial effects of the aerial parts and roots of *Agrostemma githago*, as suggested by its traditional use, have yet to be identified.

Phytochemical analysis was performed with uHPLC-DAD-ESI-qTOF-MS/MS.

Cell viability assay has been performed on skin-related cell lines, including the human melanoma cancer cell line A375 and the human epidermoid squamous carcinoma line A431. In addition, studies have been carried out on non-cancerous cell lines—fibroblasts and keratinocytes (HaCaT).

Upon incubation with extracts, the viability was determined by an MTT assay.

In saponins-rich extracts, the cytotoxicity on normal and cancerous cell lines was observed in dose-dependent manner. The highest action was observed with butanone extract.

Acknowledgments

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P71

No RIPs, no RIP. *In vitro* safety assessment of *Agrostemma githago* oil in human dermal cell lines

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Globally, vegetable oils are produced from over 350 plant species, but the majority of commercial vegetable oil production relies on about 10–12 key species.
The primary sources include *Elaeis guineensis* (palm oil), *Glycine max* (soybean oil), *Brassica napus* (rapeseed / canola oil), Helianthus annus (sunflower oil), *Arachis hypogaea* (peanut oil), *Cocos nucifera* (coconut oil), *Olea europaea* (olive oil), and *Zea mays* (corn oil).
One of the new sources of economically-important oil could be common corn-cockle. This plant has never been analyzed in this respect. In the past, it was considered a serious toxicological hazard.
The plant material was collected after formation of seeds from the experimental culture from Wroclaw Medical University Botanical Garden of Medicinal Plants. The seeds of the *A. githago* were dried and grinded with an electric mill. Seeds were extracted with the hexane via ultrasound-assisted extraction process. In next step mixtures were filtered through filter paper, and extract was concentrated by rotary evaporator. After transesterification, The fatty acids methyl esters were analysed with GC-MS/MS system.
Cell viability assay has been performed on skin-related cell lines, including the human melanoma cancer cell line A375 and the human epidermoid squamous carcinoma line A431. In addition, studies have been carried out on non-cancerous cell lines—fibroblasts and keratinocytes (HaCaT).
According to GC-MS, main constituents were: methyl palmitate, Me.C18:0, Me.C18:1n10, Me.C18:2n6, and Me.C18:3n3.
Upon incubation with fatty acids extract the viability was determined by an MTT assay. In all tested concentrations (normal and cancerous cell lines) the viability did not fall above the 80%. This demonstrates the relative safety of obtained extracts. Consequently, the use of fatty acids from corn-cockle as a composition for ointment bases appears safe.

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Stabilizing the baseline: reference gene evaluation in three invasive *Reynoutria* Species

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Accurate normalization using stably expressed housekeeping genes (HKGs) is essential for reliable relative quantification. HKGs employed as internal controls can exhibit variable expression across developmental stages, tissues, and experimental conditions, which may bias the results. Thus, systematic validation of candidate reference genes is necessary.

The *Reynoutria* species are surprisingly invasive and chemically rich, producing a diverse array of specialized metabolites of pharmacological interest. Studies on stable reference genes for knotweed species remain limited, with most efforts focused on *R. japonica* and *R. multiflora*. To enable accurate gene expression analysis related to specialized metabolism and natural product biosynthesis, we aimed to identify the most stable reference genes across commonly found species in Poland: *R. japonica*, *R. sachalinensis*, and *R. × bohemica* [1]. All twelve reference genes proposed by Wang et al. [2] for *Polygonum cuspidatum* (syn. *R. japonica*) were evaluated across three *Reynoutria* species and three tissue types (rhizomes, leaves, and flowers), with all samples collected during the peak flowering season for each specimen. Primer sets from the original study and newly designed primers were utilised in the analysis. The integrated expression stability of candidate reference genes was assessed using RefFinder (https://blooge.cn/RefFinder/), which incorporates geNorm, NormFinder, BestKeeper, and the comparative ΔCt method [3]. While gene stability differed among tissue and species groups, the analysis allowed for the identification of the two most appropriate reference genes for normalization.

This study is the first systematic attempt to identify a universally stable housekeeping gene among the commonly compared *Reynoutria* species, laying the groundwork for future transcriptomic and functional studies in this chemically rich yet underexplored genus.

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P73

From seedling to seed: tracking *C*-glycosyl flavonoid biosynthesis in *Agrostemma githago* L.

