12th INTERNATIONAL SYMPOSIUM ON CHROMATOGRAPHY OF NATURAL PRODUCTS

LUBLIN (POLAND) May 10-13, 2026

E-BOOK OF ABSTRACTS

Department of Pharmacognosy
with the Medicinal Plant Garden
& Department of Natural Products Chemistry

Medical University of Lublin

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WELCOME

Dear Colleagues,
Dear Friends,

It is our great pleasure to welcome you to the *12th International Symposium on Chromatography of Natural Products* (ISCNP 2026), held in Lublin, Poland, from May 10th to 13th, 2026.

On behalf of the Scientific and Organizing Committees, we would like to express our sincere gratitude for your participation and for contributing to the continued success of ISCNP. Over more than three decades, this Symposium has evolved into a well recognized international forum devoted to chromatographic and related analytical techniques in natural products research. What began in 1992 as a national meeting in Lublin has grown into a truly global scientific event, fostering collaboration, exchange of ideas, and the development of young researchers.

The scientific programme of ISCNP 2026 reflects both the traditions and the dynamic progress of our discipline. The abstracts collected in this volume cover a broad spectrum of topics, including modern chromatographic and bioanalytical methodologies, hyphenated techniques, metabolomics, chemotaxonomy, biological activity assessment, and applications of natural products in pharmacy, medicine, cosmetics, nutraceuticals, and food science. We are particularly pleased to note the strong participation of early career scientists, whose contributions demonstrate the vitality and future potential of our field.

Lublin, a city with a long academic tradition and a rich cultural heritage, provides a meaningful setting for this Symposium. Situated at the crossroads of cultures and ideas, it has for centuries been a place of dialogue and cooperation — values that lie at the heart of the ISCNP community. We hope that your stay here will offer not only stimulating scientific discussions, but also opportunities for personal exchange and inspiration.

We would like to thank all authors for submitting their valuable contributions, the invited speakers and session chairs for sharing their expertise, as well as our sponsors and institutional partners for their generous support, which made this Symposium possible.

We hope that the 12th International Symposium on Chromatography of Natural Products will be a rewarding and inspiring experience for all participants and will further strengthen scientific collaboration within our community.

We wish you a successful meeting and an enjoyable stay in Lublin.

Krystyna Skalicka-Woźniak
Agnieszka Ludwiczuk

Chairs of ISCNP 2026

Scientific program

Sunday, May 10th	
16.00 - 18.00	Registration
18.00 -21.00	**Welcome reception**
Monday, May 11th	
9.00 – 10.00	Registration
10.00 – 10.30	Opening ceremony
Opening lecture	Chair: *Kazimierz GŁOWNIAK* (Lublin, Poland)
10.30 – 11.15	PL 1: *Guenther BONN* (Innsbruck, Austria) "*Analytical strategies for phytochemical screening and cellular testing: Applications on Earth and aboard the ISS*"
11.15 – 12.00	**Coffee break**
Session I	Chairs: *Judith ROLLINGER* (Austria) & *Ilkay E. ORHAN* (Turkey)
12.00 – 12.40	PL2: *Leandros SKALTSOUNIS* (Athens, Greece) "*The olive tree (Olea europaea), an invaluable source of bioactive molecules*"
12.40 – 13.00	OP1: *Benedikt SCHWARZ* (Insbruck, Austria) "*From complexity to a single marker: A unified enrichment and reduction workflow for pyrrolizidine alkaloid quantification in natural products* "
13.00 – 13.20	OP2: *Phila RAHARIVELOMANANA* (French Polynesia) "*Feature-based molecular networks identification of Fagrea berteroana bioactive molecules targeting the dermal papilla cells of the hair cycle*"
13.20 – 13.40	OP3: *Martina SAVOVA* (Plovdiv, Bulgaria) "*Cycloastragenol enhances metabolic homeostasis in C. elegans through redox signalling modulation*"
13.40 – 14.30	**Lunch**

Session II: Young Scientists	Chairs: *Phila RAHARIVELOMANANA* (French Polynesia) & *Adam MATKOWSKI* (Poland)
14.30 – 14.50	YSL1: *Filip NOWACZYŃSKI* (Lublin, Poland) "*Geographical stability and environmental modulation of secondary metabolites in Marchantia polymorpha across Europe*"
14.50 – 15.10	YSL2: *Mateusz KNAP* (Cracow, Poland) "*Chromatographic characterization of betacyanin preparations with antioxidant potential obtained from Hylocereus polyrhizus fruit extracts*"
15.10 – 15.30	YSL3: *Kajetan GRZELKA* (Wrocław, Poland) "*Influence of Pulsed Electric Field (PEF) treatment on the phytochemical profile of Iris domestica Natural Deep Eutectic Solvent (NDES) extracts*"
15.30 – 15.50	YSL4: *Marek RYBAK* (Lublin, Poland) "*Linden inflorescences and elderflower as natural products with antiviral properties*"
15.50 – 16.10	YSL5: *Wojciech PAŹDZIORA* (Cracow, Poland) "*Isoflavones-rich Fabaceae plants and their impact on thyroid cells in vitro*"
16.10 – 16.45	**Coffee break**
16.45 – 18.00	**Poster session 1 (P1-P30)**

Tuesday, May 12th	
Session III	Chair: *Nikolas FOKIALAKIS* (Greece)
10.00 – 10.40	PL3: *Judith ROLLINGER* (Vienna, Austria) "*Chromatography: Breaking complexity for natural product drug discovery*"
10.40 – 11.00	OP4: *Maonian XU* (Reykjavik, Iceland) "*Chiral Chromatography and Molecular Dynamics Simulation of Usnic and Isousnic Acid Enantiomers in Lichens*"
11.00 – 11.20	OP5: *László Frici NÉMETH* (Hungary) "*Enormous saving achieved by process optimization in the purification of a high value botanical extract*"
11.20 – 11.45	**Coffee break**
Session IV	Chair: *Milen I. GEORGIEV* (Bulgaria)
11.45 – 12.25	PL4: *Ilkay E. ORHAN* (Ankara, Turkey) "*Multi-faceted approach to neuroprotective potential of selected plant phenolics: insights from in vitro, in silico, and in vivo studies*"
12.25 – 13.05	PL5: *Nikolas FOKIALAKIS* (Athens, Greece) "*Psychoactive Plants and Mushrooms: Transformative Science and Global Regulatory Transition*"
13.05 – 14.00	**Lunch**
14.00 – 16.00	**Workshop Anchem** "*Industrial-scale Purification of Psoralen from Angelicin Using CPC for Cost Efficient Production*"
16.00 – 17.15	**Coffee break & Poster session 2 (P31-P59)**
20.00	**Symposium dinner**

Wednesday, May 13th	
Session V	Chair: *Leandros SKALTSOUNIS* (Greece)
10.00 – 10.40	PL6: *Milen I. GEORGIEV* (Plovdiv, Bulgaria) "*Unveiling **a**nti-obesity and longevity potential of natural products*"
10.40 – 11.00	OP6: *Anna STASIŁOWICZ-KRZEMIEŃ* (Poznań, Poland) "*Decoding Cannabis: The Complexities Linking Chemical Composition with Biological Response*"
11.00 – 11.20	OP7: *Ljuboš UŠJAK* (Belgrade, Serbia) "*Chemical investigation of Heracleum ternatum fruits: LC-QTOF-MS/MS analysis and CPC isolation"*
11.20 – 11.40	**Coffee break**
Session VI	Chairs: *Milen I. GEORGIEV* (Bulgaria)
11.40 – 12.10	PL7: *Wenqi HUANG & Jiambo XIAO* (Ourense, Spain) "*Dietary EGCG Reshapes Metabolic-Epigenetic Interplay to Induce Transgenerational Host Defense*"
12.10 – 12.30	OP8: Łukasz ŚWIĄTEK (Lublin, Poland) "*Antiviral Potential of Natural Products* "
12.30 – 12.50	OP9: *Mariusz A. BROMKE* (Wrocław, Poland) "*Mechanism of Action of the Combination of β-Aescin and Newly Synthesized Alkylamidobetaines as Modern Components Eradicating the Biofilms of Multidrug-Resistant Candida glabrata - Lipidome and Oxidative Stress Analysis*"
12.50 – 13.10	Closing remarks
13.10 – 14.00	**Lunch**
14.00	Guided walking tour of Lublin

PLENARY LECTURES

PL-1

ANALYTICAL STRATEGIES FOR PHYTOCHEMICAL SCREENING AND CELLULAR TESTING: APPLICATIONS ON EARTH AND ABOARD THE ISS

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Plant-based products used in medicine, nutrition, and cosmetics are highly complex matrices comprising hundreds to thousands of constituents with strongly varying concentrations. This complexity creates major challenges for safety assessment, authenticity control, quality assurance, and the evaluation of biologically relevant exposure. To address these challenges, we combine modern separation science and cell-based testing.

LC-QTOF-MS is used as a key platform for phytochemical screening of plant extracts and botanical materials, enabling targeted and untargeted profiling of complex natural product mixtures.

Targeted workflows for multiple analyte classes were developed, including contaminants such as polycyclic aromatic hydrocarbons (PAHs), as well as rapid methods for the determination of hydroxytyrosol and tyrosol for the quality assurance of olive oil. The latter are particularly relevant for assessing compliance with the EFSA health claim, which requires a defined minimum content of polyphenols in olive oil. Moreover, special emphasis is placed on pyrrolizidine alkaloids (PAs), for which we developed a novel analytical strategy allowing their quantification as a sum parameter, providing an efficient and practical solution for routine screening and regulatory quality control of complex samples.

A major focus of our work is the investigation of bioavailability using different cell systems. By coupling cellular transport models with LC-QTOF-MS, we compare apical and basolateral compartments in order to determine which phytochemicals remain in the donor compartment and which are transported across the cellular barrier. This apical-versus-basolateral LC-QTOF-MS analysis provides detailed molecular insight into transport processes and compound-specific availability, thereby linking chemical composition with physiologically relevant exposure.

These workflows are further combined with cell-based bioassays to assess biological activity alongside molecular characterization. Within the interdisciplinary initiative PhytoSpace Austria, the concept is extended to cell systems under microgravity conditions on the International Space Station (ISS), with parallel ground controls, in order to study how spaceflight conditions influence cellular responses and the biological effects of plant extracts for several deseases e.g. endometriosis.

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PL-2

THE OLIVE TREE (*OLEA EUROPAEA*), AN INVALUABLE SOURCE OF BIOACTIVE MOLECULES

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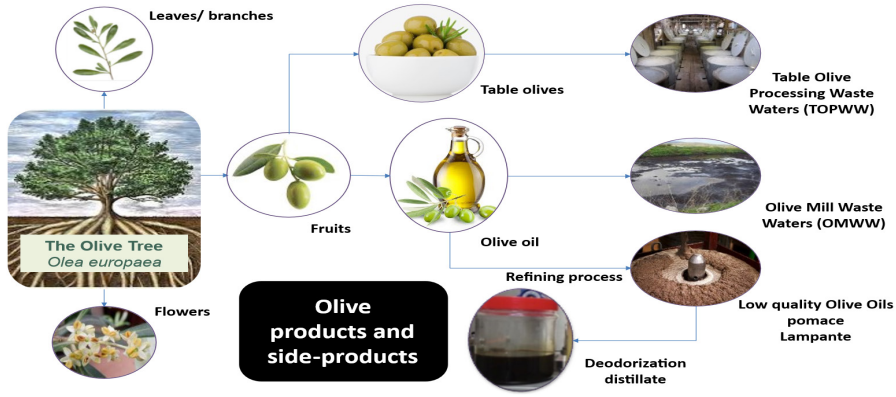
Extra virgin olive oil (EVOO), the main product of *Olea europaea* and the key ingredient of Mediterranean diet, is characterized by substantial nutritional and health beneficial value [1]. However, despite olive oil's economic and health impact, its industry is associated with environmental problems derived from the vast quantity of by-products, such as vegetation waters, olive cake, olive pulp and olive branches and leaves. [2] The amount of olive leaves produce every year exceed 18 million tons and mostly are used as animal feed, compost production or simply are burned, causing serious environmental damage. In a recent study it was found that burning of olive tree branches is a major organic aerosol source in the Mediterranean region. [3] However this material still contains high value-added compounds such as triterpenoids, secoiridois, flavonoids, phenolic alcohols, phenolic acids, and lignans which are known as olive polyphenols. All these constituents have a strong antioxidant profile and there is an increased industrial interest for possible nutraceutical, cosmetic and pharmaceutical applications. Our work is focused on finding alternative strategies to manage the residues of olive oil industry following two axes. Firstly, the development of liquid/ liquid or solid/liquid extraction followed by partition chromatography techniques for the isolation of these compounds in pilot scale. Secondly the use of these compounds such as oleuropein, as starting material for the hemi-synthesis of new analogues.



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PL-3

CHROMATOGRAPHY: BREAKING COMPLEXITY FOR NATURAL PRODUCT DRUG DISCOVERY

JUDITH MARIA ROLLINGER

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One of the most challenging tasks in natural product research is the identification of bioactive constituents from multicomponent mixtures. Sophisticated chromatographic methods for the analysis as well as for fractionation and separation of extracts are a precondition for the successful use of biochemometric approaches. MS- and NMR-based spectral correlation with bioactivities opens up new revenues for a fast and unambiguous identification of major and even minor bioactive constituents from complex mixtures. In my talk I will present some recent examples from ongoing research focusing on the discovery of resistance breaking anti-infectives and anti-inflammatory natural compounds.

Adelsberger S, Perhal AF, Bertaina L, Schwarz PF, Dirsch V, Rollinger JM, Grienke U. Biochemometric 2D NMR-based heterocovariance analysis: A targeted approach for identifying bioactive compounds in complex mixtures. *Anal. Chem.* 2025, 97, 41, 22508-22517. https://doi.org/10.1021/acs.analchem.5c02419

Gafriller J, Cruz CD, Brungs C, Kapp K, Wasilewicz A, Tammela P, Rollinger JM. Sanggenon C – A novel anti-enterococcal agent from *Morus alba* root bark. *J Ethnopharmacol.* 2025, 353:120443. https://doi.org/10.1016/j.jep.2025.120443

Wasilewicz A, Areesanan A, Kirchweger B, Nicolay S, Waltenberger E, Beniddir MA, Gründemann C, Rollinger JM, Grienke U. Combining the Strengths of MS and NMR in Biochemometrics: A Case Study on *Buddleja officinalis*. *J. Nat. Prod.* 2025; 88(5):1099-110 DOI: 10.1021/acs.jnatprod.4c00847

PL-4

MULTI-FACETED APPROACH TO NEUROPROTECTIVE POTENTIAL OF SELECTED PLANT PHENOLICS: INSIGHTS FROM *IN VITRO*, *IN SILICO*, AND *IN VIVO* STUDIES

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Alzheimer's Disease (AD) is a complex neurodegenerative disorder that necessitates the exploration of multi-targeted therapeutic strategies. We conducted comprehensive research into the neuroprotective potential of key plant phenolics, specifically rosmarinic acid (RA), gallic acid (GA), 3-hydroxytyrosol (3-HT), quercetin, oleuropein, and epigallocatechin gallate (EGCG). The research utilized a multi-faceted approach, beginning with in vitro enzyme inhibition and molecular docking. Results identified RA, EGCG, and quercetin as promising inhibitors of β-site amyloid precursor protein cleaving enzyme-1 (BACE1), with docking simulations revealing their interactions within the enzyme's non-catalytic site. In the cholinergic system, quercetin emerged as the most potent dual inhibitor of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Furthermore, *in vitro* gene expression analysis showed that oleuropein and quercetin effectively suppressed the expression of PSEN, APOE, and CLU, which are critical genes linked to AD pathogenesis. Behavioural studies provided essential *in vivo* validation of these findings. In a scopolamine-induced amnesic mouse model, 3-HT demonstrated the highest antiamnesic effect, representing the first evidence of its cognitive benefits in a living organism. Additionally, adult zebrafish models were employed to evaluate anxiolytic and cognitive-enhancing properties. While RA significantly reduced anxiety-like behaviour, GA and 3-HT notably enhanced spatial memory in Y-maze tests. 3-HT further demonstrated the ability to improve recognition memory in the novel object recognition (NOR) test.

In conclusion, these integrated results highlight the multi-target potential of dietary phenolics in neuroprotection, suggesting their viability as lead molecules or functional ingredients for supporting cognitive health and managing AD.

Acknowledgements: This research was funded by the Scientific Research Project Unit of Gazi University (Ankara, Türkiye, grant number: 02/2019-31) as well as Turkish Academy of Sciences (TÜBA) through the research budget allocated to Ilkay Erdogan Orhan.

PL-5

PSYCHOACTIVE PLANTS AND MUSHROOMS: TRANSFORMATIVE SCIENCE AND GLOBAL REGULATORY TRANSITION

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Psychoactive plants and mushrooms have been used for centuries for medicinal, spiritual, and recreational purposes. Their prohibition during the 20th century, however, significantly limited scientific research and the exploration of their therapeutic potential. In recent years, advances in biomedical research have renewed interest in these natural products, leading to a more evidence-based understanding of their biological effects.

A growing body of studies highlights the potential of Cannabis, as well as other psychoactive plants such as *Mitragyna speciosa* (kratom) and Sceletium species, and fungi including Amanita and Psilocybe species, in the treatment of neuropsychiatric disorders. These include addiction, anxiety, depression, and post-traumatic stress disorder (PTSD). The primary bioactive compounds involved are often alkaloids, which interact with key neurobiological pathways. In addition, these substances show promise in managing chronic pain and neuropathic conditions.