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While the Caryophyllaceae family is renowned for its saponins, our earlier research on *Agrostemma githago* L. revealed a considerable quantity and variety of flavonoid *C*-glycosides (FCG), including orientin, isoorientin, vitexin, and isovitexin.
C-glycosides possess various medicinal properties [1]. Although less recognized than the more prevalent *O*-glycosides, our understanding of their biosynthesis in Caryophyllaceae is even more limited. This study aims to augment understanding of the essential phases of FCG biosynthesis and its molecular regulation. We have conducted expression analyses of genes encoding the respective enzymes across various organs throughout the *A. githago* ontogenetic development. The plant material was grown in the Botanical Garden of Medicinal Plants (BGMP) glasshouse at the Wroclaw Medical University (Poland). RNA-seq [2] has identified nucleotide sequences of genes presumably involved in the *C*-glycoside biosynthetic pathway. This includes genes originating from the general phenylpropanoid pathway, extending through the flavone biosynthesis pathway to hypothetical endpoints *C*-glycosylflavones. Selected sequences were employed for PCR primer design. Our study revealed the organ-specific expression of genes encoding for two isoforms of *phenylalanine ammonia-lyase* (*PAL*), *cinnamate 4-hydroxylase* (*C4H*), two isoforms of *4-coumarate-CoA ligase* (*4CL*), two isoforms of *chalcone synthase* (*CHS*), *chalcone isomerase* (*CHI*), and *flavonoid 3'-hydroxylase* (*F3'H*).

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P74

Computational elucidation of natural anthraquinones as photosensitizers

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Photodynamic therapy (PDT) is a versatile clinical treatment used in oncology, antimicrobial therapy, and dermatology, relying on light-activated photosensitizers (Ps) to induce selective photochemical damage in diseased tissues. The effectiveness of PDT depends on the efficient formation of the Ps's triplet excited state through intersystem crossing (ISC), typically accelerated by the presence of heavy atoms. However, their inclusion raises concerns about toxicity, cost, and environmental impact, prompting a shift toward heavy-atom-free alternatives. Accordingly, recent developments focus on π–conjugated frameworks that enhance ISC through orthogonal charge transfer in donor-acceptor dyads [1] or utilize singlet fission to generate multiple triplet excitons from a single photon [2]. Aromatic systems also enable efficient two-photon absorption, improving tissue penetration and spatial resolution within the therapeutic window [3].
Nature provides valuable molecular scaffolds for such applications. For instance, coumarins have been modified into efficient, mitochondria-targeting dyes [4]. Anthraquinones,another class of photoactive phytochemicals, are also gaining interest, though their optimization is currently limited by a lack of fundamental photophysical data [5].
To bridge this gap, the study applied molecular modeling to explore the photophysical behavior of selected anthraquinones. Key properties, such as one- and two-photon absorption, excited-state dynamics, and DNA intercalation potential were assessed, taking into account acid-base speciation arising from the acidity of aromatic hydrogens. These insights support the positioning of anthraquinones as viable templates for heavy-atom-free photosensitizers with the potential to address critical challenges in PDT, including hypoxia resilience, microbial resistance, and affordability.

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Experimental and computational elucidation of the antioxidant properties of flavonoids from the *Malvaceae* Family: the impact of glycosylation and glucuronidation

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Oxidative stress, driven by an imbalance between reactive oxygen species and antioxidant defenses, underpins chronic diseases such as cancer, cardiovascular disorders, and neurodegeneration. Natural flavonoids, with their safety and multifunctional bioactivity, present viable alternatives to synthetic antioxidants. This study elucidates the antioxidative mechanisms and efficacy of three flavonoid derivatives from the *Malvaceae* family [1] - gossypetin, gossypin, and hibifolin - through integrated experimental assays and computational analyses.
Quantitative evaluation using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [2] radical scavenging and ferric-reducing antioxidant power (FRAP) [3] assays demonstrated that gossypetin exhibits superior activity ($TEAC_{DPPH}$ = 111.5 mM/g; $TEAC_{FRAP}$ = 155.2 mM/g), while gossypin ($TEAC_{DPPH}$ = 41.7 mM/g; $TEAC_{FRAP}$ = 126.3 mM/g) and hibifolin ($TEAC_{DPPH}$ = 40.0 mM/g; $TEAC_{FRAP}$ = 94.7 mM/g) showed moderate efficacy. Computational modeling, encompassing quantum mechanical calculations and based on acid-base speciation analysis, revealed that gossypetin's hydroxyl-rich backbone enhances radical scavenging *via* hydrogen atom transfer, whereas glucosyl (gossypin) and glucuronyl (hibifolin) substitutions introduce steric hindrance, limiting hydroxyl group accessibility and reducing anti-DPPH activity. However, these glycosidic modifications preserved electron transfer capacity in FRAP, highlighting structure-dependent antioxidative mechanisms.
These findings position gossypetin as a potent therapeutic candidate for oxidative-stress-mediated pathologies, while gossypin and hibifolin, with their enhanced solubility and stability, are suited for aqueous formulations and nutraceuticals requiring extended shelf-life. The study underscores the importance of targeted structural optimization in designing antioxidants for specific biomedical and industrial applications.

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P76

Genetic transformation of Japanese knotweed (*Reynoutria japonica* Houtt.) with wild strains of *Rhizobium rhizogenes*

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Reynoutria japonica Houtt. (also known as *Fallopia japonica* (Houtt.) and *Polygonum cuspidatum*), commonly named Japanese knotweed, is a plant that belongs to the Polygonaceae family. *R. japonica* has been used in traditional Asian natural medicine, and its rhizome is recognized in the Chinese Pharmacopoeia as a medicinal raw material with numerous applications as it contains phenolic derivatives in high concentrations. *R. japonica* can be a rich source of phenolic compounds, but its biomass from wild habitats does not meet the standards of pharmaceutical-grade plant raw material due to heavy metals contamination. Therefore, to obtain plant material that is free of harmful contaminants and rich in phenolic compounds from Japanese knotweed tissue, *in vitro* cultures are a useful tool [1]. Moreover, the establishment of hairy root plant cultures through genetic transformation using wild strains of *Rhizobium rhizogenes* bacteria could be a potent method for acquiring *R. japonica* underground organ cultures that would be rich in phenolic derivatives.
The research aimed to obtain hairy roots of *R. japonica* and establish their growth efficiency in different conditions along with the ability of polyphenolic compounds synthesis.
Genetic transformation of *R. japonica* plants was performed by using a wild strain of *R. rhizogenes* A4 (ATCC 31798). This type of transformation is used to produce fast-growing, metabolite-rich secondary root cultures, in which bacterial oncogenes can act as endogenous, continuous elicitors. The presence of T-DNA (*rol*A, *rol*B, *rol*C, and *rol*D genes) in clone RJ10 [2] was verified by PCR analysis of the DNA isolated from transformed hairy root cultures of *R. japonica* clone RJ10 as compared to the non-transformed plants. Biomass growth rates were determined. The total content of phenolic compounds and level of phenolic acids, flawan-3-ols and flavonoids were assessed with the use of DAD- HPLC. The results obtained for the RJ10 clone were compared to those obtained for non-transformed plants grown *in vitro* in a liquid medium.
This is the first scientific report to demonstrate the successful genetic transformation of Japanese knotweed and its suitability for the synthesis of biologically active phenolic compounds [2].

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P77

Transformed roots of *Reynoutria japonica* in bioreactors - new perspectives for obtaining secondary metabolites with biologically active properties

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Phenolic compounds belong to an important class of plant secondary metabolism products, which have important physiological functions throughout the plant life cycle and significant applications in medicine. Representatives of such metabolites are catechins, in which, the Japanese knotweed (*Reynoutria japonica* Houtt.), the subject of this study, is rich [1]. Catechins have strong antioxidant, anti-inflammatory, anticancer, and antibacterial activity. Numerous studies show that the use of natural compounds, such as phytochemicals, is a promising strategy for the treatment of many diseases.

Therefore, the presented study aimed to establish hairy root cultures of *R. japonica* in balloon and spray bioreactors and to compare their growth and secondary metabolites content in relation to standard conditions - shaken cultures of *R. japonica*. The transformed roots culture of *R. japonica* (clone RJ10) was established after Japanese knotweed transformation with the wild-type strain of *Rhizobium rhizogenes* A4 (ATCC 31798) [2]. To determine how bioreactor use affects the metabolism and physiology of *R. japonica*, the material grown *in vitro* in this study was compared with the material from the rhizomes of the plants grown in *ex-vitro* conditions. Biometric measurements and antioxidant properties were determined using CUPRAC and DPPH methods. Detailed analysis of specific secondary compounds abundance was performed using DAD-HPLC. Bactericidal properties (minimal inhibitory and bactericidal concentrations) against *Staphylococcus aureus* ATCC 25923 of *R. japonica* clone RJ10 extracts from hairy roots grown in the above-mentioned conditions were evaluated using microdilution methods.

The results showed an increased accumulation of flavan-3-ols (catechins) in hairy root tissue from bioreactors compared to standard shaken culture. In addition, hairy roots exhibited enhanced antioxidant activity and bactericidal properties compared to roots grown *ex vitro*.

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P78

Development of coupled gas chromatography and photoacoustic detection methods for measuring endogenous ethylene in plants

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We created a method based on coupled gas chromatography (GC-FID) and photoacoustic detection (ETD-300) for the measurement of ethylene production in plants with induced endogenous levels. Both of these types of devices have been used in the past to measure ethylene production in plants, but a detailed comparison of the results of these approaches and an attempt to merge them into a single method have not yet been published. We aimed to develop a method, that would combine the advantages of both approaches. While the use of GC has been proven for a long time to be very effective and requiring small amounts of sample gas, photoacoustic detection provides a more sensitive alternative. Our method, unlike typical methods using photoacoustic ethylene detection, involves measuring plant fresh mass and thus we determine the results per unit of fresh plant weight. This allows us to recognize whether the change in ethylene production was due to an increase in plant fresh weight induced by the test compound and/or whether the treatment affects ethylene production *per se*. Our method is also unique in that unlike typical methods where plants are grown on treated media, we grow them on untreated media and the treatment is applied at a precise time interval. This allows us to eliminate the effect of the treatment on plant germination and thus we can more accurately simulate the effect of the treatment under field conditions. Another advantage is that the subsequent use of the two methods on the same sample allows us to recognize machine errors that would otherwise be attributed to biological variability. Because of this approach, we are also able to save the samples for further hormonal measurements by mass spectrometry. The method has been successfully tested for use with *Arabidopsis* plants treated with compounds known from the literature to affect ethylene production (ACC - see attached graphs, AVG, AIB, ethephon, and others). Although the machines do not provide numerically identical values, the observed trends are identical and the values are in the same order.