This emerging evidence has contributed to a shift in regulatory approaches, with several authorities revising frameworks to allow controlled medical access. The move toward legalization or decriminalization reflects both scientific progress and increasing societal acceptance.

Within this context, projects such as NEUROHEALTH aim to systematically investigate the therapeutic potential of psychoactive alkaloids using advanced analytical and computational tools. Overall, ongoing research is reshaping scientific and regulatory perspectives, supporting the development of more adaptive and evidence-based policies.

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PL-6

UNVEILING ANTI-OBESITY AND LONGEVITY POTENTIAL OF NATURAL PRODUCTS

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Obesity presents a significant challenge to the modern society, imposing a substantial burden on health systems and individuals alike. Nowadays, more than a billion people globally are considered obese, a condition that does not solely refer to weight management but rather to mitigating its far-reaching consequences on healthspan and lifespan [1]. Individuals diagnosed with obesity are predisposed to comorbidities including type 2 diabetes, cardiovascular disease, and metabolic complications often referred to as metabolic syndrome. Most importantly, obesity is associated with a reduction in disease-free years, an excess risk of premature death, and accelerated aging [1, 2]. On the other hand, we live in a rapidly aging world, with an expectancy of more than a 2 billion people aged 65 years (or older) by 2050, alongside a rising proportion of age-related diseases. Thus, both obesity and ageing are, per se, different sides of the same coin and represent healthcare burden on our society [1, 2]. The development of strategies targeting both of these processes now becomes a challenge for science. Central to this pursuit is the recognition that metabolic health serves as a cornerstone for both healthy weight maintenance and prolonged lifespan [2]. Additionally, key molecular pathways attributed to nutrient signalling that are implicated in obesity progression, intersect with those fundamental to longevity, suggesting potential shared targets for intervention [2].

Utilizing the model nematode organism Caenorhabditis elegans, along with combining omics and molecular pharmacology approaches, our research focuses on the discovery of natural products that target shared and highly conserved pathways [1-5]. Through our approach, we aim not only to mitigate the adverse effects of obesity but also to uncover novel strategies for promoting healthy aging and longevity.

Acknowledgements: MIG acknowledges financial support from the Bulgarian National Science Fund under a contract number КП-06-Н83/4.

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PL-7

DIETARY EGCG RESHAPES METABOLIC-EPIGENETIC INTERPLAY TO INDUCE TRANSGENERATIONAL HOST DEFENSE

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Parental diet is a key determinant of offspring health and immune function, in part through epigenetic regulation. However, the mechanism by which specific dietary bioactive compounds reshape metabolic-epigenetic networks to drive transgenerational adaptive responses remains poorly understood. Here, we investigate whether and how epigallocatechin-3-gallate (EGCG), a well-characterized dietary bioactive compound, modulates heritable host defense through metabolic-epigenetic crosstalk. In mice, EGCG administration led to a decrease in Escherichia coli burden across multiple tissues in paternal and male offspring in a sex-specific manner, accompanied by metabolic and pro-inflammatory factor changes. In *Drosophila melanogaster*, early-life EGCG exposure increased survival upon *Pseudomonas aeruginosa* or *Staphylococcus aureus* infection and persisted for two subsequent generations. Mechanistically, EGCG reduced intestinal amino acids, thereby moderately inducing activation of activating transcription factor 4 (ATF4), which in turn enhanced maternal glycolysis and immune adaptation. Tyrosine supplementation abolished the enhanced host defense and metabolic changes. Furthermore, ATF4-induced activation of glycolysis promoted ovarian lactate production, serving as a substrate for increased global H3K27 acetylation in the offspring. Together, these findings suggest that dietary bioactive compounds modulate metabolic and gene regulatory processes, with functional evidence supporting a role for amino acid metabolism and lactate in linking metabolic remodeling to enhanced resistance to infection in the offspring. This work provides mechanistic insight into how diet can shape heritable immune function through metabolic-epigenetic interplay.

Keywords: Epigenetics; Flavonoids; Histone acetylation; Host Defense; Metabolic reprogramming; Phytotherapy; Transgenerational inheritance;

ORAL PRESENTATIONS

OP-1

FROM COMPLEXITY TO A SINGLE MARKER: A UNIFIED ENRICHMENT AND REDUCTION WORKFLOW FOR PYRROLIZIDINE ALKALOID QUANTIFICATION IN NATURAL PRODUCTS

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Pyrrolizidine alkaloids (PAs) comprise a structurally diverse class of plant derived toxins whose reliable quantification remains analytically demanding due to their variability, low abundance, and complex sample matrices. Accurate and efficient PA determination is, however, critical for safety assessment in phytopharmaceuticals, phytocosmetics, and food products. [1,2]

We present a fast analytical workflow, that substantially simplifies the quantification of retronecine-type PAs. The method integrates two key innovations. First, selective enrichment using nano zirconium silicate enables efficient capture of both PA free bases and N-oxides, significantly improving sensitivity and matrix tolerance. [3] Second, the enriched PA fraction undergoes a one-pot reduction and hydrolysis using lithium aluminium hydride, quantitatively converting retronecine type PAs into the common necine base retronecine. This transformation collapses structurally diverse PAs into a single measurable marker, enabling quantification while markedly reducing chromatographic complexity and the number of required analytical reference standards.

Method performance was evaluated in an authentic botanical matrix (*Radix Primulae*) spiked with defined PA standards. The workflow demonstrated high total recovery (95.1%), excellent linearity across 10 - 90 µg L^{-1} (R^2 = 0.9977), and satisfactory sensitivity (LOD 4.9 µg L^{-1}, LOQ 14.7 µg L^{-1}).

By combining selective enrichment with the hydrolysis and reduction process, this approach provides a robust, sensitive, and cost-efficient strategy for comprehensive monitoring of retronecine based PAs in botanical materials, offering clear advantages over conventional multi analyte methods.

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OP-2

FEATURE-BASED MOLECULAR NETWORKS IDENTIFICATION OF *FAGREA BERTEROANA* BIOACTIVE MOLECULES TARGETING THE DERMAL PAPILLA CELLS OF THE HAIR CYCLE

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Fagraea berteroana (Gentianaceae), locally called "*pua*", is a small tree, whose flowers and fruits are traditionally used in Polynesian cosmetopoeia. Unconventionally, its ground fruits were used on cadavers to prevent hair loss [1]. Aiming to understand the effect of the fruits of *F. berteroana* on the hair growth cycle and identify bioactive metabolites, we focused our study on this plant part extract.

Bioguided fractionation of the EtOAc extract of the fruits of *F. berteroana* (FEAE) by Combiflash led to the selection of sub-fraction FF1, which showed significant hair follicle dermal papilla cells (HFDPCs) proliferation after both 24 and 48h of treatment.

Further investigation of molecular mechanisms revealed a 17% and 50% increase (p-value < 0.001) in β-catenin protein expression and CCND1 gene expression, respectively, compared to vehicle, of FF1. These findings suggested an upregulation of the Wnt pathway in dermal papilla cells [2].

LC-MS/MS dereplication analysis within feature-based networking of FF1 fraction allowed to annotate several classes of compounds including mainly subclasses of terpenoids such as iridoids and secoiridoids (dihydroactinidiolide, boonein, loganic acid or swertiamarin), triterpenoids (cis/trans-p-coumaroyloxy maslinic acid and cis/trans-p-coumaroyloxy corosolic acid). Compounds such as flavonoids and coumarins were isolated from FF1 and identified by NMR. These obtained results will be discussed.

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OP-3

CYCLOASTRAGENOL ENHANCES METABOLYC HOMEOSTASIS IN *C. ELEGANS* THROUGH REDOX SIGNALING MODULATION

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Research into plant-derived bioactive compounds has increasingly focused on their capacity to influence oxidative balance and metabolic regulation in the context of obesity. Disruptions in cellular redox status are closely linked to excessive lipid accumulation and metabolic stress, largely mediated by conserved defense pathways such as those governed by the transcription factor SKN-1, the functional homolog of mammalian Nrf2. Activation of this pathway promotes the downstream expression detoxification enzymes, serve as indicators of cellular stress adaptation. The triterpenoid cycloastragenol is recognized for its protective effects against cellular damage and aging [1]. However, its involvement in redox-sensitive metabolic processes remains to be clarified.

In the present work, we examine how cycloastragenol influences signaling pathways related to lipid homeostasis and oxidative stress responses in a 2% glucose-induced obesity model using *Caenorhabditis elegans*, with a focus on SKN-1 signaling and nutrient sensing network with key players as *C. elegans* homologues of AMPK – *aak-2* and Sirtuin 1 – *sir2.1*, respectively.

Obtained results revealed that cycloastragenol stimulates AAK-2/SIR-2.1 signaling along with *far-3* expression in *C. elegans* [1]. In alignment with our preliminary studies these observations indicate that anti-obesogenic activity of cycloastragenol is mediated through activation of AAK-2 and SKN-1 signaling pathways and *far-3*.

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OP-4

CHIRAL CHROMATOGRAPHY AND MOLECULAR DYNAMICS SIMULATION OF USNIC AND ISOUSNIC ACID ENANTIOMERS IN LICHENS

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Lichens are symbiotic microbial consortia composed of lichen-forming fungi (LFF), photosynthesizing microalgae and other microbes [1]. LFF contain unique biosynthetic genes and produce specialized metabolites with interesting bioactivity [2]. Usnic acid (UA) enantiomers in lichens are potent antibiotic compounds, and they might differ drastically in bioactivity depending on the models in tests [3]. In contrast, the isousnic acid (isoUA) enantiomers are rarely characterised about their presence and bioactivity. This calls for a validated chiral method for the separation of the two pairs of enantiomers in lichens. The current study developed two chiral methods: one in polar organic mode [4], and the other one in normal phase condition. In polar organic mode, UA enantiomers are well resolved, but not for isoUA ones. Simultaneous separation of two pairs of enantiomers is currently only feasible with the normal phase method. We found the significant impact of acidic additives on the enantiomeric resolution. Molecular Dynamics simulation was also performed to elucidate the mechanisms in chiral separation, for which the hydrogen-bonding between the analytes and stationary phase plays an important role.

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OP-5

ENORMOUS SAVING ACHIEVED BY PROCESS OPTIMIZATION IN THE PURIFICATION OF A HIGH VALUE BOTANICAL EXTRACT

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This work presents the transition from a conventional purification workflow to centrifugal partition chromatography (CPC), enabling improved selectivity, recovery of valuable by-products, and enhanced overall process efficiency.

The purification of a high-value botanical extract containing the target compound (T1) is challenged by the presence of structurally related impurities. Among these, a second active pharmaceutical ingredient (T2) must be completely removed to its mutagenic properties, despite its intrinsic commercial value.

The crude extract can be divided into three fractions:

1) an early-eluting fraction rich in T2 and its isomers, along with additional valuable APIs recoverable via crystallization during acetonitrile regeneration,

2) a high-purity T1 fraction (>99.0%, T2 <0.10% by HPLC), where complete removal of T2 is critical due to its mutagenic properties,

3) a late fraction containing non-detectable residues from CO_2 extraction, including plant matrix and solvent impurities, further reduced by molecular distillation.

The objective of this work was to improve the efficiency and economic viability of the three-step purification process. Implementation of the CPC-based workflow resulted in a 50% reduction in solvent consumption and a 40% increase in T1 yield, while also enabling the recovery of valuable by-products and improving overall process sustainability.

OP-6

DECODING CANNABIS: THE COMPLEXITIES LINKING CHEMICAL COMPOSITION WITH BIOLOGICAL RESPONSE

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Cannabis sativa L. is a complex natural product widely investigated for its therapeutic potential; however, its translation from plant material to a predictable clinical effect remains limited. A key challenge lies in the lack of a clear relationship between analytical data and biological response. Although major cannabinoids such as Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are well characterized and included in the European Pharmacopoeia monograph, the overall biological activity is modulated by interactions with other constituents, including terpenes and flavonoids, contributing to the so-called “entourage effect.”

This work focuses on the analytical aspects of cannabis, while also addressing limitations related to regulatory frameworks, social perception, and the lack of standardized pharmaceutical formulations, and presents strategies to improve solubility and support the effective application of cannabis extracts and cannabis-derived compounds.

The gap between chemical composition and biological activity is further influenced by formulation-related factors, particularly the limited bioavailability of cannabinoids. Our studies address this issue by exploring approaches to enhance solubility and performance, including supercritical CO_2 processing, hot-melt extrusion, and freeze-drying, alongside the development of delivery systems for oral, mucosal, and topical administration. These strategies resulted in improved solubility, dissolution rate, and membrane permeability, contributing to enhanced bioavailability.

This perspective highlights that the inherent complexity of cannabis, combined with the limitations of current analytical approaches, prevents reliable prediction of biological response. Bridging this gap requires integrated strategies combining advanced analytical techniques with bioanalytical and formulation-oriented studies, which are essential for the development of safe, effective, and reproducible cannabis-based therapies.

OP-7

CHEMICAL INVESTIGATION OF *HERACLEUM TERNATUM* FRUITS: LC-QTOF-MS/MS ANALYSIS AND CPC ISOLATION

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Heracleum ternatum Velen. (Apiaceae), a member of the *H. sphondylium* L. group, inhabits the mountains of the Balkan Peninsula and north and central Anatolia, as well as north and central Apennines [1]. The aim of this work was to perform chemical analysis of polyphenols and furanocoumarins of the fruits of *H. ternatum* collected on Mt. Durmitor in Montenegro. Dried plant material was successively extracted with dichloromethane and methanol. Qualitative analysis of the dry methanol extract was performed by LC-QTOF-MS/MS. Isolation of compounds from the dry dichloromethane extract was done by normal-phase centrifugal partition chromatography (CPC), using hexane – ethyl acetate – methanol – water (6:5:6:5) system, and/or by semi-preparative HPLC, using peak-based fraction collection. The total of 15 kaempferol, quercetin and methylquercetin glycosides were detected in the methanol extract. Kaempferol 3,7-di-*O*-rhamnoside (kaempferitrin), quercetin 7-*O*-rhamnosyl 3-*O*-glucoside (vincetoxicoside A), quercetin 7-*O*-rutinoside (rutin) and quercetin 7-*O*-rhamnoside (quercitrin) were identified using standard compounds. Furthermore, 9 furanocoumarins were detected in the methanol extract. Isolation of furanocoumarins was performed from the dichloromethane extract (5 g), which contained notably higher amounts of these compounds compared to the methanol extract. Bergapten (40.67 mg) was isolated solely using the CPC, while byakangelicol (12.91 mg), heraclenin (10.56 mg), isopimpinellin (3.04 mg) and phellopterin (1.15 mg) were isolated after additional semi-preparative HPLC separation. The compounds were identified using standards and/or based on one-dimensional ^{1}H-NMR and two-dimensional ROESY NMR spectra. It can be concluded that *H. ternatum* fruits represent a source of compounds that may be of importance for pharmaceutical industry.

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OP-8

ANTIVIRAL POTENTIAL OF NATURAL PRODUCTS

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Human viral diseases remain one of the most serious threats to public health. The limited number of antiviral drugs, narrow spectrum of activity, toxicity, and the emergence of viral resistance are common problems in antiviral chemotherapy [1]. Moreover, in many human viral infections, no effective drugs are available. Approximately 44% of currently approved antiviral drugs are natural products, semi-synthetic natural product analogs, or synthetic compounds based on natural-product pharmacophores [2]. That is why natural products remain a significant bioresource for antiviral screening and further antiviral drug development.

In recent years, we have evaluated several natural products for their antiviral activity, including various types of propolis, honeys, and plant extracts, e.g., from *Spathodea campanulata*, *Ficus sur*, *Justicia secunda*, *Geranium* spp., *Oenanthe* spp., *Stachys sylvatica*, and *Micromeria nervosa*. The methodology included assessment of impact on virus-induced cytopathic effect (CPE), virus infectivity (titration), and viral load (qPCR).

Among these natural products, some showed significant activity against viruses. *S. campanulata* leaves methanolic extract (500 µg/mL) suppressed the formation of HHV-1-induced CPE in the infected VERO cells, reduced the HHV-1 infectious titer by 5.11 log, and HHV-1 viral load by 1.45 log. This difference in virus infectivity and viral load reduction indicated that late stages of viral replication were inhibited, as further confirmed by molecular docking to viral enzymes, which indicated inhibition of HHV-1 protease. *M. nervosa* extracts displayed potent antiviral activity against HHV-1 and adenovirus Ad5, reducing CPEs and viral titers, with qPCR revealing decreased HHV-1 load. *In silico* analysis suggested binding of HHV-1 glycoprotein D. The *S. sylvatica* ethanolic extract showed noticeable antiviral activity against the human coronavirus 229E (HCoV-229E) replication in MRC-5 cells, reducing viral load by 1.56 log, corresponding to 97.24% viral inhibition. These results confirm the antiviral potential of natural products.