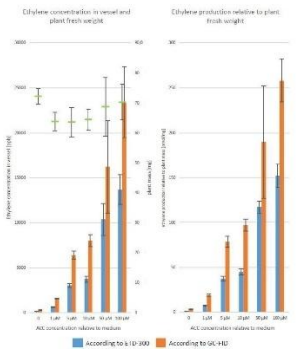

Figure Increase of ethylene production in *Arabidopsis* plants treated with different doses of ACC measured by the coupled method. Values according to GC-FID are in orange, according to ETD-300 in blue.

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Development and Validation of an HPLC-UV Analytical Method for Cannabinoid Determination in Oils and Cosmetics

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The increasing availability and popularity of cannabis-based commercial products necessitate standardised analytical methods for precise cannabinoid quantification. This study developed and validated a robust HPLC-UV method for quantifying CBD, CBN, THC, and their acidic precursors (CBDA, THCA) in oils and cosmetic products. Initially validated for MCT oil, the method is being extended to hemp seed oil, olive oil, and cannabinoid-infused cosmetics, showing promising results.

Sample preparation was optimized using various solvents, dilution ratios, and extraction volumes to maximize recovery. Chromatographic separation was achieved on an Agilent 1260 Infinity HPLC with a C18-AR column (30°C), using isocratic elution (acetonitrile/0.5% acetic acid, 75:25 v/v), 1.5 mL/min flow, and 220 nm UV detection.

Validation was conducted according to International Council for Harmonisation (ICH) Q2(R2) [1] guidelines for cannabinoids in MCT oil. Analytical performance parameters, including accuracy, intra- and inter-day precision, linearity, sensitivity, limit of detection (LOD), and limit of quantification (LOQ), were assessed. The method demonstrated acceptable accuracy (101%–114%), precision (RSD <15%), and linearity (R^2 = 0.99) over cannabinoid concentrations ranging from 0.03 to 0.5 mg/mL. Sensitivity assessments revealed an LOD of 0.01 mg/mL for all cannabinoids and LOQs of 0.03 mg/mL for CBD and CBN, and 0.06 mg/mL for THC. The method was adapted for hemp seed oil with modified sample prep and chromatographic conditions. While validation is ongoing, early results suggest suitability for olive oil and cosmetics. This HPLC-UV method shows strong potential for routine quality control of cannabinoid products.

Refernece

Committee for Medicinal Products for Human Use. ICH Q2(R2) Guideline on validation of analytical procedures. 2023.

P80

Exploring the triterpenoid and steroid profiles of *Gingko biloba* leaves: implications for their potential functions

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Ginkgo biloba L., commonly known as ginkgo, is one of the oldest living species of gymnosperm trees. It is native to East Asia but is widely cultivated across the United States and Europe, where it is often planted in cities as an ornamental tree due to its high tolerance to pollution. The seeds, leaves, and nuts of ginkgo have long been used in traditional Chinese medicine to treat various ailments, including dementia, asthma, bronchitis, and kidney and bladder disorders. In Europe, ginkgo leaf extracts or powdered leaves are among the best-selling herbal preparations, commonly used as dietary supplements for memory improvement and for treating mild age-related dementia and peripheral vascular disease. Ginkgo leaves contain two main groups of bioactive constituents: phenolic compounds, including flavonoids and proanthocyanidins; and terpenoids, primarily diterpene and sesquiterpene lactones, such as ginkgolides and bilobalides. However, data on the presence of other classes of terpenoids, such as steroids and triterpenoids, in ginkgo leaves are scarce. The study aimed to analyse triterpenoid and steroid content of *G. biloba* leaves collected at the mature stage (October-November) from both young and old trees growing in two locations in Warsaw, Poland. The leaves were dried and extracted using diethyl ether through an ultrasound-assisted technique. An additional objective was to determine the content of ginkgo leaf cuticular waxes, which were obtained through short-time immersion in chloroform. All extracts were fractionated by preparative adsorption chromatography and analyzed using gas chromatography-mass spectrometry (GC-MS). The results revealed the presence of two typical phytosterols, campesterol and sitosterol (along with its derivative, sitosterol acetate), as well as cycloartanol (the saturated form of a sterol precursor), and two steroid ketones, tremulone and sitostenone. The same compounds were also detected in the leaf cuticular waxes. Regarding triterpenoid compounds, the occurrence of oleanane- and ursane-type constituents was confirmed in ginkgo leaves, specifically oleanolic acid, and its methyl ester, as well as α-amyrin, ursolic acid, and ursonic (3-oxo-ursolic) acid. Small amounts of triterpenoid acids were also detected in leaf cuticular waxes. Although the relatively small content of triterpenoids is unlikely to significantly influence the pharmaceutical properties of ginkgo leaf extract, it cannot be excluded that they may act synergistically with some phenolic compounds. Their presence in leaf cuticular waxes could be important for the mechanical properties of the leaf surface layer, enhancing resistance to external factors, including abiotic and biotic stresses.

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Unveiling the triterpenoid diversity in oaks: a comparative study of *Quercus robur* and *Quercus rubra*

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Quercus trees (commonly known as oaks) belong to one of the most significant genera in the Northern Hemisphere. The primary parts of these trees utilized in various industries are wood and bark; acorns are used as animal feed; and leaves are employed to prepare infusions for medicinal or nutritional purposes. *Quercus* species are known for their complex phytochemical composition, which includes bioactive compounds responsible for antioxidant, antitumoral, anti-inflammatory, antidiabetic, and antimicrobial properties. The study aimed to compare the steroid and triterpenoid profiles of two representative oak species: *Quercus robur* and *Quercus rubra*. *Q. robur* is native to Europe, western Asia, and northern Africa, and is widespread in Poland both in natural and urban habitats. *Q. rubra* (red oak), native to North America, was introduced to Europe in the 17th century, and it is currently classified in Poland as a moderately invasive alien species. Leaf samples of *Q. robur* and *Q. rubra* were collected at fully maturity from a suburban forest in Wawer, Poland, The samples were air-dried, powdered, and subjected to ultrasound-assisted extraction using diethyl ether. The extracts were then fractionated by preparative adsorption chromatography and analyzed by gas chromatography-mass spectrometry (GC-MS).

A similar steroid profile was identified in leaf extracts from both oak species, with sitosterol as the predominant compound, followed by campesterol and stigmasterol, as well as two steroid ketones, tremulone and sitostenone. The triterpenoid composition was complex, comprising compounds from the lupane, oleanane, ursane, taraxerane, and friedelane types. Notably, taraxerol and taraxerone were detected exclusively in *Q. robur* samples, whereas fernenol and simiarenol were present only in *Q. rubra*. Additionally, the total triterpenoid content was significantly higher in *Q. rubra* (accounted for approx. 2 mg/g d.w.), exceeding that of *Q. robur* (0.8 mg/g) by 2.5-fold. In *Q. robur*, lupeol and taraxerol were the predominant triterpenoids, while in *Q. rubra*, fernenol, friedelin, and simiarenol were the most abundant. Extracts from both species contained small amounts of betulinic, oleanolic, and ursolic acids. The results of this study highlight significant distinctiveness in the triterpenoid profiles of *Q. robur* and *Q. rubra.* These distinctions could be valuable for chemotaxonomic classification of the species. Given the complex composition of triterpenoids, which exhibit various bioactivities, it can be concluded that extracts from *Quercus* leaves may have potential as medicinal nutraceuticals or could be utilized in the development of novel functional foods.

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Some descriptors to distinguish specialty coffees processed by spontaneous and induced methods

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We investigated some descriptors for distinguishing of various coffee processing methods, including spontaneous methods (natural, washed, and honey) and induced (anaerobic fermentation and carbonic maceration). Fatty acid (FA) composition and sensory properties of specialty coffees from Ethiopia, Kenya, Rwanda, Burundi, Guatemala, Nicaragua, and Peru were analysed. During the fermentation coffee process, endogenous plant enzymes were degraded by mucilage macromolecules bound to the beans and accelerated the release of compounds into the fermentation waters. As naturally occurring microorganisms interacted with the sugars of the coffee fruit, carbon dioxide was released. After the oxygen was removed, the microorganisms were forced to consume the sugars in the fruit for energy. This chemical reaction released enzymes and completely changed the chemical composition and ultimately the taste of the coffee. Fatty acid methyl esters (FAMEs) obtained from the extracts of green beans were identified by gas chromatography-mass spectrometry (GC–MS) and quantified on gas chromatography. The FA composition and sensory scores, following the Specialty Coffee American Association protocols, were used to differentiate 19 coffee samples based on the processing method. We evaluated 10 important attributes of the coffee: fragrance/aroma, flavour, aftertaste, acidity, body, balance, overall, uniformity, clean cup, sweetness, and total score. Principal component analysis (PCA) discriminated between coffees processed by spontaneous fermentation and those by induced anaerobic fermentation. Notably, Peruvian coffee processed by carbonic maceration exhibited the highest linoleic acid content (48.04%), while the same coffee processed naturally showed the highest palmitic acid content (38.96%). All coffee samples scored over 80 points for uniformity, clean cup, and sweetness. The highest total sensory scores for spontaneously processing coffees were 88.75 for washed Kenyan and 87.75 for washed Ethiopian coffee, with other coffees scoring between 82.75 and 85.00. For the best specialty coffees processed by induced methods, scores ranged from 87.25 to 88.00 in seven out of nine coffees. The dominance of certain attributes varied among geographical areas and processing methods, even within the same farm. Coffees from different geographical regions processed with innovative methods (i.e., anaerobic fermentation and carbonic maceration) were among those with the highest qualities.