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OP-9

MECHANISM OF ACTION A COMBINATION OF β-AESCIN AND NEWLY SYNTHESIZED ALKYLAMIDOBETAINES AS MODERN COMPONENTS ERADICATING THE BIOFILMS OF MULTIDRUG-RESISTANT *CANDIDA GLABRATA - LIPIDOME AND OXIDATIVE STRESS ANALYSIS*

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Alkylamidopropyl betaines (ABBs) are surfactants with a tertiary amide linker, characterized by the presence of a stable, quaternary nitrogen atom and a hydrolyzable or biodegradable moiety [1]. Aescin is a natural surfactant, a triterpene saponin, isolated from the seeds of the horse chestnut tree. Previous studies on the antibiofilm activity of newly synthesized alkylamidopropyl betaines against *C. glabrata* have shown the C9-substituted AAB to be the most effective. However, little is known about the effect of combining these compounds on *C. glabrata* cells [2]. The aim of this study is to shed light on the molecular mechanism of action of these compounds, which, due to their complementary antibiofilm activities, demonstrate promising efficacy.

Three strains of C*. glabrata* were used in the study: *C. glabrata* ATCC 90030 as the reference strain, and two drug-resistant clinical strains, *C. glabrata* 2586 and *C. glabrata* 2853. Yeast blastospores were treated with AAB-C9, β-escin, and a combination of these two compounds. Cells harvested after 6 hours were extracted using the Matyash method, and untargeted lipidomic analysis was performed on the resulting lipid extract. Additionally, oxidative stress was analyzed using fluorescence microscopy and molecular probes.

The lipidomic study revealed a reduction in the amounts of sterol and phospholipid intermediates (PE, PC) in cells treated with AAB C9 and β-escin. The combination of applied compounds increased oxidative stress in the yeast cells. This stress was not high and mainly affected budding and young cells. The tested compounds contribute to increased mitochondrial oxidative stress: increased detection of superoxide anion (O_2^-) and lipid oxidation.

The obtained results indicate that oxidative stress does occur in the tested reference strain and in clinical strains. However, this does not rule out the mechanism of action of the compounds, which, as further research indicates, may be multifactorial.

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YOUNG SCIENTISTS LECTURES

YSL-1

GEOGRAPHICAL STABILITY AND ENVIRONMENTAL MODULATION OF SECONDARY METABOLITES IN *MARCHANTIA POLYMORPHA* ACROSS EUROPE

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Marchantia polymorpha L. is a cosmopolitan liverwort and a key model species among early land plants, recognized for its rich diversity of biologically active secondary metabolites, particularly sesquiterpenoids and macrocyclic bisbibenzyls. Recent work has highlighted both the chemical complexity and bioactivity potential of this species [1]. The present study aimed to assess the extent of geographical variation in secondary metabolite composition of *M. polymorpha* across Europe, with particular emphasis on populations from Iceland in comparison with continental European regions. Plant material was collected from multiple locations in Iceland as well as from Poland, Sweden, Norway, Bulgaria and Romania. The samples were cultivated under non-axenic conditions on pot soil in a climate chamber. Homogenization was performed first with diethyl ether and subsequently with a methanol/chloroform mixture (2:1). Volatile constituents were analyzed using GC-MS, while non-volatile metabolites were examined by HPLC-MS/Q-TOF. Across all analyzed samples, a highly consistent qualitative metabolite profile was observed. The chemical composition was dominated by cuparane-, chamigrane- and thujopsane-type sesquiterpenoids as well as the macrocyclic bisbibenzyl marchantin A and its isomers. Icelandic populations showed metabolite patterns closely matching those of continental Europe, despite their climatic isolation. Differences between regions were mainly quantitative and involved variations in the relative abundance of individual sesquiterpenoids, bisbibenzyl isomers and selected minor phenolic constituents, rather than the occurrence of distinct chemotypes. These findings demonstrate pronounced chemical stability of *M. polymorpha* across broad geographical and environmental gradients, indicating robust biosynthetic pathways with limited sensitivity to regional ecological conditions. The results underline the suitability of this liverwort as a reliable source of natural products for comparative phytochemical and bioprospecting studies.

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YSL-2

CHROMATOGRAPHIC CHARACTERIZATION OF BETACYANIN PREPARATIONS WITH ANTIOXIDANT POTENTIAL OBTAINED FROM *HYLOCEREUS POLYRHIZUS* FRUIT EXTRACTS

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Betacyanins are nitrogen-containing pigments formed by the condensation of betalamic acid with cyclo-3,4-dihydroxyphenylalanine (cyclo-DOPA) [1]. Their characteristic coloration arises from an extended system of conjugated double bonds generated by the linkage of betalamic acid to cyclo-DOPA-5-*O*-glucoside. These betalain compounds are present in most plants of the order *Caryophyllales* and are widely recognized for their antioxidant activity, primarily attributed to their ability to scavenge free radicals and donate electrons due to their conjugated structure [2].

In this study, the fruit pulp of *Hylocereus polyrhizus* (Weber) Britton & Rose was extracted and subsequently pre-purified using column chromatography on weak anion exchange resin and hydrophobic C18 sorbent. Selected fractions were further purified by high-performance liquid chromatography (HPLC). Part of the pre-purified extract was also heated at 70-75 **°C** in citric buffer at pH 3. The betacyanin profile of the obtained preparations was characterized using liquid chromatography coupled with diode-array detection and electrospray ionization mass spectrometry (LC-DAD-ESI-MS) in selected-ion monitoring (SIM) mode. Antioxidant activity was evaluated *in vitro* using the Trolox Equivalent Antioxidant Capacity (TEAC) assay with ABTS radical cations.

The results of the chromatographic analysis revealed diverse betacyanin profiles for the individual preparations. Betanin and their acylated derivatives were identified in extracts. After heating the pre-purified extract, the predominant products in the betacyanin profile were monodecarboxylated pigments, with smaller amounts of bidecarboxylated derivatives. Moreover, a positive correlation between the degree of purification and antioxidant capacity was observed. Crude extracts exhibited lower free radical scavenging activity than fractions with a high degree of purification. These findings highlight the potential of betacyanins as bioactive compounds with significant health-promoting properties and suggest possibilities for their use in future research on industrial and functional foods.

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YSL-4

INFLUENCE OF PULSED ELECTRIC FIELD (PEF) TREATMENT ON THE PHYTOCHEMICAL PROFILE OF *IRIS DOMESTICA* NATURAL DEEP EUTECTIC SOLVENT (NDES) EXTRACTS.

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Our aim was to determine the best conditions for extraction of phytochemicals from *Iris domestica* roots employing reversible electroporation as a non-lethal extraction method. Natural Deep Eutectic Solvents (NDES, (choline chloride:xylose 1:2 + 30% H_2O, choline chloride:glucose + 1:2 30% H_2O, and choline chloride:ethylene glycol 1:2) were used as extraction media. Pulsed Electric Field (PEF) treatment was applied to the roots at a constant electric field strength (E), pulse duration (t), and frequency (f), while the number of delivered pulses varied between experiments. In the control group, the plants were dried, their roots were then separated, weighed, and subjected to conventional ultrasound-mediated methanolic extraction. All obtained extracts were dissolved in 80% HPLC-grade methanol and concentrated down to a final volume of 2 mL. HPLC-MS analysis was performed using UHPLC-HR-MS (Dionex UltiMate 3000RS, Thermo Scientific) coupled to a quadrupole time-of-flight high-resolution mass spectrometer (HR/Q-TOF/MS, Compact, Bruker Daltonik GmbH). Chromatographic separation was carried out on a Kinetex C18 reverse phase column. The mobile phase consisted of solvent A 0.1% formic acid in Milli-Q water, v/v) and solvent B (0.1% formic acid in acetonitrile, v/v) with a flow rate of 0.3 mL/min. Concentrations of the major compounds were calculated from acquired spectra based on calibration curves of the main compounds and compared using statistical tests.

The most effective extraction was achieved using NDES containing ethylene glycol combined with the delivery of 33 electrical pulses. Under these conditions, the broadest range, as well as the highest concentrations of several main active compounds (irisflorentin, 3′-hydroxytectoridin, iridin, resveratrol, psi-tectorigenin, irigenin) were achieved here in comparison with other protocols and solvents. It's worth mentioning here, that this sample showed higher concentrations of iridin (5.66 µg/mL vs 0.32 µg/mL) and 3′-hydroxytectoridin (0.65 µg/mL vs 0.12 µg/mL) than those obtained via conventional methanolic extraction (control), yet allowed for plant survival, which highlights the potential of presented extraction method. In summary, we found that use of NDES as the extraction medium had largely positively influenced the efficiency of electroporation-assisted extraction, however the extraction protocol and solvent composition require further optimization.

YSL-5

LINDEN INFLORESCENCES AND ELDERFLOWER AS NATURAL PRODUCTS WITH ANTIVIRAL PROPERTIES

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Linden (*Tilia cordata*) inflorescences are commonly used to relieve the symptoms of colds, coughs, and sore throats [1], although no reports of their antiviral effects have been published. Elderberry (*Sambucus nigra*) fruit juice has demonstrated anti-influenza properties [2], whilst extracts from the leaves and flowers have shown virucidal activity against the dengue virus [3]. An infusion from *S. nigra* flowers, *Hypericum perforatum* herb, and *Saponaria officinalis* roots has demonstrated anti-herpetic activity, whilst the compound preparation Sinupret®, which contains elderflower, possesses antiviral properties against influenza, parainfluenza, Coxsackie, RSV, rhinoviruses, and adenoviruses [4]. However, there are few studies on the antiviral activity of extracts from these plants used as single medicinal products. In view of the above, the aim of the present study was to evaluate their antiviral potential against herpesvirus type 1 (HHV-1), Coxsackievirus B3 (CVB3), and rhinovirus 14 (HRV-14).

T. cordata (TC) extract at a concentration of 62.5 µg/ml significantly inhibited the cytopathic effect (CPE) induced by HHV-1 infection in VERO cells, with only slight signs of cell rounding. Subsequent titration revealed a 2.73-log reduction in HHV-1 infectious titer, whilst qPCR analysis showed a 2.59-log reduction in viral load. At a lower dose, 31.25 µg/ml, the effect of TC on HHV-1-induced CPE was slight, with a titer reduction of only 0.58 log. Elderflower extract also demonstrated a noticeable, albeit weaker, antiviral effect against herpes at a concentration of 250 µg/ml, reducing CPE induced by HHV-1, lowering the infectious titer of the virus by 1.69 log and the viral load by 1.35 log. Both extracts had little effect on cells infected with CVB3 or HRV-14.

Linden inflorescence extract demonstrated a significant inhibitory effect on HHV-1 replication. Interestingly, the consistent reduction in infectious titer (2.73 log) and viral load (2.59 log) in samples treated with TC 62.5 µg/ml may indicate an inhibitory effect on the early stages of the replication cycle.

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YSL-6

ISOFLAVONES-RICH FABACEAE PLANTS AND THEIR IMPACT ON THYROID CELLS IN VITRO

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Isoflavones, phytoestrogens abundant in legumes, show structural similarity to 17β-estradiol and may interact with estrogen receptors [1]. The thyroid gland is a hormone-sensitive tissue because both normal thyrocytes and thyroid cancer cells express estrogen receptors [2]. Estrogen signaling is involved in the regulation of proliferation, apoptosis, and the redox balance of thyroid cells and may play a role in the development and progression of thyroid cancer [3].

The aim of the study was to evaluate the isoflavone composition of Genista tinctoria, Ononis arvense, Trifolium medium and Trifolium pratense, and to assess their effect on the proliferation and viability of thyroid cancer and normal cells in vitro.

Qualitative and quantitative analysis of isoflavones in selected legume species was performed using HPLC-UV-VIS. Plant material was extracted under optimized conditions using a fractional extraction scheme [4]. Biological activity of the extracts was assessed using a panel of human thyroid cell lines representing various histological types and grades of malignancy: follicular carcinoma (FTC-133), papillary carcinoma (TPC-1), and normal thyroid follicular epithelial cells (Nthy-ori 3-1). The effect of the extracts on viability and cell proliferation was assessed using the MTT and crystal violet assay, respectively.

TPC-1 cells were the most sensitive to the examined extracts, with IC50 112.04, 118.6, 158.26, and 16.99 µg/mL for Trifolium medium, T. pratense, Ononis arvense, and Genista tinctoria, respectively. Importantly, the extracts showed relatively low toxicity to normal thyroid cells (Nthy-ori 3-1). The strongest antiproliferative effect was noted for FTC-133 cells exposed to the highest tested concentration of Trifolium pratense and Trifolium medium extracts, with cell proliferation decrease to 40.57 ± 3.20 and 43.57 ± 1.84% of control, respectively.

These results suggest that isoflavone-rich plants may modulate thyroid cell behavior in vitro and represent a potential source of compounds with chemopreventive activity in thyroid cancer.

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POSTERS

P-1

LC/MS PROFILING OF PHENOLIC COMPOUNDS IN LEAVES OF *MAGNOLIA KOBUS* DC. AND EVALUATION OF THEIR ANTIOXIDANT POTENTIAL

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Magnolia kobus DC is a medium-sized tree native to Japan but also found in China and Korea. Its dried flower buds are used as sedative and analgesic components in TCM herbal medicinal products [1]. Leaf samples of *M. kobus* DC. were collected from two different locations in Poland, namely the Warsaw University Botanical Garden (MKW) and the Rogów Arboretum (MKR), and subjected to ultrasound-assisted extraction using 30, 50 and 70% (*V/V*) EtOH. Prepared extracts were analysed using coupled chromatographic (RP-LC), spectroscopic (PDA) and mass spectrometric (QTOF/ESI-MS/MS) techniques as described by Zgórka et al. (2023) [2]. The results showed diverse quantitative profiles of the main phenolic compounds, including phenolic acids, phenylethanoids, flavonoids and lignans. Total phenolic content (TPC), calculated as gallic acid equivalent (GAE), increased significantly with the elution strength of the extraction solvent and was the highest in extracts obtained with 70% EtOH (34.34 and 24.60 mg GAE/g dry weight, for MKW and MKR, respectively). The antiradical capacity of these herbal preparations, as assessed by the DPPH$^{\bullet}$ assay, was strongly correlated with the total phenolic acid and flavonoid content, which was about three and a half times and more than two times higher in MKW and MKR, respectively. MKW extract exhibited higher antiradical activity (IC_{50} ~12.13 µg/mL) than MKR extract (IC_{50} ~19.02 µg/mL). This study demonstrated a correlation between environmental factors in the plant habitat and the concentration and diversity of polyphenols synthesised in the leaves of the same magnolia taxon.

Keywords: Magnolia kobus DC., LC-MS phenolic profiling, antioxidant properties

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P-2

MATERIA MEDICA OPOLIENSIS – A RELATIONAL DATABASE INTEGRATING MULTI-SYSTEM KNOWLEDGE OF NATURAL MEDICINAL RESOURCES AND PHYTOTHERAPY

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Materia Medica Opoliensis (MMO) is a relational factographic-bibliographic database developed at the University of Opole. It integrates data on phytotherapy, pharmacognosy, and natural medicinal substances across all medical systems recognised by the WHO. No comparable database combining Western medicine, Traditional Chinese Medicine (TCM), Kampo, Ayurveda, Siddha, Unani, and Tibetan medicine within one relational architecture has been reported in the available literature to date. MMO, with over 250,000 records, is the largest currently available tool of this type.

The database was built on over 100 interconnected relational tables linked by a dense network of relations, enabling multi-dimensional querying and cross-system filtering. Data were obtained from international pharmacopoeial databases and official compendia (FP, Ph. Eur., Pharmacopoeia of the People's Republic of China, Ayurvedic Pharmacopoeia of India), peer-reviewed publications, and academic textbooks. All WHO-recognised medical systems are treated as equal – a principle not found in any existing database. Media content analyses were also applied to Polish herbal periodicals; the complete run of "Wiadomości Zielarskie" (1956–2002) was encoded in full.

At present the database holds 10,146 species, 13,377 medicinal substances, 2,581 disease entities, 2,353 pharmacological mechanisms, 38,973 indications and contraindications, and over 22,000 chemical compounds. Users can search substances by pharmacological activity, screen natural–synthetic drug interactions, design herbal compositions with cross-system comparison, or use the built-in therapy assistant. MMO is freely accessible at https://mmo.uni.opole.pl.

No other publicly available resource covers a comparable scope. To the best of the authors' knowledge, MMO is the first database to integrate phytotherapy data from all WHO-recognised medical systems at this scale.

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P-3

EFFECT OF PLANT GROWTH REGULATORS ON THE BIOCHEMICAL POTENTIAL ACTIVITY OF EXTRACTS FROM *ACHILLEA PTARMICA* L. IN VITRO CULTURES

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Background: Achillea ptarmica L. is an underexplored species of the Achillea genus, producing diverse secondary metabolites with antioxidant, anti-inflammatory and antimicrobial activities [1]. In vitro plant culture enables controlled biomass production independent of seasonal variation and allows optimization of conditions to enhance secondary metabolite production [2]. This study aimed to optimize culture conditions to improve A. ptarmica shoot biomass growth and phenolic compounds production which can influence on tyrosinase activity.

Methods: The cultures initiated from seeds were maintained on Murashige and Skoog (MS) medium supplemented with different plant growth regulators (PGRs): 1-naphthaleneacetic acid (NAA; 0.1 mg/L) combined with cytokinins: thidiazuron (TDZ, 0.5 mg/L), 6-benzyladenine (BA, 1 mg/L) or zeatin (Zea, 1 mg/L) and gibberellic acid (GA_3, 0.2 mg/L) as well as PGR-free as the control. Cultures were maintained over 4 week growth periods (n = 5) [3]. Extracts from lyophilized biomass were prepared in 50% ethanol. Total phenolic content (TPC) was measured using the Folin–Ciocalteu assay and tyrosinase inhibition using mushroom tyrosinase assay [4] together with the HPLC-MS/MS fingerprinting of the obtained extracts..