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Development of *in vitro* cultures from *Astragalus thracicus* Griseb and *Astragalus aitosensis* (Ivanisch.) and evaluation of their bioproduction potential

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With more than 2200 taxonomically classified species, genus *Astragalus* (Fabaceae) is one of the largest genera of dicotyledonous plants. *Astragalus* species are widespread mainly in the temperate regions of the northern hemisphere, growing in arid and semiarid regions up to 1700 meters above sea level.
Nearly 133 species grow in Europe, of which 29 in Bulgaria. Several of them are endemic and thus included in the Red List of Bulgaria under vulnerable, endangered or critically endangered status.
In this research we investigated the peculiarities in establishment and development of *in vitro* cultures of two endangered Astragalus species - *A. thracicus* and *A. aitosensis*.
Astragalus thracicus (Griseb) is a tertiary relict and Balkan endemic species. Nowadays it is found only in three habitats in Bulgaria, along it could be found in some limited areas of Greek and Turkish regions of Thrace.
Astragalus aitosensis (Ivan.) is an endemic relic plant in Bulgaria. Nowadays the plant is found only in the suburbs of Aytos, a small Bulgarian town.
In a series of *in vitro* experiments, we investigated and optimized the basic growth conditions, suitable for growing and maintaining of stable and long-lasting callus cultures. The parameters, which we investigated were: effect of scarification over the seed germination rate, type and concentration of gelling agent, hormone regiments included, type and concentration of carbon sources.
Additionally, we measured behaviour and flavonoid biosynthetic potential of callus cultures after stress induction with elicitors like methyl jasmonate (MeJ), salicylic acid (SA) and yeast extract (YE). In case of biotic stress, we concluded that growth index of callus cultures from *A. thracicus* decreased inversely proportional to the concentration of elicitors applied, as SA showed the strongest growth-inhibiting effect. Synthetic potential of the same callus cultures was evaluated according HPLC/MS measure of three flavonol aglycons – kaempferol, quercetin and methylquercetin. The highest amount of kaempferol (9.12 µg/g dry weight) and quercetin (5.72 µg/g dry weight) was detected when callus cultures were exposed for 72 hours in medium supplemented with 50 mg/l YE. For the synthesis of the third aglycon, methylquercetin, the best inductive properties (equivalent to 4.69 µg/g dry weight) were measured in media supplemented with 200 µmol/l MeJ for 72 hours. The fastest kaempferol synthesis was found at 24 hours in the medium supplemented with 1000 µmol/l SA, quantified as 7.61 µg/g dry weight.

Based on all these experiments, the study resulted with developing of a protocol for micropropagation.

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Active ingredients from hemp roots

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Cannabis sativa L., is a crop with many industrial and pharmaceutical applications. The cannabinoids and terpenoids contained in the flowers of industrial hemp are of great pharmaceutical interest. Although hemp roots were historically used to treat fever and inflammations [1], their application in contemporary medicinal practices is rare..

In our study, we plan to investigate the spectrum of natural components in hemp roots as well as the influence of different parameters on the spectrum. The parameters that will be observed fall into one of three categories: cultivation, processing, and extraction. The cultivation includes different growing locations with varying climatic conditions and soil compositions. Critical steps during processing, that could potentially influence the spectrum of components are drying, storing, and shredding. For the extraction process, conventional solvent extraction is supposed to be compared to more recent techniques like natural deep eutectic solvents (NADES) or supercritical CO_2-extraction.

At the outset of our research, we conducted a thin-layer chromatography (TLC) analyses on the roots to assess the presence of specific classes of natural compounds. By using different extraction methods, solvents, and detection reagents we tested for the following substance groups: alkaloids, anthraglycosides, arbutin, coumarins, flavonoids, phenol carboxylic acids, terpenes, valepotriates and essential oils [2]. These tests have revealed positive reactions in some of these natural substance groups (arbutin, essential oils, phenol carboxylic acids, terpenes). In the course of these investigations, we noticed one zone in particular in several different TLCs (R_f = 0,43). This zone was isolated using preparative thin-layer chromatography and checked for purity using another TLC. A final analysis using FT-ICR-MS allows the identification of the isolate, but an evaluation of the extensive results is still ongoing.