Results: The tissue growth rate as well as metabolite production depended on the applied media variant. The highest biomass increase and TPC (0.47 mg GAE/g DW on TDZ-containing medium and 0.43 mg GAE/g DW on TDZ + GA_3 medium vs 0.29 mg GAE/g DW in control) were reached on media supplemented with TDZ and GA_3. These extracts showed the strongest tyrosinase inhibition (~27% at 200 µg/mL) vs ~25% in control. Soil-grown plants showed lower activity: TPC was 0.40 mg GAE/g DW (leaf) and 0.45 (herb), while inhibition reached ~5% and ~23%, respectively. The HPLC-MS/MS profile of the tested samples was diverse, however the phenolic compounds, including the derivatives of caffeic acid constituted the differentiating group of components.

Conclusions: MS media enriched with TDZ exerted the strongest stimulatory effects on biomass growth and phenolic biosynthesis. Optimized in vitro cultures of A. ptarmica represent a scalable and sustainable platform for the production of bioactive phenolics with potential pharmaceutical and cosmetic applications.

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P-4

INTESTINAL PERMEABILITY OF MICROBIOTA-DERIVED METABOLITES FROM LAVENDER WATER EXTRACT ASSESSED BY CACO-2 CELLS AND CHROMATOGRAPHY

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Lavender flowers contain phenolic acid glycosides, including *E*- and *Z*-isomers of melilotoside and 4-methoxymelilotoside, which undergo metabolic transformation in the gastrointestinal tracts. Previous studies demonstrated that gut bacteria cleave the sugar moiety of these compounds, with *Z*-isomers undergoing subsequent cyclization to form the corresponding lactones, coumarin, and herniarin [1]. The present study evaluated the permeability of these compounds and their metabolites using the Caco-2 intestinal barrier model. In permeability experiments, *Z*-melilotoside (**1**) and *Z*-4-methoxymelilotoside (**2**) were detected on the acceptor side when tested as part of the lavender extract (LOI), whereas the *E*-isomers (**3** and **4**) were not detected within 24 hours. These findings suggest that intestinal absorption is strongly dependent on metabolic conversion and indicate that coumarin and herniarin are likely the key compounds responsible for intestinal permeation (**Fig**. **1**).

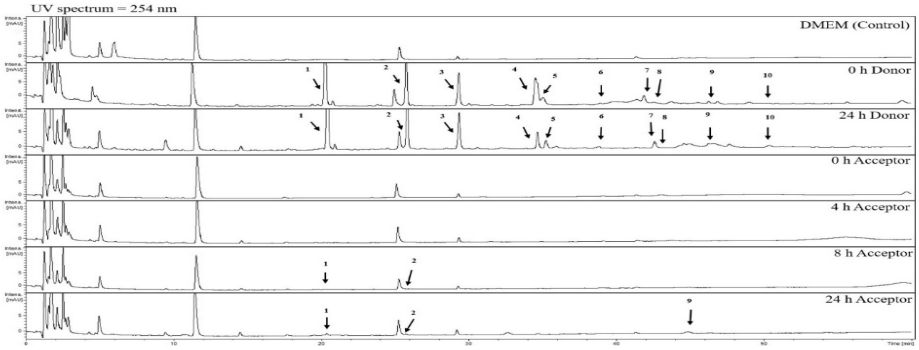

Figure 1: The UHPLC-DAD-IT-MS chromatograms of donor and acceptor side samples collected during the Caco-2 permeability assay of LOI. Peaks: **1** - Z-melilotoside, **2** - Z-4-methoxymelilotoside, **3** - E-melilotoside, **4** - E-4-methoxymelilotoside, **5** - apigenin-O-glucuronide-O-hexoside, **6** - unidentified compound, **7** - quercetin glycoside derivative, **8** - luteolin-7-O-glucuronide, **9** - apigenin-7-O-glucopyranoside, **10** - apigenin-7-O-glucuronide. The Dulbecco's Modified Eagle Medium (DMEM) was used as the control.

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P-5

SCALE-UP OF ROSMARINIC ACID PRODUCTION IN *SALVIA ATROPATANA* SHOOT CULTURE USING TEMPORARY IMMERSION SYSTEMS

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Salvia atropatana (Lamiaceae) is a medicinal plant native to the Middle East and traditionally used for the treatment of inflammation, wounds, infections, and diabetes [1]. Its pharmacological potential is associated with a rich phenolic profile, with rosmarinic acid (RA) recognized as the predominant bioactive compound. The present study aimed to scale up *S. atropatana* shoot culture using temporary immersion system systems (Plantform and RITA) to enhance RA production.

Shoots were cultivated in both bioreactors in Murashige and Skoog medium [2] with 0.1 mg/L indole-3-acetic acid and 1 mg/L 6-benzylaminopurine. After 40 days of culture, biomass growth was evaluated by determining the dry weight growth index (DW_GI). Polyphenolic compounds in 80% methanolic extracts of the obtained plant material were analyzed by high-performance liquid chromatography (HPLC) using an Agilent Technologies 1290 Infinity system equipped with an Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm). The mobile phase consisted of acetonitrile (A) and 0.1% formic acid (B) with the following gradient elution profile: 0–5 min, 10% A; 5–20 min, 18% A; 20–25 min, 38% A; 25–30 min, 100% A.

Intensive biomass accumulation was observed in both systems, with DW_GIs of 35, and a higher proliferation rate in the RITA bioreactor (7.8 *vs.* 6.6). Phytochemical profiling by UPLC-DAD/ESI-MS revealed nine phenolic constituents, including caffeic acid, caffeoyl-threonic acid, prolithospermic acid, salvianolic acid K, two isomers of salvianolic acid F, and rosmarinic acid together with its derivatives (rosmarinic acid hexoside and methyl rosmarinate). RA was the dominant metabolite, exceeding 30 mg/g DW in both bioreactor cultures. This level was more than 4.5 times higher than that detected in soil-grown plants and approximately two times higher than in shoots cultivated on solid medium [3]. Overall, both temporary immersion systems efficiently supported large-scale rosmarinic acid production in *S. atropatana* shoot culture, reaching approximately 300 mg/L within a 40-day culture cycle.

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P-6

PHENYLPROPANOID PATHWAY ACTIVATION IN METHYL JASMONATE-ELICITED *SALVIA BULLEYANA* SHOOTS

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Salvia bulleyana Diels. (Lamiaceae), a medicinal plant native to the mountainous regions of China, has traditionally been used to treat cardiovascular disorders, liver diseases, and inflammatory conditions [1]. Its biological activity is mainly associated with phenolic acids, particularly rosmarinic acid (RA) [2]. The present study investigated the effect of methyl jasmonate (MJA) elicitation on RA biosynthesis in *S. bulleyana* shoot cultures, focusing on phytochemical analysis and expression of selected genes of the phenylpropanoid pathway.

The shoots were cultured in liquid Murashige and Skoog medium [3] supplemented with 0.1 mg/L indole-3-acetic acid and 1 mg/L meta-topolin. After 30 days, methyl jasmonate (50 μM) was added to the medium, while control cultures received an equivalent amount of ethanol. Biomass was determined 1, 3, and 5 days after elicitation. Freeze-dried plant material was extracted with 80% methanol. Rosmarinic acid content was quantified using HPLC-DAD with an Agilent Technologies 1290 Infinity chromatographic system equipped with a Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 5 μm). The mobile phases consisted of acetonitrile (A) and water with 0.1% formic acid (B). Expression of three key genes of the phenylpropanoid pathway - phenylalanine ammonia-lyase (*PAL*), tyrosine aminotransferase (*TAT*), and rosmarinic acid synthase (*RAS*) was analysed using quantitative real-time PCR method.

Phytochemical analysis confirmed rosmarinic acid as the dominant metabolite in *S. bulleyana* culture. Methyl jasmonate significantly stimulated RA accumulation, with the highest level detected after 3 days of elicitation (over 40 mg/g DW), about 20% higher than in non-elicited shoots. The strongest transcriptional response was observed one day after elicitation, with *TAT* and *RA*S showing 4-fold and 2-fold increases in relative expression, respectively, compared with the control, whereas *PAL* showed no significant change These results indicate that methyl jasmonate can effectively enhances rosmarinic acid biosynthesis in *S. bulleyana* shoot cultures.

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P-7

PHYTOCHEMICAL PROFILING OF PHENYLETHANOID IN *CLERODENDRUM COLEBROOKIANUM* HAIRY ROOT CULTURES

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Clerodendrum colebrookianum Walp. (Lamiaceae) is a perennial shrub native to India, Malaysia, and Indonesia and used in traditional medicine to treat inflammation, hypertension, diabetes, hyperlipidemia, and cancer [1]. Phytochemical studies have reported terpenoids, steroids, phenylethanoids, flavonoids, and lignans in this species [2]. The present study aimed to establish hairy root cultures of *C. colebrookianum* and evaluate their potential for the production of bioactive metabolites.

Hairy roots were induced by infecting stem explants at the nodal region with *Rhizobium rhizogenes* strain A4. Two clones (L1 and L2) were selected for further evaluation of growth and secondary metabolite production and cultivated in WP medium [3]. Phytochemical profiling of hydromethanolic extracts was performed using UPLC-DAD/ESI-MS operating in the negative ion mode. Qualitative analysis was carried out using an Agilent Technologies 1290 Infinity LC system equipped with an InfinityLab Poroshell 120 EC-C18 column (4.6 × 150 mm, 4 µm). Compounds were identified based on retention times, UV spectra, and mass spectral data compared with authentic standards and literature data.

Clone L1 showed slightly higher biomass accumulation than L2, with a dry weight growth index of 6.34 and 5.69, respectively. UPLC-DAD/ESI-MS analysis of hairy root extracts revealed nine phenylethanoid glycosides (acteoside, isoacteoside, acteoside isomer, campneoside I, leucosceptoside A, **β**-hydroxyacteoside, lipedoside A, eukovoside/cistanoside C, martinoside) and one flavonoid (hispidulin glucuronide). Acteoside was the predominant metabolite. Its content exceeded 52 mg/g DW in clone L1 and reached 32 mg/g DW in L2. In contrast, clone L2 accumulated higher amounts of **β**-hydroxyacteoside, campneoside I, isoacteoside, and martinoside. Total phenolic content reached over 65 mg/g DW in L1 and 48 mg/g DW in L2.

These results indicate that hairy root cultures of *C. colebrookianum* constitute a promising system for the production of phenylethanoid glycosides, with metabolite accumulation dependent on clonal variability.

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P-8

METABOLIC REPROGRAMMING OF MACROPHAGES DURING INFECTION: IMPACT OF ANTIBIOTICS AND NATURAL PRODUCTS ON AMINO ACIDS, CERAMIDES, AND TCA CYCLE INTERMEDIATES

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Understanding host–pathogen interactions and the efficacy of antimicrobial treatments requires detailed insight into immune cell function and metabolic adaptation. Traditionally, infection and macrophage responses have been assessed using immunological biomarkers and bacterial clearance assays. However, metabolomics has emerged as a powerful approach to capture dynamic biochemical changes that reflect cellular activity and physiological states. Because metabolites are direct products of cellular processes, their profiles provide a sensitive readout of how macrophages respond to infection and therapeutic interventions.

Upon encountering pathogens, macrophages undergo profound metabolic reprogramming to support immune functions, including increased glycolysis, remodeling of the tricarboxylic acid (TCA) cycle, and alterations in lipid metabolism [1-2]. Ceramides, a class of sphingolipids, play key roles in this process by regulating inflammatory signaling, organizing membrane microdomains, and enhancing phagocytosis through phagosome-lysosome fusion [3]. At the same time, pathogens can exploit host metabolism, for example, by manipulating the TCA cycle or utilizing amino acids as nutrient sources, to enhance their intracellular survival and impair immune defense mechanisms [4].

In this study, metabolic profiling of amino acids, ceramides, and TCA cycle intermediates was performed using mass spectrometry with internal standards. The results demonstrated pronounced changes in metabolite levels across all three classes following treatment of infected macrophages with antibiotics and natural products. These findings underscore the significant impact of therapeutic interventions on macrophage metabolism and provide insight into metabolic mechanisms shaping infection outcomes and treatment responses.

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P-9

HOT-MELT EXTRUSION STABILIZED BY CYCLODEXTRINS FOR SOLUBILITY ENHANCEMENT OF OLEANOLIC ACID

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Oleanolic acid (OA) is a natural triterpene with a broad spectrum of biological activity, whose therapeutic application is significantly limited by its low aqueous solubility [1]. One of the most effective strategies to overcome this limitation is amorphization, which increases the free energy of the system, thereby enhancing solubility and, consequently, bioavailability. Hot-melt extrusion (HME) is an efficient method for obtaining amorphous forms, enabling the preparation of amorphous solid dispersions (ASD).

The aim of this study was to evaluate the effect of cyclodextrins on the physicochemical properties and solubility of HME extrudates containing OA. Systems with OA and PVP VA64 were prepared both without cyclodextrins and with HP-γ-CD (equimolar to OA) at OA:polymer molar ratios of 1:5, 1:10, and 1:15, at 160 °C and 20 rpm [2]. The obtained systems were characterized using FTIR spectroscopy and X-ray powder diffraction.

FTIR analysis indicated the presence of intermolecular interactions between OA and PVP VA64 after the extrusion process. Equilibrium solubility studies showed a significant increase in OA solubility for all systems compared to the pure compound. The highest values were observed for HP-γ-CD-containing systems, with approximately a 35-fold increase compared to corresponding systems without cyclodextrins. No statistically significant differences were found between systems with and without HP-γ-CD at the 1:15 ratio.

In cyclodextrin-containing systems, solubility increased with decreasing polymer content (from 0.57 to 1.85 mg/mL), suggesting an additional stabilization mechanism dependent on polymer proportion.

These results indicate that combining HME with cyclodextrins is a promising strategy for enhancing the solubility of OA beyond polymer-based systems alone.

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P-10

FUNCTIONAL MODULATION OF EPITHELIAL-MESENCHYMAL TRANSITION AND TUMOR MICROENVIRONMENT SIGNALLING BY DIOSMETIN IN BREAST CANCER IN VITRO MODEL

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Metastasis remains the leading cause of mortality in breast cancer and is closely linked to epithelial–mesenchymal transition (EMT) and tumor microenvironment (TME) remodeling. Diosmetin (DT), the active metabolite of diosmin, a widely used venoactive drug and dietary supplement - has emerged as a potential anticancer agent. Building on our previous findings demonstrating that DT enhances doxorubicin efficacy, this study investigated its role in modulating tumor cell plasticity and tumor–stroma interactions. EMT was induced in MCF-7 cells, while a stromal model was established by TGF-β–mediated activation of BJ fibroblasts into a cancer-associated fibroblast (CAF)-like phenotype. Additionally, doxorubicin-induced senescence was generated in fibroblasts. Migration assays and quantitative real-time PCR were used to assess functional and transcriptional changes. EMT induction resulted in decreased CDH1 expression and increased VIM, MMP2, MMP9, IL-6, and HIF-1A levels, accompanied by enhanced migration. DT attenuated these changes by partially restoring epithelial marker expression and reducing mesenchymal, inflammatory, and proteolytic gene expression, leading to decreased EMT-driven migration. In fibroblasts, DT reduced CAF-like activation, as evidenced by decreased ACTA2, HGF, MMPs, and IL-6 expression, and modulated hyaluronan-related genes. Moreover, DT partially alleviated doxorubicin-induced senescence. Collectively, DT acts as a context-dependent modulator of EMT and TME signalling. Given its origin as a metabolite of a clinically used compound and its previously demonstrated chemosensitizing properties, DT represents a promising candidate for repurposing strategies aimed at limiting metastatic progression in breast cancer.

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COMPREHENSIVE CHROMATOGRAPHIC PROFILING AND BIOACTIVITY ASSESSMENT OF *ALCHEMILLA SPECIOSA* BUSER FOR DERMATOLOGICAL APPLICATIONS

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The genus *Alchemilla* (Rosaceae) comprises species widely used in traditional medicine, particularly for the treatment of skin disorders. Despite extensive ethnopharmacological relevance, *Alchemilla speciosa* Buser remains poorly characterized in terms of its phytochemical composition and biological activity. This study focuses on the comprehensive chromatographic profiling of *A. speciosa* aerial parts and the evaluation of their dermatologically relevant bioactivities.

Phytochemical analysis was performed using chromatographic techniques, including liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and gas chromatography–mass spectrometry (GC-MS), enabling detailed identification of secondary metabolites. Quantitative spectrophotometric assays were used to determine total phenolic, flavonoid, and phenolic acid contents. The antioxidant potential of extracts and fractions was assessed using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging assays, as well as metal chelation capacity.

To evaluate dermatological relevance, enzyme inhibition assays targeting collagenase, elastase, and tyrosinase were conducted. Antibacterial activity was tested against skin-associated microorganisms, including *Cutibacterium acnes*. Additionally, cytotoxicity and anti-lipid peroxidation effects were investigated in normal human skin fibroblasts.

Chromatographic analyses revealed a rich profile of bioactive constituents, including phenolic acids, flavonoids, and pentacyclic triterpenes. The studied extracts demonstrated strong antioxidant activity and moderate inhibition of collagenase and tyrosinase. Noticeable antibacterial activity (MIC 12.5-200 µg/mL) and low cytotoxicity (≥80% cell viability at 62.5 µg/mL) were observed. Selected fractions exhibited high therapeutic indices (TI ≥ 10) and effectively reduced H_2O_2-induced lipid peroxidation in fibroblasts.

These results highlight the value of chromatographic approaches in the characterization of underexplored plant species and identify A. speciosa as a promising source of bioactive compounds with potential applications in dermatology and cosmetology.

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CHROMATOGRAPHIC PROFILING OF *ALCHEMILLA PERISTERICA* AND *A. CRINITA* EXTRACTS IN RELATION TO THEIR ANTI-HHV-1 ACTIVITY

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Species of the genus *Alchemilla* (Rosaceae) are recognized as rich sources of phenolic compounds with diverse biological activities; however, many taxa remain insufficiently explored. In this study, *Alchemilla peristerica* and *Alchemilla crinita* were investigated with a particular focus on comprehensive chromatographic characterization and evaluation of their antiviral potential against Human Herpesvirus type 1 (HHV-1).

Phytochemical profiling of selected extracts was performed using liquid chromatography-tandem mass spectrometry (LC-MS) and gas chromatography–mass spectrometry (GC-MS), enabling the identification of major groups of secondary metabolites.

Cytotoxicity was assessed in VERO cells using the MTT assay after 72 h incubation to determine maximum non-toxic concentrations (MNTC), which were established at 15.6 µg/mL for AP2OE, AC4OE, and AP2MAW, and 62 µg/mL for AC4MAW. The antiviral activity was subsequently evaluated at non-cytotoxic concentrations by monitoring virus-induced cytopathic effect (CPE) and determining the reduction in infectious viral titter using the endpoint titration method.

Despite the presence of phytochemically diverse constituents revealed by chromatographic analysis, the tested extracts did not demonstrate significant antiviral activity against HHV-1, defined as a reduction in viral titer of at least 3 log units.

The results highlight the importance of combining advanced chromatographic techniques with biological assays in the evaluation of natural products. Although *A. peristerica* and *A. crinita* extracts were not effective inhibitors of HHV-1 replication under the tested conditions, their detailed chemical characterization contributes to the growing knowledge of the genus and provides a foundation for further studies on their biological potential.

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KAZAKH FLORA UNDER THE LENS: PHYTOCHEMICAL PROFILE AND ANTIMICROBIAL ACTIVITY OF *COTONEASTER MULTIFLORUS* AND *C. MELANOCARPUS* EXTRACTS

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Cotoneaster melanocarpus (CME) and *C. multiflorus* (CMU) are medicinal shrubs of the Rosaceae family, increasingly studied for their rich secondary-metabolite profiles [1]. The present study aimed at characterizing the chemical composition of CME and CMU extracts and evaluating their antimicrobial activity.

First, 70% hydroethanolic (v/v) CME and CMU extracts were prepared from aerial parts and fruits collected in Almaty, Kazakhstan. Then, the extracts were qualitatively analysed using HPLC-ESI-QTOF-MS. Their antimicrobial activity was evaluated using microbroth dilution method by determining the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) against five reference pathogenic Gram-positive bacteria, five Gram-negative bacteria, two yeasts and three probiotic bacteria.

Chemical analysis identified 20 compounds (organic acids, phenolic acids, flavonoids, proanthocyanidins) across four extracts with common quinic, citric, chlorogenic acid, rutin, and hyperoside. CME plant extract uniquely contained neochlorogenic acid, procyanidin B2, coumaroyloquinic acid isomer I, caffeoylomalic acid, kaempferol rutinoside, caffeoyloquinic acid glucoside; fruit extract had kaempferol 3-O-glucoside, quercetin pentoside. CMU plant extract featured vanillyl glucoside, coumaroyloquinic acid isomer II, quercetin malonyl glucoside, quercetin; fruit extract had vanillic acid glucoside, cryptochlorogenic acid, epicatechin. Both CME and CMU extracts exhibited the highest antimicrobial activity against *Staphylococcus* spp. and *B. cereus* (MIC = 1 - 4 mg/ml). Low antibacterial activity was observed against *C. difficile*, *L. monocytogenes* (MIC = 16 mg/ml), and probiotic *Lactobacillus* and *Bifidobacterium* (MIC ≥ 16 mg/ml).

The study demonstrates that CME and CMU extracts rich in phenolic compounds exhibited the strongest antimicrobial activity against *Staphylococcus* spp. and *Bacillus cereus.* High insensitivity for probiotic bacteria highlights the potential of CME and CMU extracts as candidates for prebiotic applications.

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P-14

BEYOND A CUP OF MOUNTAIN TEA: PHYTOCHEMICAL DISTINCTIVENESS AND IMMUNOMODULATORY DIVERGENCE ACROSS THREE *SIDERITIS* SPECIES

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Plants of the genus *Sideritis* L. (Lamiaceae), commonly referred to as mountain teas, have a long-standing tradition of use in the Mediterranean region, particularly for the management of upper respiratory tract infections and catarrhal conditions. This traditional application is acknowledged in the European Medicines Agency (EMA) herbal monograph established for *Sideritis scardica*. Despite growing interest in the biological properties of *Sideritis* species, the mechanisms underlying their anti-inflammatory and immunomodulatory activity remain insufficiently characterized. Moreover, the genus exhibits considerable interspecific phytochemical variability, which may lead to substantial differences in biological potential among species.

The present study aimed to characterize the phytochemical profiles of three Sideritis L. species using HPLC-DAD-MS/MS^n and to evaluate the effects of their infusions and ethanolic extracts on proinflammatory cytokine and chemokine release (TNF-α, IL-6, IL-1β, IL-8) and reactive oxygen species (ROS) generation in human immune cell models, namely neutrophils isolated from peripheral blood and THP-1-derived macrophages.

HPLC-DAD-MS/MS^n analysis revealed distinct phytochemical fingerprints among the investigated species, reflecting marked interspecific diversity in secondary metabolite composition. Extracts and their subfractions demonstrated species- and preparation-dependent efficacy in suppressing proinflammatory cytokine secretion and ROS production by human immune cells relative to stimulated controls. Notably, the observed differences in biological activity corresponded to the variation in phytochemical profiles, suggesting that species identity and extraction method are critical determinants of the immunomodulatory potential within the genus.

In conclusion, the studied *Sideritis* species represent a valuable yet heterogeneous source of bioactive compounds with anti-inflammatory properties. The demonstrated link between phytochemical composition and immune cell modulation underscores the importance of species-level characterization when assessing the therapeutic value of mountain teas. These findings contribute to the pharmacological rationale for the traditional use of *Sideritis* in respiratory conditions and provide a foundation for further investigation into species-specific applications in evidence-based phytotherapy.

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FROM MEDITERRANEAN TRADITION TO MOLECULAR TARGETS: FLAVONOL GLYCOSIDES AND ELLAGITANNINS FROM *CISTUS* L. AS SELECTIVE MODULATORS OF RESPIRATORY INFLAMMATION

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Upper respiratory tract infections (URTIs) rank among the leading causes of outpatient medical visits worldwide, and the associated mucosal inflammation represents a principal target for symptomatic therapy. Herbal medicinal products continue to hold an established position in this therapeutic context. *Cistus creticus* L. and *Cistus ladanifer* L. have a long-standing history of use in Mediterranean traditional medicine for the management of URTIs. This traditional application has recently been formalized at the European level through the adoption of the EMA herbal monograph on *Cisti cretici herba* (2025)[1]. However, the individual bioactive constituents responsible for the anti-inflammatory activity of *Cistus* preparations, along with their respective molecular targets, have not been systematically characterized[2,3].

The aim of the present study was to perform a bioassay-guided isolation and structural elucidation of the principal polyphenolic constituents of *C. creticus* and *C. ladanifer* 60% ethanolic extracts and to delineate their individual anti-inflammatory activity, with particular emphasis on mediator selectivity and modulation of adhesion molecule expression, in complementary human neutrophil and endothelial cell models.

HPLC-DAD-MS/MS^n analysis revealed distinct phytochemical fingerprints of the investigated *Cistus* species. The isolated polyphenols displayed pronounced mediator-selective activity: myricetin 3-*O*-rhamnoside was the most potent TNF-α suppressor (15.4 ± 2.0% of stimulated control at 50 μM), whereas punicalagin preferentially inhibited IL-8 release (33.2 ± 4.5%). In TNF-α-stimulated endothelium (HUVECs), an acylated kaempferol derivative exhibited the broadest activity, reducing VCAM-1, and ICAM-1 expression to 25.9 ± 6.5%, and 24.9 ± 5.7% of stimulated control, respectively. All tested flavonoids selectively inhibited CD11b/CD18 without affecting CD11a/CD18 expression, while myricetin 3-*O*-rhamnoside enhanced selectin CD62L expression, indicating attenuation of the inflammation-driven neutrophil activation phenotype.

Collectively, the anti-inflammatory activity of *Cistus* preparations arises not from a single lead compound but from the mechanistically complementary actions of co-occurring flavonol glycosides and ellagitannins, providing a compound-level rationale for the traditional use of *Cistus* species in the treatment of respiratory inflammation.

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PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL EVALUATION OF HYDROETHANOLIC EXTRACTS OBTAINED FROM FIVE *ACHILLEA* SP.

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The purpose of this work was to identify and quantitatively determine the phenolic compounds of the hydroethanolic extracts obtained from *A. atrata, A. setacea, A. nobilis, A. erba-rotta, A. erba-rotta susp. moschata* and to investigate their antioxidant, and inhibitory properties towards mushroom and murine tyrosinase.

The secondary metabolites present in the extracts were identified by HPLC–ESI-Q-TOF-MS/MS. The fingerprinting confirmed the presence of several phenolic compounds like: caffeic, protocatechuic, chlorogenic, hydroxybenzoic, gentisic, quinic, dicaffeoylquinic and feruloylquinic acids. Flavonoids and their glycosides were also detected in the samples and among them rutoside, hyperoside, isorhamnetin hexoside, luteolin, quercetin, apigenin and kaempferol.

Based on the information presented above the content of the aforementioned compounds may have a significant meaning in the total tyrosinase inhibitory potential of the analyzed extracts. Mushroom tyrosinase activity was determined according to the method described by Uchida et al. [1,2]. Additionally, a murine tyrosinase model was employed, based on enzyme preparations derived from B16F10 cell lysates, as described by Strzępek-Gomółka et al. [3] Inhibition assays using mushroom tyrosinase showed that *A. erba rotta* extracts reduced enzyme activity by up to 55% at the highest tested concentration (200 µg/mL). However, these extracts were less effective against murine tyrosinase in contrast, *A. atrata* extracts showed significantly stronger inhibitory activity against murine tyrosinase, reaching up to 68% inhibition at a concentration of 100 µg/mL. This is particularly relevant, as murine tyrosinase, being of mammalian origin, is more similar in structure and function to the human enzyme.

The antioxidant activity of the analyzed samples was evaluated using the DPPH assay. The highest radical scavenging capacity was observed for extracts *A. erba rotta* (IC_{50}=0.0115) and *A. erba-rotta ssp. Moschata* (IC_{50} = 0.0146), compared to the reference standard, vitamin C (IC_{50} = 0.0018). The remaining samples showed lower activity, indicating variability among the tested materials. The results are consistent with the qualitative compositional analysis, suggesting that the identified compounds may contribute to the observed antioxidant effects.

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P-17

NEUROPROTECTIVE POTENTIAL OF SPECIOFOLINE IN A 6-HYDROXYDOPAMINE ZEBRAFISH LARVAL MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized by progressive dopaminergic neurodegeneration, and existing therapies fail to halt disease progression. Plant-derived alkaloids represent a structurally diverse source of compounds with neuroprotective potential. Speciofoline is a tetracyclic oxindole alkaloid isolated from *Mitragyna inermis*, a plant of the Rubiaceae family. Structurally related oxindole alkaloids from the same genus have demonstrated neuroprotective activity in experimental PD models through anti-neuroinflammatory and autophagy-inducing mechanisms. The neuroprotective potential of speciofoline itself has not been investigated in any experimental model of PD.

At 3 days post-fertilization (dpf), zebrafish larvae were co-exposed to 6-hydroxydopamine (6-OHDA; 250 µM) and speciofoline at concentrations of 15, 31, and 62 µg/mL for 72 hours. Locomotor activity was subsequently assessed using the DanioVision (Noldus) system under a standardized light/dark protocol: 10 min acclimation in light, followed by 10 min light-phase and 10 min dark-phase recording. Total distance traveled served as the primary endpoint.

6-OHDA exposure markedly reduced locomotor activity relative to untreated controls, consistent with dopaminergic impairment. Co-treatment with speciofoline at 62 µg/mL reversed this hypoactivity, while lower concentrations (15 and 31 µg/mL) were without significant effect, indicating a concentration-dependent neuroprotective response.

These results provide the first evidence of neuroprotective activity for speciofoline in an experimental PD model, and support further investigation of its mechanistic targets within dopaminergic and broader neuromodulatory signaling pathway.

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NEUROPROTECTIVE POTENTIAL OF LYCOPODIUM DEUTERODENSUM IN A 6-HYDROXYDOPAMINE ZEBRAFISH LARVAL MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is characterized by progressive dopaminergic neurodegeneration, and existing therapies fail to slow down disease progression. Lycopodiaceae are known to produce structurally diverse alkaloids, including lycopodine-type compounds, some of which exhibit central nervous system activity. Alkaloids of the *Lycopodium* have been shown to exert neuroprotective effects through mechanisms including acetylcholinesterase inhibition and modulation of neuronal survival pathways, with activity in experimental models of neurodegenerative disease. *Lycopodium deuterodensum*, a lycophyte with broad geographic distribution, contains alkaloids of this structural class, however, the biological activity of its extracts has not been investigated in experimental models of PD.

At 3 days post-fertilization (dpf), zebrafish larvae were co-exposed to 6-hydroxydopamine (6-OHDA; 250 µM) and an ethyl acetate extract of *L. deuterodensum* at concentrations of 7, 15, and 31 µg/mL for 72 hours. Locomotor activity was subsequently assessed using the DanioVision (Noldus) system under a standardized light/dark protocol: 10 min acclimation in light, followed by 10 min light-phase and 10 min dark-phase recording. Total distance traveled served as the primary endpoint.

6-OHDA exposure markedly reduced locomotor activity relative to untreated controls, consistent with dopaminergic impairment. Co-treatment with the extract at 15 µg/mL reversed this hypoactivity, indicating a neuroprotective effect at this concentration. The lowest concentration tested (7 µg/mL) was without effect, while the highest concentration (31 µg/mL) was associated with increased larval mortality, precluding locomotor assessment and indicating a narrow therapeutic window for this extract.

These results provide the first evidence of neuroprotective activity for a *L. deuterodensum* extract in an experimental PD model. Further studies are warranted to characterize the alkaloid composition responsible for the observed effect and to assess dopaminergic neuron integrity at the neuroprotective concentration.

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BIOACTIVITY-GUIDED FRACTIONATION OF *MELISSA OFFICINALIS* USING CENTRIFUGAL PARTITION CHROMATOGRAPHY REVEALS DIFFERENTIATED IN VITRO AND IN VIVO EFFECTS

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Melissa officinalis L. (Lamiaceae) is a well-established medicinal plant widely used in traditional therapies and increasingly incorporated into modern cosmetic formulations[1]. Despite its long-term use and favorable safety profile, the contribution of individual constituents within its complex phytochemical matrix to biological activity remains insufficiently characterized.

The aim of this study was to evaluate the biological activity of a crude lemon balm extract and fractions obtained using centrifugal partition chromatography (CPC), combining in vitro enzyme-based assays with in vivo evaluation [2].

CPC was applied as a support-free liquid–liquid separation technique enabling efficient fractionation without irreversible adsorption, which preserves the native composition of metabolites and at the same time offers scalability and reproducibility. The in vitro activity was assessed based on the inhibition of tyrosinase using both pre-column and post-column assays coupled with HPLC-MS, enabling localization of active constituents within the chromatographic profile. In vivo evaluation was performed in a zebrafish model.

The results demonstrated that fractionation led to differentiation of biological responses, with selected CPC-derived fractions exhibiting enhanced activity compared to the crude extract. The integration of chromatographic separation with on-line and off-line bioassays enabled effective tracking of active fractions within the complex mixture.

In conclusion, this study highlights CPC as an effective tool for the investigation of plant-derived mixtures and demonstrates the value of integrating advanced analytical techniques with biological assays. The findings provide new insights into the functional complexity of *Melissa officinalis*, supporting its further exploration in pharmaceutical and cosmetic applications.

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INTEGRATED PHYTOCHEMICAL AND MOLECULAR DOCKING STUDY OF VERNONIA CAMPANEA FOR ANTIMICROBIAL AND ANTIMALARIAL DRUG DISCOVERY

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The increasing resistance of Plasmodium falciparum and pathogenic bacteria to existing therapeutics necessitates the identification of novel bioactive compounds using integrated experimental and computational approaches. This study investigated Vernonia campanea, an ethnomedicinal plant widely used in Uganda, as a potential source of antimicrobial and antiplasmodial agents. Stem bark extracts were prepared using dichloromethane:methanol (1:1) and fractionated using chromatographic techniques. Structural elucidation of isolated compounds was achieved using one- and two-dimensional NMR spectroscopy. Four compounds were identified, including dibutyl phthalate, octadec-1-ene, stigmasterol, and a substituted naphthalene derivative reported for the first time from Vernonia species. Biological evaluation showed that stigmasterol exhibited the highest antibacterial activity against Staphylococcus aureus (zone of inhibition: 29 mm; MIC: 12.5 µg/mL; MBC: 25.0 µg/mL), comparable to ciprofloxacin. Molecular docking studies targeting Plasmodium falciparum lactate dehydrogenase revealed strong binding affinities for stigmasterol (–7.4 to –8.1 kcal/mol), with key interactions involving LEU152, VAL151 and TYR141. These findings highlight the potential of V. campanea as a source of bioactive compounds and a basis for further collaborative research in drug discovery.