The TLC enabled the first conclusions to be drawn about the detectable natural substance groups in hemp roots. Further complex extraction and analysis methods will be used to break down the individual chemical compounds and possibly identify other substances that cannot be detected by TLC.

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P85

Novel bioactive Amaryllidaceae alkaloids from *Zephyranthes citrina* analysis with *in silico* tools

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Amaryllidaceae alkaloids represent a structurally diverse group of natural compounds with a wide range of biological activities, including antitumor and antiviral. Among these are compounds such as lycorine, haemanthamine, and galanthamine, which possess bioactive properties and potential antitumor therapeutic applications [1].

This research focuses on Amaryllidaceae alkaloids, chosen based on preliminary evidence of their cytotoxic and antitumor properties. The primary objective of this study is to investigate the anticancer potential of two novel alkaloids, zephicitrine and 6-oxonarcissidine, which were isolated from *Zephyranthes citrina* [2]. For this purpose, the molecular docking study was performed for two targets: Mcl-1 (myeloid cell leukemia-1) and CDK4 (cyclin-dependent kinase 4) proteins. The Bcl-2 protein family is highly expressed in various cancers, including certain forms of leukemia. It can control apoptosis, promoting cell survival by interfering at an early stage in the cascade of events leading to the release of cytochrome c from mitochondria. The translational inhibition of the Mcl-1 protein in tumor cell lines could induce their apoptosis. Mcl-1, which belongs to the Bcl-2 protein family, plays a major role in suppressing apoptosis in cancer cells. To study the effects of cell cycle inhibition, the enzyme protein CDK4 was chosen, which plays a key role in cell cycle regulation. It is part of a larger family of cyclin-dependent kinases (CDKs), which are responsible for transducing signals that determine cell progression through different stages of the cell cycle. CDK4 in particular is involved in controlling the transition from the G1 phase (the initial phase of the cell cycle) to the S phase (the phase of DNA replication).

Moreover, the ADMET analysis was performed for these compounds. The properties were evaluated to further assess their drug-likeness. There are key parameters to determine the pharmacokinetic profile and safety of new chemical compounds, especially potential drugs. The preliminary results appear to be promising, but further *in vitro* validation is necessary.

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New compounds isolated from *Zephyranthes citrina* as potential anti-inflammatory agents - analysis of interaction with COX and LOX enzymes and determination of ADMET profile

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Inflammation constitutes a fundamental biological response that plays a critical role in the pathogenesis of numerous diseases, including autoimmune disorders, cardiovascular conditions, and chronic inflammatory syndromes. The enzymes cyclooxygenases (COX-1 and COX-2) and lipoxygenases (LOX) are key mediators in the inflammatory cascade, catalysing the conversion of arachidonic acid into prostaglandins and leukotrienes, which contribute to pain, oedema, and tissue damage. Given the well-established role of COX and LOX inhibitors in managing inflammation, the search for novel bioactive compounds targeting these pathways remains an important focus of pharmaceutical research. Non-steroidal anti-inflammatory drugs (NSAIDs), which primarily inhibit cyclooxygenases (COXs), are widely utilised in clinical practice, while specific lipoxin (LXA) inhibitors and leukotriene receptor antagonists have demonstrated efficacy in conditions such as asthma and allergic inflammation. Furthermore, dual COX/LOX inhibitors are being explored as potential alternatives with improved safety profiles.

The present study investigates the anti-inflammatory potential of two newly isolated alkaloids, 6-*O*-ethylzephyranine E and 6-*O*-ethylzephyranine F, extracted from *Zephyranthes candida* [1]. Molecular docking simulations were employed to analyse their interactions with COX and LOX enzymes, evaluating their binding affinities, key molecular interactions, and selectivity in comparison to recognised anti-inflammatory inhibitors. To further evaluate their potential as drug candidates, an *in silico* ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis was conducted, providing insights into their pharmacokinetic properties, including bioavailability, metabolic stability, and potential toxicity risks.

The preliminary findings suggest that both alkaloids exhibit strong binding affinities for COX and LOX enzymes, indicating their potential as anti-inflammatory agents.

Furthermore, their favourable ADMET profiles support their suitability for further experimental validation. These results provide a foundation for subsequent *in vitro* and *in vivo* studies, contributing to the ongoing search for novel, plant-derived anti-inflammatory compounds with therapeutic potential.

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Preliminary assessment of the neuroprotective potential of plant-derived benzoquinone, rapanone

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According to the WHO, neurological diseases affect one-third of the population worldwide. Among these, dementia (a group of neurodegenerative disorders) is one of the five most prevalent. Conventional therapy mainly includes acetylcholinesterase inhibitors, such as donepezil or plant-derived galantamine. Furthermore, various compounds of plant origin show neuroprotective potential [1]. These include curcumin, quercetin, and many others, among which are compounds with a quinone moiety: tanshinones or embelin. Still, both prevention and treatment of dementia remain unsatisfactory due to the poor efficacy of the substances that are in use. As a result, the search for new options is an important part of scientific efforts.