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DESIGNING GRAPE POMACE FOR VALORIZATION: WHY STABILIZATION MATTERS

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Grape pomace represents a major winery by-product with increasing potential for cosmetic, and agricultural applications. Efficient valorization requires stabilization, to prevent degradation and enable storage however, these procedures may alter matrix structure and compound accessibility[1]. This study compared three stabilization strategies—convective drying, freezing, and microwave-assisted processing—applied to grape pomace obtained from ten *Vitis vinifera* L. cultivars grown under identical agro-technical conditions. Convective drying was adopted as the reference method based on pharmacopoeial guideline[2]. Phytochemical composition of grape pomace was analyzed using HPLC, LC–MS, GC–MS methods, while mixed-effects statistical models, performed using the MathWorks MATLAB environment, enabled simultaneous assessment of stabilization effects and compounds-cultivars associations.

Stabilization significantly influenced pomace physiochemical properties. Freezing strongly reduced lightness (L*), primarily through matrix-level optical changes rather than pigment degradation. Compound-specific responses were noted particularly among phenolic acids with hydroxybenzoic acids decreasing under freezing, and enrichment observed for several hydroxycinnamic acids relative to convective drying. Procyanidin and anthocyanin composition was cultivar-dependent and largely irrespective of stabilization strategy. Microwave-assisted processing enhanced the detectability of fatty acids (linoleic acid ethyl ester, linoleic acid trimethylsilyl ester, palmitic acid trimethylsilyl ester, and stearic acid ethyl ester) and sterols (campesterol, stigmasterol, and β-sitosterol), indicating increased matrix accessibility. Inter-cultivar variability in hydroxycinnamic and hydroxybenzoic subclasses was also observed, indicating a strong genetic influence on phenolic profiles. Hydroxycinnamic acids were generally better preserved or enhanced across processing methods.Within this group *p*-coumaric acid dominated across all cultivars. The caffeic and 5-hydroxyferulic acids occurred at lower proportions. Ferulic acid showed cultivar-specific accumulation, particularly in two cultivars: Johanniter and Pinot Noir. Hydroxybenzoic acid was the predominant constituents across all cultivars, with the highest absolute abundance in Pinot Noir, Cabernet Cortis, and Johanniter. Protocatechuic acid was the second most abundant compound identified in all samples.

The results demonstrate that stabilization is not a neutral preparatory step but a defining technological intervention that alters compound accessibility and visual properties[3]. The valorization potential of grape pomace therefore depends on the selection of an appropriate sample stabilization method and the intended application, rather than on the application of a single universal processing approach.

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P-22

PHYTOCHEMICAL INVESTIGATION AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF *BERBERIS JULIANAE* FRUITS

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Berberis julianae Schneid. is an evergreen shrub native to Central China and widely cultivated as an ornamental plant in temperate regions. Despite the growing interest in the genus *Berberis* as a source of biologically active compounds with neuroprotective properties, the fruits of *B. julianae* remain poorly investigated [1, 2]. In particular, their phytochemical composition, fractionation studies, and acetylcholinesterase inhibitory activity have not yet been thoroughly explored.

Therefore, the aim of this study was to investigate the phytochemical composition of *B. julianae* fruits and to evaluate their potential as a source of acetylcholinesterase (AChE) inhibitors.

The crude extract of *B. julianae* fruits was first analyzed by LC–MS and subsequently fractionated using centrifugal partition chromatography (CPC); the obtained fractions were evaluated for acetylcholinesterase (AChE) inhibitory activity using a fluorometric assay, and the most active fractions were further chemically characterized.

CPC fractionation of the crude extract yielded 12 fractions. Among them, fraction 7 exhibited complete acetylcholinesterase inhibition (100%), while several others showed moderate activity. LC–MS analysis of the most active fractions tentatively indicated the presence of isoquinoline alkaloids that could impact the total activity of the extract.

This study provides the first phytochemical investigation of *B. julianae* fruits, highlights their potential as a promising source of natural acetylcholinesterase inhibitors and provides evidence on the presence of isoquinoline alkaloids in this plant organ.

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P-23

CENTRIFUGAL PARTITION CHROMATOGRAPHY FOR THE ISOLATION OF TWO ACETYLENIC COMPOUNDS FROM *CONYZA BONARIENSIS*

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Acetylenic compounds, widely found in living organisms, hold significant biological and chemical importance due to the presence of acetylene unsaturation and conjugation with functional groups [1]. *Conyza bonariensis*, a species belonging to the Asteraceae family, produces notable amounts of acetylenic compounds, which have been reported to exhibit various biological activities [2]. Therefore, identifying efficient methods for isolating these compounds is of particular importance. This study focuses on isolating acetylenic compounds from *Conyza bonariensis* using Centrifugal Partition Chromatography (CPC), a liquid-liquid chromatographic technique based on the partitioning of analytes between two immiscible liquid phases, one acting as the stationary phase and the other as the mobile phase [3]. Hexane extracts of the aerial parts and roots were obtained through accelerated solvent extraction (ASE) from the powdered plant material. Subsequent GC/MS analysis identified 19 compounds in the root extract and 13 in the aerial parts extract, with dominant acetylenic compounds detected exclusively in the root fraction. As a result, CPC fractionation was performed on the root extract (90 mg) using the Arizona U solvent system in a volume ratio of 4:1:4:1 (hexane: ethyl acetate: methanol: water; v/v/v/v). Using this approach, the two major acetylenic compounds present in the root extract, 4(Z)-lachnophyllum lactone and (2Z)-lachnophyllum methyl ester, were successfully isolated and subsequently characterized by NMR analysis. These results demonstrate the efficiency of CPC for the isolation of target acetylenic compounds, even when working with limited amounts of crude extract.

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P-24

CHROMATOGRAPHIC PROFILING AND ISOLATION OF SELECTED COMPOUNDS FROM THE EXTRACT OF ARCTIUM LAPPA LEAVES USING UHPLC-DAD-IT-MS/MS, COLUMN CHROMATOGRAPHY, AND PREPARATIVE HPLC

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Arctium lappa L. (Asteraceae), commonly known as burdock, is a medicinal plant widely used in traditional medicine in Europe and Asia. Different parts of the plant contain various bioactive secondary metabolites, including lignans, sesquiterpene lactones, phenolic acids, and flavonoids, associated with antioxidant, anti-inflammatory, and antimicrobial activities. Despite numerous studies on the roots and seeds, the chemical composition of A. lappa leaves remains less explored.

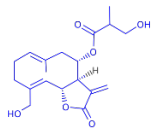
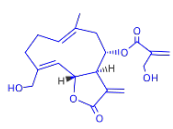
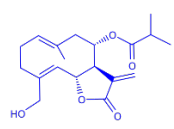
The aim of this study was to characterize the chemical composition of an extract obtained from Arctium lappa leaves and its solvent-partitioned fractions.

Dried and powdered leaves were extracted with acetone:methanol:water (3:1:1). The crude extract was subjected to liquid–liquid fractionation to obtain fractions of different polarity: dichloromethane (DCM), diethyl ether, ethyl acetate, n-butanol, and an aqueous residue. Chemical profiling was performed using UHPLC-DAD-IT-MS/MS. The DCM fraction was further purified by silica gel column chromatography using an ethyl acetate–methanol gradient, followed by preparative HPLC.

UHPLC-DAD-IT-MS/MS analysis enabled the tentative identification of 20 compounds belonging to different classes of secondary metabolites, including phenolic acids, flavonoids, and sesquiterpene lactones. Based on the chromatographic profiles, the DCM fraction enriched in less polar constituents was selected for further purification. Chromatographic separation resulted in the isolation of three sesquiterpene lactones: arctiopicrin, onopordopicrin, and 8-O-isobutyroylsalonitenolide.

The results demonstrate that extract of Arctium lappa leaves contain diverse secondary metabolites, and that the DCM fraction is particularly rich in sesquiterpene lactones. These findings expand current knowledge on the chemical composition of A. lappa leaves and indicate their potential as a source of bioactive compounds. The isolated compounds will be further characterized and evaluated for biological activity.

***Table 1**: Identified secondary metabolites from Arctium lappa leaves extract*

arctiopicrin	onopordopicrin	8-*O*-isobutyroylsalonitenolide
		

P-25

ISOLATION AND STRUCTURAL CHARACTERIZATION OF PHENOLIC ACIDS AND FLAVONOIDS FROM LAVENDER FLOWERS USING SEQUENTIAL SOLVENT FRACTIONATION AND PREPARATIVE HPLC

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A comprehensive phytochemical study was conducted on Lavandula angustifolia flowers to isolate and characterize phenolic acids and flavonoids. The dried plant material was extracted with 70% ethanol, and the resulting extract was sequentially partitioned with solvents of increasing polarity: dichloromethane (DCM), ethyl acetate (AcOEt) and n-butanol (n-BuOH). The DCM fraction was not analyzed further. The n-BuOH fraction underwent direct isolation using preparative C18 HPLC. The AcOEt and H_2O_{res} fractions were initially fractionated on silica gel. Subfractions of AcOEt were further purified on preparative C_{18} HPLC, while water residue (H_2O_{res}) subfractions were sequentially purified using preparative C_{18} and biphenyl HPLC columns.

This workflow led to the isolation of 18 compounds, primarily phenolic acids and flavonoids. The main structural scaffolds identified included apigenin, luteolin, tricetin, as well as cis- and trans-isomers of o-coumaric and 4-methoxy-o-coumaric acids. Structural elucidation of all isolates was confirmed using UHPLC-DAD-IT-MS/MS and NMR spectroscopy.

The combination of solvent partitioning, silica gel fractionation, and sequential preparative HPLC enabled efficient separation of closely related flavonoid and phenolic acid constituents, demonstrating a robust approach for comprehensive chemical profiling of lavender flowers.

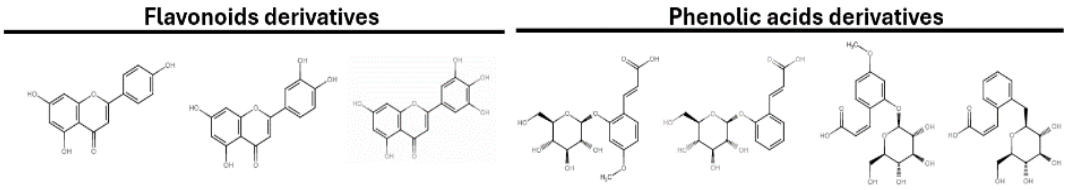

***Figure 1**: Main skeletons of isolated compounds*

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P-26

WHEN APOLLO LANDS ON A LEAF: METABOLOMIC RESPONSES OF HOST PLANTS TO FEEDING BY PARNASSIUS APOLLO LARVAE

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In this study, we analyzed the metabolic responses of two sedum species—*Hylotelephium maximum* (L.) Holub and *Sedum album* L.—to feeding by the oligophagous caterpillars of the Apollo butterfly (*Parnassius apollo* L.).

The Apollo butterfly is an endangered species associated primarily with montane and alpine habitats across Europe. Once widespread, its numbers have undergone severe declines, with many local populations disappearing. As a result, multiple restitution and reintroduction projects have been initiated, where successful breeding requires a deep understanding of the insect's interactions with its host plants. Apollo larvae feed exclusively on a limited number of Crassulaceae genera, including *Hylotelephium* and *Sedum*. To date, nothing has been known about potential plant defense mechanisms that might influence the growth, survival, or overall fitness of Apollo caterpillars. Plants typically respond to herbivory through chemical defenses that deter, impair, or kill the herbivore and insects may evolve tolerance or resistance to these compounds. To investigate the conditions favorable for larval development, we compared the metabolic profiles of intact and herbivoredamaged plants using Liquid Chromatography–HighResolution Mass Spectrometry (LCHRMS) combined with chemometric analyses.

Significant but contrasting metabolic responses were observed between the two host species. In *H. maximum*, herbivory induced a strong increase in tricarboxylic acid (TCA) cycle intermediates, such as citric and *cis*aconitic acids, potentially indicating mobilization of defenserelated metabolic pathways. Several flavonoids, including saponarin, kaempferol, afzelin, and catechin gallate also increased. Conversely, a marked decrease in azelaic acid was detected, despite this compound being a key signaling molecule involved in priming systemic acquired resistance in plants. In contrast, *S. album* showed a slight increase in azelaic acid, accompanied by a rapid decrease in several flavonoids after the first day of feeding. These results provide the first evidence that plant species serving as Apollo caterpillar hosts exhibit distinct metabolic responses to herbivory. Such differences may, in turn, influence larval performance and overall reproductive success. Our findings highlight that hostplant chemistry should be considered in conservation and breeding programs aimed at restoring *P. apollo* populations.

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P-27

HPLC PROFILING AND FRACTIONATION OF SYMPHYTUM OFFICINALE ROOT EXTRACT WITH EVALUATION OF ANTI-INFLAMMATORY ACTIVITY IN SKIN CELL MODELS

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Symphytum officinale L. (comfrey) root is a medicinal plant traditionally used in topical preparations for the treatment of inflammation, minor injuries, and musculoskeletal pain. Its therapeutic effects are attributed to a complex phytochemical composition, including phenolic compounds, lignans, and pyrrolizidine alkaloids. The present study aimed to characterize the chemical profile of comfrey root extract and its fractions and to evaluate their anti-inflammatory activity in skin models [1-3].

A 70% ethanolic extract of *S. officinale* root was subjected to chromatographic profiling using UHPLC-DAD-MS^n and fractionation using preparative HPLC. The obtained fractions were further evaluated for their biological activity in human skin cell models, including keratinocytes and normal human dermal fibroblasts. The anti-inflammatory potential was assessed by measuring the production pf pro-inflammatory cytokines, including IL-6 and IL-8, using ELISA assay.

The chromatographic analysis revealed a complex chemical profile of the extract, allowing the separation of multiple fractions differing in their phytochemical composition. Biological evaluation demonstrated that selected fractions modulated inflammatory responses in skin cells, indicating the presence of compounds with potential anti-inflammatory activity. These findings support the traditional use of comfrey root in topical formulations and highlight the value of combining chromatographic fractionation with cell-based assays to identify bioactive constituents of medicinal plant extracts.

The authors declare no conflict of interest. The presented research was financially supported by the NCN research grant Preludium Bis 2 No. 2020/39/O/NZ7/01109.

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P-28

NATURAL PRODUCTS FROM *LACTUCA BIENNIS* (MOENCH) FERNALD (ASTERACEAE, CICHORIEAE)

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The roots and aerial parts of *Lactuca biennis* (Moench) Fernald (*tall blue lettuce, blue wood lettuce*) that belongs to the North American members of the *L. canadensis* clade, were collected in the Garden of Medicinal Plants of the Maj Institute of Pharmacology, Polish Academy of Sciences in Kraków. The original seeds of the plant were supplied by the Montreal Botanical Garden.

The aim of the present study was to isolate and identify specialized natural products as potentially bioactive compounds from *L. biennis*, a previously chemically unexplored plant.

The ethanol extracts from the plant material were subjected to column chromatography on silica gel (Merck, Art. 7754) and eluted successively with n-hexane-EtOAc (up to 100% EtOAc) and then EtOAc-MeOH (up to 10% MeOH) using mobile phase gradients. The separated fractions were monitored by TLC (Merck, Art. 1.05553) and analytical RP-HPLC. Further separations were performed by prep. TLC and semiprep. RP-HPLC (Delta-Pak C-18 column, particle size 15µm, 25x100mm, dual wavelength UV/VIS detector, H_2O-MeOH mixtures, flow rate of 3 ml min^{-1}). The procedures led to the isolation of ten sesquiterpenoids – (11*β*,13-dihydrolactucin (**1**), lactucin (**2**), 8-deoxylactucin (**3**), jacquinelin (**4**), crepidiaside A (**5**), crepidiaside B (**6**), cichorioside B (**7**), 9-*α*-hydroxyzaluzanin C, macroclinoside A, 3*β*,14-dihydroxy-11*β*,13-dihydrocostunolide-3-*O*-*β*-glucopyranoside), one sterol – stigmasterol, three lignans – (+)syringaresinol, lactucaside, dihydrodehydrodiconiferyl alcohol-9-*O*-*β*-glucopyranoside and three apocarotenoids – (6*S*,9*S*) roseoside, icariside B_1, 3*β*-hydroxy-*β*-ionone 3-*O*-*β*-glucopyranoside.

All compounds were characterized by direct comparison of their spectral data with those of the reference compounds previously isolated in our laboratory or with literature data. All NMR spectra were recorded on a Bruker AVANCE III 400.

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P-29

ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF *GAULTHERIA PROCUMBENS* L. FRACTIONATED LEAF EXTRACTS AND WINTERGREEN ESSENTIAL OIL IN IMMUNE CELLS AND HUMAN PLASMA *IN VITRO*

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Gaultheria procumbens L. (Ericaceae) is an evergreen plant native to North America and a source of traditional anti-inflammatory and antipyretic herbal remedies [1]. Its bioactive constituents include a methyl salicylate-rich essential oil (EO), glycosylated salicylates, and other polyphenols, such as flavonoids, proanthocyanidins, and monocaffeoylquinic acids [1]. However, differences in biological effectiveness among various preparations of *G. procumbens*, which are usually used to treat inflammation (e.g., leaf extracts or EO), remain unclear. Therefore, this study aimed to compare the antioxidant and anti-inflammatory potential of polyphenol-rich extracts and EO obtained from the same batch of plant material in several *in vitro* models of immune cells.

Leaf extracts were prepared by fractionated solvent partitioning (diethyl ether, ethyl acetate, *n*-butanol) following methanol-water extraction of the leaves, while EO was obtained by hydrodistillation. Phytochemical profiles of the extracts and EO were analysed using UHPLC-PDA-ESI-MS/MS and GC-FID-MS, respectively, and the total phenolic content was determined spectrophotometrically and by HPLC-PDA. Biological activity was tested *in vitro* in human plasma exposed to peroxynitrite-induced oxidative stress, in concanavalin A-stimulated PBMCs, in THP1-Blue™ NF-κB monocytic cells, and in human polarised macrophages.

Five major phenolic groups were identified in the extracts, while methyl salicylate (99.8% v/w) was confirmed as the dominant volatile compound. Fractionation markedly enriched the extracts in polyphenols (up to 646.7 mg/g dw), mainly proanthocyanidins, salicylates, and quercetin derivatives. All preparations were biocompatible (1–50 µg/mL). The leaf extracts, particularly the ethyl acetate fraction (EAF), reduced protein oxidation and lipid peroxidation, and improved plasma antioxidant status more effectively than EO. Moreover, only EAF at 50 µg/mL decreased IL-6; all leaf extracts increased IL-10 in stimulated PBMCs, whereas EO was ineffective. In contrast, some leaf extracts and EO at 5-100 µg/mL reduced NF-κB activation and promoted M0→M1 macrophage polarisation across the wide concentration range (5–100 µg/mL).

These findings indicate that leaf extracts primarily drive antioxidant effects, while both plant extracts and EO contribute to the anti-inflammatory activity of *G. procumbens*; however, their therapeutic targets and advantages need *in vivo* verification.

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P-30

LEAVES OF SELECTED *BETULA* L. SPECIES AS A SOURCE OF POLYPHENOLIC COMPOUNDS: PHYTOCHEMICAL ANALYSIS BY HPTLC, UHPLC-PDA-ESI-MS^n AND HPLC-PDA

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The genus *Betula* L. (Betulaceae) comprises numerous tree species widely distributed throughout the temperate regions of the Northern Hemisphere and is recognized as a source of biologically active secondary metabolites. Leaves of *B. pendula* Roth and *B. pubescens* Ehrh. (*Betulae folium*) are listed as medicinal plant materials in the Polish Pharmacopoeia XIII and the ESCOP monograph, and have long been used in phytotherapy owing to their diuretic, anti-inflammatory, and antioxidant properties [1,2]. These activities are attributed primarily to the presence of polyphenolic constituents, including flavonoids, as well as triterpenes characteristic of the genus *Betula*. However, many other *Betula* species remain insufficiently investigated with regard to their phytochemical composition and potential use as alternative leaf raw materials [3]. Therefore, the present study aimed to identify potential alternative leaf raw materials within the genus *Betula* by comparing the qualitative and quantitative polyphenolic profiles of leaf extracts obtained from *B. alleghaniensis*, *B. lenta,* and *B. grossa* with those of the pharmacopoeial species *B. pendula*.

Methanol–water (7:3, v/v) extracts were prepared from dried leaves using accelerated solvent extraction (ASE). Polyphenolic profiles and levels were evaluated by HPTLC, UHPLC-PDA-ESI-MS^n and HPLC-PDA. The resulting chromatographic fingerprints revealed clear interspecific differences in the qualitative composition of flavonoids. More than 20 compounds were identified; the leaf extracts of *B. alleghaniensis, B. lenta,* and *B. grossa* contained predominantly di- and triglycosides of kaempferol, whereas the pharmacopoeial species *B. pendula* was characterized by the predominance of quercetin and myricetin monoglycosides. LC-PDA analysis demonstrated that the total polyphenol content was within the range of 51.4–70.3 mg/g DW of extract, calculated as the sum of flavonoids, catechins and caffeoylquinic acid derivatives. The highest polyphenol content was found in *B. lenta* leaf extract (70.3 mg/g), followed by *B. pendula* (55.5 mg/g), *B. alleghaniensis* (53.3 mg/g) and *B. grossa* (51.4 mg/g). Flavonoids constituted the major fraction of the polyphenolic constituents, accounting for up to 84% of the total polyphenol content, with the highest level observed in *B. lenta* leaf extract.

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NATURE-INSPIRED DERIVATIVES FROM *JATROPHA CURCAS* EXHIBIT INHIBITION OF ASEXUAL BLOOD STAGES IN MALARIA PARASITES DURING LATE-RING AND EARLY-TROPHOZOITE PHASES.

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Abstract

Purpose: The present work aims to leverage traditional ethnobotanical knowledge combined with modern medicinal chemistry and computational biology to identify novel antimalarial pharmacophores that selectively target the early intraerythrocytic developmental stages of malaria parasites.

Methods: The extracts of *J. curcas* were obtained through maceration with ethanol and water. These extracts were evaluated *in vitro* for their antiplasmodial activity against both chloroquine-sensitive (3D7) and multidrug-resistant (Dd2) strains of *P. falciparum*, using SyBr Green fluorescence-based assay. Furthermore, bioactive compounds were successfully isolated using various chromatographic techniques. The pharmacodynamics of these active compounds were thoroughly investigated, with a focus on their killing kinetics and stage-specific actions. molecular interactions were analysed through docking strategies focusing on potential malaria drug targets.

Results: The hydroethanolic extract and *n*-hexane fraction of leaves showed significant activity with IC_{50} values of 2.75 to 31.38 µg/mL against two strains. Among 30 isolated compounds, Curcusone B and B exhibited EC_{50} values ranging from 0.58 to 1.24 μM against the two strains. Both compounds function as slow-acting inhibitors with remarkable efficacy against late-ring and early-trophozoite stages. Notably, Curcusone C demonstrated an EC_{50} of 9.75 μM against early rings, But Curcusone B demonstrated significantly efficacy on early trophozoites (EC_{50} < 1 μM) and strong inhibition on late rings (0.37 μM). Microscopic analysis confirmed parasiticidal effects, evidenced by morphological changes in parasites and the absence of viable parasites. Docking studies indicated effective interactions with *Pf*Rad51 and *Pf*Hsp90.

Conclusion: These findings highlight the potential of Curcusone B and C as lead candidates for antimalarial drug development, particularly critical in addressing resistant strains in Africa. Their unique stage-specific activity profiles provide valuable insights for developing targeted therapies, aligning with global health priorities.

Keywords: Malaria, *Jatropha curcas,* Antiplasmodial Efficacy, Docking Studies, pharmaco-dynamics.

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QUALITATIVE AND QUANTITATIVE INVESTIGATION OF *NARCISSUS* ALKALOIDS IN UNINVESTIGATED *NARCISSUS* SPECIES BY LC-QTOF-MS AND TLC WITH BIOAUTOGRAPHY

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Amaryllidaceae alkaloids are natural compounds present in Amaryllidaceae family. They exhibit numerous pharmacological activities, like: cholinesterase inhibitors applied in Alzheimer's disease, antidepressive, antitumor, antiviral, antibacterial and antimalarial [1]. Chemically, there are complex natural structures which can be divided into several subtypes like: galanthamine-like, lycorine, lycoramine, haemanthamine, crinine, homolycorine, pseudolycorine, narciclassine, etc. [2].

In the presented paper 2 uninvestigated *Narcissus* sp. (*N.* cv. 'Flower Parade' and *N.* cv. 'February Gold') have been investigated using comprehensive and fully validated analytical procedures including: Pressurized Liquid Extraction, Solid-Phase Extraction, HILIC-ESI-QTOF-MS followed by TLC-bioautography using automated spraying chamber. The alkaloids have been analyzed both qualitatively and quantitatively. Numerous potent alkaloids have been determined including: buphanidrine, pluviine, tortuosine, haemanthamine, galanthine, lycorine, *nor*-galanthamine, *epi*-galanthamine and lycoramine in *N.* cv. 'Flower Parade', whereas in *N.* cv. 'February Gold' dihydrobicolorine, tortuosine, 1-*O*-acetylo-*nor*-pluviine, galanthamine-*N*-oxide, *nor*-galanthamine and galanthamine were detected. Quantitative assays were performed in EIC mode using calibration curve assessment. Moreover, TLC-bioautography was used for detection of prominent acetylcholinesterase inhibitors in investigated bulbs of two *Narcissus* cultivars.

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PHYTOCHEMICAL CHARACTERIZATION AND ANTIPROLIFERATIVE ACTIVITY OF METABOLITES FROM *SENNA DIDYMOBOTRYA*

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Cancer is a non-communicable disease characterized by uncontrolled cell proliferation and is responsible for over 10 million deaths annually worldwide [1]. Natural products remain a major source of anticancer agents and drug leads [2]. *Senna didymobotrya* (Caesalpiniaceae) is widely used in traditional medicine for the management of cancer and related conditions; however, its bioactive metabolites are not fully characterized [3]. This study aimed to identify phytochemical constituents and evaluate their antiproliferative activity against lung cancer cells. Leaves were extracted by maceration, and antiproliferative activity was evaluated using the MTT assay against NCI-H441 lung cancer cells [4]. Chemical profiling was carried out using UHPLC–MS/MS coupled with Global Natural Products Social (GNPS) molecular networking for compound annotation [5]. Compounds were isolated using column chromatography and structurally characterized using nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). A total of 26 metabolites were identified, including anthraquinones such as chrysophanol and physcion, and the flavonoid rutin. Among these, rutin exhibited the highest antiproliferative activity against NCI-H441 cells. These findings highlight the potential of *S. didymobotrya* as a source of bioactive compounds for anticancer drug discovery and provide scientific support for its traditional medicinal use.

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MODERN SYNTHETIC AND NATURAL ANTIADHESIVE AND ANTIBIOFILM COMPONENTS AFFECT PROTEOME AND METABOLOME OF MULTIDRUG-RESISTANT *CANDIDA GLABRATA*

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β-Aescin is a natural surfactant, a triterpene saponin, isolated from the seeds of the horse chestnut tree. Previous studies on the antibiofilm activity of newly synthesized alkylamidobetaines (AABs) have shown the C9-substituted AAB to be the most effective against *C. glabrata*. However, little is known about the effect of combining these compounds on *C. glabrata* cells. The aim of this study is to shed light on the molecular mechanism of action of these compounds, which, due to their complementary antibiofilm activities, demonstrate promising efficacy.

Two strains of C*. glabrata* were used in the study: *C. glabrata* ATCC 90030 as the reference strain, and a drug-resistant clinical strains, *C. glabrata* 2586. Yeast blastospores were treated with AAB-C9, β-aescin, and a combination of these two compounds. Cells were harvested after 6 hours and extracted proteins were analysed in LCMS-based non-targeted shotgun proteomics and metabolite analysis.

More than 50 proteins were found which were statistically significantly differentially accumulated when treated with AAB C9 + β-aescin against controls. Especially interesting findings are represented by increased content of detox and redox stress protection protein S-(hydroxymethyl)glutathione dehydrogenase (CAGL0L01111g) or hyphally-regulated cell wall protein N-terminal domain-containing protein (CAGL0C01133g). In *Candida albicans* this is a GPI-anchored cell wall proteins required for hyphal growth and virulence. Cells treated with AAB C9 + β-aescin had significantly lower content of the protein, which was previously observed to be involved in innate immune cell evasion through conferring resistance to neutrophil killing. Our measurements of redox protection metabolites suggest involvement of the pathway in the mitigation of the oxidative stress elicited due to the treatment, which might contribute to weaker biofilms.

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NMR-BASED METABOLOMICS FOR THE DIFFERENTIATION AND AUTHENTICATION OF *HERNIARIA* L. SPECIES.

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Herniaria L. species (Caryophyllaceae) are medicinal plants traditionally used for urinary tract disorders. According to the EMA herbal monograph (EMA/HMPC/554043/2018), *Herniariae* herba may originate from *H. glabra* L., *H. incana* Lam, or *H. hirsuta* L. Despite being used interchangeably, these taxa exhibit chemical variability. While previous LC-MS studies focused on *H. glabra*, *H. polygama* J. Gay, and *H. incana*, this study extends the metabolomic characterization of the genus by incorporating *H. hirsuta* commercial samples.

The aim was to evaluate the chemical profiles of *Herniaria* L. species (commercial, wild-grown and cultivated) using ^{1}H NMR-based metabolomics. Herbal materials were extracted with boiling water (1:25), followed by ethanol-induced precipitation and subsequent partitioning between water and ethyl acetate. The aqueous fractions, enriched in polar metabolites, were analyzed via ^{1}H NMR. Spectral data were processed and subjected to multivariate statistical analysis.

PLS-DA modelling revealed significant clustering and differentiation between the studied species. The results indicated major differences in coumarin, saponin and flavonoid profiles [1, 2]. Specifically, multivariate analysis identified a set of chemical shifts as Variable Importance in Projection (VIP) markers, which allowed for the clear separation of *H. hirsuta* from other taxa. These markers correspond to specific patterns in the sugar moieties and aglycone signals of flavonoids and triterpenoid saponins. The study demonstrates that NMR-based fingerprinting, combined with PLS-DA, serves as a rapid and non-targeted tool for the authentication of *Herniaria* herba materials. This approach provides a reliable strategy for quality control of herbal products containing different *Herniaria* L. species.

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NEUROCYTOTOXIC ACTIVITY OF RAPANONE, A PLANT-DERIVED BENZOQUINONE

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According to WHO statistics CNS cancers, although not the most prevalent, are among the most lethal with a mortality rate of approximately 77% [1]. Furthermore, this type of cancer ranks 12th in terms of oncological deaths worldwide. Consequently, the search for agents capable of reducing the incidence and/or mortality associated with CNS cancers is an important direction of global research.

Since some of the chemotherapeutics currently in use, e.g. paclitaxel, vincristine, and vinblastine, are of plant origin, it is reasonable to explore plant resources in the search for new bioactives. The Primulaceae family comprises species rich in bioactive compounds such as triterpene saponins, polyphenols, and benzoquinones. The latter represents a group of highly bioactive phytochemicals. One of the prominent structures belonging to this group is rapanone, occurring in *Ardisia crenata* Sims, a homologue of embelin, a well-known cytotoxic agent with XIAP-inhibitory activity. Both compounds exhibit anti-inflammatory and antioxidant properties [2].

Previously, our team demonstrated the partially selective cytotoxic *in vitro* potential of rapanone towards melanoma, prostate, and thyroid cancer as well as colorectal carcinoma cell lines [3,4]. As a continuation of these study, the aim of present work was to assess its cytotoxic activity against CNS cancer cel lines, including human U-87, U-251, SH-SY5Y, and murine Neuro2A, as well as non-cancerous, C8-D1A, cell line. Cell viability was evaluated by MTT or MTS method after 24 h and 48 h of incubation.

Rapanone exhibited high cytotoxic activity against U-87, U-251 cell lines with IC_{50} values below 10 µg/mL. In the case of SH-SY5Y, the IC_{50} after 24 h was below 12 µg/mL. Interestingly, rapanone was non-toxic towards C8-D1A and Neuro2A cells.

Overall, rapanone again exhibited high cytotoxic potential – this time against CNS cancer cell lines. Furthermore, the effect was much weaker in the non-cancerous astrocytes used as a control. To the best of our knowledge, this is the first study evaluating neurocytotoxic potential of rapanone.

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CYTOTOXIC ACTIVITY OF *POPULUS TRICHOCARPA* BUDS

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Polyphenolic compounds are important natural antioxidants with potential significance for maintaining long-term human health and preventing disease development. Buds of plants from the *Populus* genus are considered a promising source of bioactive compounds due to their rich phytochemical composition. In this study, contracted methanolic extract and a series of its fractions were prepared from *Populus trichocarpa* buds. Fractions were separated from the previous extract by using C-18E SPE column in methanol-water elution system in the concentration range of 20–90% methanol. The obtained extracts were subjected to phytochemical analysis, including the determination of total phenolic and flavonoid contents, and their effects on L929 mouse fibroblast viability were evaluated using the MTT assay according to ISO 10993-5. The results showed that both the methanol concentration used for extraction and the tested extract concentration influenced the cellular response. Cytotoxicity was compared based on estimated IC_{50} values calculated from the MTT results. The strongest cytotoxic effect was observed for extracts prepared with higher methanol concentrations, especially the 80% methanolic extract. Extracts obtained with 40–60% methanol also showed marked cytotoxic activity, whereas extracts prepared with 20%, 30% and 90% methanol did not reach IC_{50} within the tested concentration range. These findings indicate that extraction solvent composition significantly affects the phytochemical and biological properties of *Populus trichocarpa* bud extracts.