Rapanone is a naturally occurring simple benzoquinone with antioxidant, anti-inflammatory, and cytotoxic properties [2]. It is an orange pigment found in some species of plants belonging to the Primulaceae family, such as *Ardisia crenata* Sims.

The goal of the study was to assess its cholinesteraseinhibitory activity indicating the neuroprotective potential of the compound.

To achieve this, the inhibitory activity of rapanone against acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) was tested by *in vitro* enzymatic assays. Galantamine was used as a reference. In addition, *in silico* molecular docking was conducted to simulate the interaction between rapanone and the enzymes.

The study results exhibited anticholinesterase activity of rapanone, with IC_{50} = 1.43 mg/mL in AChE assay, and 2.79 mg/mL in BChE assay. Galantamine blocked the enzymes with IC_{50} = 0.06 mg/mL, and 0.34 mg/mL, respectively. This is the first study presenting the inhibitory activity of rapanone against AChE, and BChE.

The anticholinesterase potential of rapanone, revealed for the first time, encourages further experiments to verify its neuroprotective activity.

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Analysis of the barley and its mutant *chlorina f2*^{f2} chloroplast proteome

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Barley (*Hordeum vulgare*) is the one of the most important cereal crops and an important model organism for studying chloroplasts and their substructures. Its suitability for this research is further enhanced by the availability of numerous mutant lines. One of the most widely used mutants is *chlorina f2*^{f2}, which is characterized by a strongly reduced content, or complete absence, of chlorophyll b. The mutant *chlorina f2*^{f2} has played a crucial role in numerous studies investigating the importance of chlorophyll and the effects of its deficiency on the function of photosynthetic complexes, thylakoid organization, chloroplast structure, and overall plant physiology. Interestingly, no complex proteomic study on *chlorina f2*^{f2} has yet been published despite this fact. In this study, we isolated chloroplasts from seven-day-old wild-type barley cultivar Morex leaves and the *chlorina f2*^{f2} mutant. Thylakoid membranes were subsequently extracted from these chloroplasts, and comprehensive proteomic analyses of both compartments were performed. Our results demonstrate a substantial downregulation of proteins associated with photosynthesis, chlorophyll biosynthesis, and thylakoid organization, findings that align with previously documented physiological and biochemical characteristics of the *chlorina f2*^{f2} mutant. Additionally, we observed a significant downregulation in several hormone biosynthetic pathways.. Follow-up analyses of phytohormone levels and key metabolites further supported these findings. Taken together, our data significantly expand the current understanding of *chlorina f2*^{f2} physiology. Rather than merely a model for chlorophyll b deficiency, *chlorina f2*^{f2} should be considered a more complex system that also reflects alterations in plant hormonal homeostasis and stress-related signalling pathways. From this point of view, it represents a valuable resource for future studies focused on the interplay between pigment biosynthesis, photosynthetic efficiency, and hormonal regulation in plants.

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Wild and cultivated inulin-rich medicinal plants as a natural source for skin microbiome-friendly cosmetics

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Inulin, a naturally occurring fructan-type polysaccharide, is gaining increasing attention in the field of cosmetic science due to its multifunctional properties and favorable interactions with the skin microbiome. Commonly extracted from chicory roots (*Cichorium intybus* L.), inulin is also found in a variety of wild and cultivated medicinal and edible plants growing in Poland, including elecampane (*Inula helenium* L.), Jerusalem artichoke (*Helianthus tuberosus* L.), dandelion (*Taraxacum officinale* coll.), burdock (*Arctium* spp.), and balloon flower (*Platycodon grandiflorus* /Jacq./ A.DC.).
These species, traditionally valued for their digestive, anti-inflammatory, expectorant, antispasmodic, and choleretic effects, are now recognized for their potential to promote skin health. Inulin acts as a natural humectant and prebiotic, fostering the growth of beneficial skin microorganisms, while inhibiting the proliferation of pathogenic strains. This selective modulation of the skin microbiome helps maintain barrier integrity and mitigate conditions such as acne, atopic dermatitis, and skin dryness.
The inclusion of inulin-rich plant extracts or purified inulin in dermocosmetic and natural skincare formulations offers both functional and marketing advantages. In addition to its microbiome-balancing properties, inulin improves product texture, stability, and moisture retention, making it particularly beneficial for formulations targeting sensitive or allergy-prone skin.
This presentation highlights the botanical diversity of inulin-containing species in Poland, their phytochemical profiles, and ethnobotanical relevance. It also explores their incorporation into modern cosmetic applications, supported by emerging data on microbiome-skin interactions. The growing consumer demand for sustainable, plant-based, and microbiome-friendly products reinforces the importance of these underutilized herbal resources in green cosmetic innovation.

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List of Participants

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