Table 1. Estimated IC_{50} values of extract and its fractions toward L929 cells in the MTT assay

Extract	Estimated IC_{50} [µg/mL]	Cytotoxic effect
20%-30%, 90% MeOH	**>1000**	**low / not reached**
40% MeOH	~316	marked
50% MeOH	~360	marked
60% MeOH	~283	marked
70% MeOH	**~879**	**moderate**
80% MeOH	~126	strongest
Previous methanolic extract	~531	moderate

Table Legend: IC_{50} values were estimated from MTT assay results and represent the concentration of extract required to reduce L929 cell viability by 50% compared with untreated control cells. For extracts that did not decrease viability below 50% at the highest tested concentration, IC_{50} was expressed as >1000 µg/mL

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ANTIOXIDANT POTENTIAL OF GEORGIAN *POPULUS* SPECIES BUDS EXTRACTS

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Polyphenolic antioxidant components are important for maintaining long-term human health and preventing disease development, especially tumors. One of the promising sources on natural antioxidants are buds extracts of *Populus* genus due to rich amount of polyphenols. In present research, antioxidant properties and phytochemical profile of aqueous ethanol extract (70:30, ethanol:water, V/V)[1] from Georgian *Populus* species buds was revealed. Investigated species included *P. alba, P. ×canascens, P. euphratica, P. ×hybrida*, *P. nigra* and *P. tremula*. Antioxidant properties were analyzed in various ways (DPPH[1], FRAP[1] and ABTS tests[1]) and referred to the phytochemical profile (including the estimations of total phenolic content (TOP)[1] and flavonoid content (FC)[1]. Results were presented in Table 1. The samples from buds of *Populus nigra*, *P. euphratica*, and *P. tremula* were most abundant in phenolics and exhibited the strongest antioxidant properties, while those of *P. ×canascens* and *P. ×hybrida* were noticeably weaker. In our best knowledge, this is the first investigation of antioxidant properties of selected *Populus* species buds from Georgia.

Table 1: Antioxidant activity and phytochemical profile of hydroalcoholic *Populus* buds extracts.

Species	Antioxidant activity			Composition	
	DPPH	FRAP	ABTS	TOP	FC
P. nigra	291.4 ±1.4	4.8 ±0.3	2.0 ±0.1	224.3 ±2.0	76.2 ±2.2
P. euphratica	254.3 ±3.6	4.9 ±0.1	2.0 ±0.0	185.1 ±2.7	89.9 ±1.9
P. tremula	185.0 ±6.4	4.5 ±0.0	1.8 ±0.1	180.7 ±1.6	33.3 ±1.1
P. alba (syn. *P. nivea*)	242.4 ±9.1	4.6 ±0.1	1.6 ±0.0	171.9 ±1.7	<2
P. ×canascens	67.3 ±0.3	2.6 ±0.1	0.4 ±0.0	73.3 ±1.0	<2
P. ×hybrida	117.4 ±1.6	2.4 ±0.1	0.7 ±0.0	73.8 ±1.7	8.1 ±0.2

Table legend: DPPH - radical scavering activity, activity expressed as mg of gallic acid equivalents per gram of dry extract (mgGAE/g); **FRAP** - ferric reduction antioxidant power, activity expressed as mmol of Fe^{2+} equivalents per gram of dry extract (mmolFe^{2+}/g); **ABTS -** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical cation decolorization assay, activity expressed as mg of trolox equivalents per gram of dry extract (mgTrolox/g); **TOP** - total phenolic content, expressed as mg of gallic acid equivalents per gram of dry extract (mgGAE/g); **FC** - flavonoid content, expressed as mg of quercetin equivalents per gram of dry extract (mgQUE/g).

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Literature (used methodology):

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PRUNUS SPINOSA BRANCH EXTRACT – POLYPHENOLIC PROFILING AND IN VITRO EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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Various parts of the rosaceous shrub *Prunus spinosa* L. (blackthorn) are traditionally used in phytomedicine to treat inflammatory conditions in the skin, intestines, as well as urinary and circulatory systems. Despite their historical usage and prior studies, the scientific validation of their molecular mechanisms and efficacy, especially for branches, remains limited [1]. This study aims to analyse the phytochemical profile of the branch extract and preliminarily assess its antioxidant and anti-inflammatory potential.

Detailed qualitative and quantitative survey of ethanol-water (3:1) branch extract used LC-MS/MS and HPLC-PDA techniques. A total of 67 polyphenols were detected, mostly A-type procyanidin oligomers (16 dimers and 7 trimers), along with B-type dimers (4) and trimers (4), numerous phenolic acids and flavonol glycosides. The total polyphenol and proanthocyanidin levels reached ca. 352.4 and 320 mg GAE/g dw, respectively.

In vitro, the extract potently scavenged ROS (strongest effects against $O_2^{\bullet-}$, then HClO and $HO^{\bullet}$, weaker towards H_2O_2; lowest against $ONOO^-$, compared to Trolox), showing notable selectivity. The extract's antioxidant capacity was further confirmed in vitro in $ONOO^-$-stressed human plasma: protein nitration and lipid peroxidation were reduced and endogenous antioxidant defence was restored by the extract at 1-50 μg/ml, with effects similar to those of Trolox. The extract also potently inhibited hyaluronidase in vitro, a pro-inflammatory enzyme involved in extracellular matrix degradation, showing greater potency than heparin. The extract's cytocompatibility was confirmed in L929 mouse fibroblasts at a concentration range of 0.008-500 μg/mL. Further studies are underway to explore anti-inflammatory effects of the extract, including its potential to modulate NF-κB activation and inhibit COX-2.

These findings position blackthorn branches as a rich source of proanthocyanidins with promising bioactivity, highlighting the need for in-depth research into their biological potential, molecular mechanisms, and targets, both in vitro and in vivo.

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PHYTOCHEMICAL DIVERSITY AND BIOACTIVITY IN *SAMBUCUS*

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Sambucus nigra is a well-recognized European species cultivated for the medicinal and food applications [1]. Its flowers and leaves are rich sources of rutin and chlorogenic acids, as well as valued pharmaceutical raw material used as diuretics, anti-inflammatory agents, and for the support of immune system [2]. However, other species in the genus remain under-investigated. The present study provides a comparative assessment of flower and leaf extracts from seven *Sambucus* species (*S. nigra*, *S. canadensis*, *S. caerulea*, *S. racemosa*, *S. williamsii*, *S. sibirica* and *S. kamtschatica*) to identify alternative valuable raw materials.

Phytochemical UHPLC-PDA-ESI-MS/MS profiling identified 38 constituents, primarily chlorogenic acid derivatives and flavonol glycosides. Quantitative analysis revealed substantial interspecific variation, with total phenolic content accounting for 106-651 mg/g of dry extracts. Multivariate statistical tools were applied to integrate and interpret the chemical data. Principal Component Analysis and Hierarchical Cluster Analysis distinguished species-specific phenolic patterns, separating rutin-dominant species from those enriched in flavonol glucosides.

Moreover, the extracts displayed strong antioxidant activity against various reactive oxygen species (ROS) of physiological significance, with several samples showing $OH^{\cdot}$ and HClO-scavenging capacity comparable to ascorbic acid. Calculation of average *z*-scores enabled standardized ranking of the extracts scavenging capacity. In a model of ex-vivo-stimulated human neutrophils, all extracts significantly inhibited ROS release and elastase-2 secretion in a dose-dependent manner, with the Area Under the Curve estimates accepted as a global measure of the inhibitory effects.

Overall, the results demonstrated considerable phytochemical diversity within the genus *Sambucus* and identified *S. williamsii* and *S. sibirica* as particularly promising for wider pharmaceutical applications. They represent viable complements or alternatives to *S. nigra* for the production of phenolic-rich extracts with antioxidant and anti-inflammatory potential.

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P-41

PHENOLIC PROFILES IN *SORBUS* INFLORESCENCES: IMPACT OF VARIABILITY ON BIOLOGICAL EFFECTS *IN VITRO*

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Genus *Sorbus* is a broad taxon comprising over 250 species widespread over the northern hemisphere. Many of these species yield ethnomedicinal herbal materials known for their anti-inflammatory, antidiabetic, diuretic, and vasoprotective effects [1]. Previous research has shown that inflorescences of species representing the subgenus *Sorbus sensu stricto* are particularly rich in polyphenolic compounds, demonstrating significant potential for producing bioactive extracts [2]. While the inflorescence of *S. aucuparia* has been the most extensively studied to date, several other species have displayed the potential for similar or even greater performance [2].

Here, we present the qualitative and quantitative chromatographic profiles (HPLC-PDA, LC-MS/MS) of 40 primary phenolic compounds in *Sorbus* inflorescences derived from five selected species (*S. aucuparia*, SA; *S. commixta*, SC; *S. decora*, SD; *S. gracilis*, SG; *S. koehneana*, SK), collected in two harvest seasons to assess their value as sources of polyphenolic extracts. In addition, antioxidant and antidiabetic activity tests and statistical tools (Principal Component Analysis, PCA) are employed to identify the primary variability and activity markers.

The results reveal that the antioxidant potential of *Sorbus* inflorescences is mainly influenced by the harvest season, with all species exhibiting similar effectiveness. In contrast, their antidiabetic effects (inhibition of α-glucosidase and advanced glycation end-products formation in vitro) were more species-specific. These findings correlate with the accumulation of specific phenolic compounds, reflecting the plant responses to environmental stressors or variations in flowering development associated with climatic conditions (seasonal variability). This also highlights possible taxonomic markers for particular species (species variability). The first category of markers includes acetylated flavonols and monomeric, dimeric, and trimeric flavan-3-ols, while the second category encompasses various mono- and diglycosides of quercetin and kaempferol.

The selected markers may be useful in standardization procedures to ensure the quality and desired biological effects of products derived from *Sorbus* inflorescences.

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POLYPHENOLIC-PROTEIN-POLYSACCHARIDE COMPLEX AS ACTIVITY DETERMINANT OF ROWANBERRIES

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Sorbus aucuparia fruits (rowanberries) exhibit a variety of biological activities, including antioxidant, anti-inflammatory, and antidiabetic effects, often attributed to polyphenols. However, this fraction alone does not fully explain the overall activity of rowanberries, indicating a potential role of other constituents [1]. Among these, polysaccharides could be important, as polyphenolic-protein-polysaccharide (PPP) complexes found in many medicinal/edible plants are often reported to exhibit functional properties similar to, or even superior to those of their other components or raw extracts [2].

This study aims to preliminarily evaluate the extent to which PPP complexes contribute to the biological activity of rowanberries by comparing the effects of isolated PPP with those of raw extract (SA) and a fraction that lacks PPP (SAF). First, the composition of PPP, SA, and SAF was analyzed using chromatographic methods (HPLC, LC-MS/MS), revealing various levels of phenolics, proteins, and saccharides in each fraction. In bioactivity testing, PPP significantly inhibited the activity of NF-κB in the LPS-stimulated THP1-Blue™ NF-κB cells (at 1-25 μg/mL). PPP also reduced the secretion of pro-inflammatory cytokines (TNF-α, IL-2) in the concanavalin A-stimulated PBMCs, with effects similar to those of dexamethasone or diclofenac. Additionally, PPP inhibited the activity of α-amylase and α-glucosidase in vitro, with effects similar to those of acarbose and 20 times stronger, respectively. Compared with PPP, SA activity was moderate, while that of SAF was significantly lower, confirming that PPP is a primary activity vector in rowanberries and an auspicious candidate for further studies on its potential to treat metabolic diseases and as a functional food ingredient.

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P-43

PRODUCTION OF ALANTOLACTONE IN MULTIPLE SHOOTS OF *BUPHTHALMUM SPECIOSISSIMUM*, A RARE ALPINE ENDEMIC

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Buphthalmum speciosissimum L., a rare plant endemic to the southern Alps, belongs to the family Asteraceae (tribe Inuleae, subtribe Inulinae). Its medicinal potential has only recently begun to be explored (1-3). HPLC-DAD analysis, followed by ^{1}H NMR spectroscopic analysis, of the chloroform extract from in vitro grown *B. speciosissimum* multiple shoots revealed three major constituents. Based on their UV and ^{1}H NMR spectra, these compounds were identified as sesquiterpene lactones: inuviscolide, 4,5-epoxy-4,5-*cis*-inunolide, and alantolactone. The lactones are specialized terpenoid metabolites which are widely distributed in the tribe Inuleae and are known for their anti-inflammatory, cytotoxic, antibacterial, and immunomodulatory properties [4].

The multiple shoots of *B. speciosissimum* were cultivated using three different multiplication media and two kinds of culture vessels. Optimum for both shoot multiplication and biomass accumulation was Murashige and Skoog (MS) basal medium supplemented with 5.37 μM naphthaleneacetic acid (NAA) and 13.32 μM benzyladenine (BA). The type of culture vessel used did not affect the biomass accumulation. The analyzed multiple shoots contained from 0.3% to 1.6% of alantolactone, calculating on a dry weight basis. Composition of the nutrient medium was the major factor that influenced accumulation of alantolactone in the plant tissue.

The obtained results provide new insights into the phytochemical profile of *B. speciosissimum* and indicate that this rare Alpine species may represent a promising source of biologically active secondary metabolites.

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QUANTITATIVE DETERMINATION OF POLYPHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY OF EDIBLE PRUNUS FRUITS.

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The *Prunus* fruits are widely consumed as edible fruits in everyday diets. They are also well established in ethnopharmacology for the management of various health disorders. Fruits of the *Prunus* genus are rich in polyphenolic compounds, revealing antioxidant potential. This study aimed to optimize the extraction of polyphenols using a fractional factorial design and to quantitatively analyze the fruits of three *Prunus* species native to Polish flora, namely *Prunus padus, P. serotina, and P. spinosa* by HPLC-UV-VIS. The optimal extraction conditions were determined to be 30-minute reflux extraction with 75% ethanol and a plant material-to-solvent ratio of 1:100 (w/v). HPLC analysis revealed significant differences in polyphenolic composition between Prunus fruits. In *P.* serotina and *P. padus* cyanidin-3-O-rutinoside was the predominant anthocyanin, while *P. spinosa* exhibited more diverse anthocyanin profile, including also the presence of cyanidin-3-O-glucoside and peonidin-3-O-glucoside, not noted in the two former species. The highest anthocyanin content was observed in *Prunus serotina* (1972.3 mg/100 g d.m.), whereas *Prunus spinosa* exhibited the greatest content of other polyphenols (230.08 mg/100 g d.m.). The obtained extracts were further evaluated for antioxidant activity using the DPPH and FRAP assays. The highest activity was observed in *P. padus*, with 13.30 ± 0.50 mmol TE/100 g dw (DPPH) and 33.67 ± 0.33 mmol Fe^{2+}/100 g dw (FRAP). *P. serotina* and P. spinosa showed moderate but comparable activity, half as weak as P. padus (7.40 ± 0.15 mmol TE/100 g dw and 16.12 ± 0.21 mmol Fe^{2+}/100 g dw and 7.31 ± 0.27 mmol TE/100 g dw and 16.11 ± 0.38 mmol Fe^{2+}/100 g dw, respectively).

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CULTIVAR- AND GROWTH-STAGE-DEPENDENT VARIABILITY OF TRITERPENE SAPONINS IN ROOTS AND LEAVES OF *BETA VULGARIS* L.

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Beta vulgaris L. is an important crop and a promising source of specialized metabolites with nutritional and biological relevance. Among them, triterpene saponins are of particular interest due to their structural diversity and broad range of reported bioactivities, including antimicrobial, anti-inflammatory, anticancer, and antidiabetic effects [1-2]. However, information on the variability of saponin accumulation in beet tissues during plant development remains limited, especially with regard to the combined effects of genotype, plant organ, and harvest stage.

The aim of this study was to evaluate the qualitative and quantitative variability of triterpene saponins in roots and leaves of selected *B. vulgaris* cultivars collected at different developmental stages. Three beet cultivars, Round Dark Red, Cylindra, and Snow Ball, together with Swiss chard (Rhubarb Chard), were analyzed across seven harvest stages during the growing season. Saponin profiling was performed using liquid chromatography–electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS).

A total of 32 triterpene saponins were identified. Their total content varied markedly depending on genotype, organ type, and harvest date, ranging from 386 to 10,414 mg/kg fresh weight in leaves and from 1,170 to 23,298 mg/kg fresh weight in roots. Two-way ANOVA confirmed significant effects of cultivar, harvest stage, and their interaction on total saponin levels. Multivariate analyses further separated samples according to organ, genotype, and seasonal stage, indicating distinct and dynamic saponin profiles during plant development.

Roots showed a broader quantitative range and generally higher total saponin levels than leaves, with a clear maximum in the middle of the growing season. In contrast, leaf saponin accumulation typically peaked from mid to late season, depending on cultivar. This variability was largely driven by a limited number of dominant compounds displaying coordinated seasonal trends.

Overall, the results demonstrate that saponin accumulation in *B. vulgaris* is strongly influenced by plant organ, genotype, and developmental stage. These findings provide new insight into beet metabolic diversity and may support the targeted selection of cultivar, tissue, and harvest time for the efficient use of beet biomass as a source of bioactive saponins for food, nutraceutical, and pharmaceutical applications.

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P-46

A NEW HUMULANE-TYPE SESQUITERPENOID FROM AERIAL PARTS OF COMMON FLEABANE

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Pulicaria dysenterica (L.) Bernh. (Common fleabane; Asteraceae, subtribe: Inuleae-Inulinae) has been used to treat dysentery and as an insect repellent. However, the plant constituents responsible for the potential antibacterial activity have not yet been identified. In a search of previously unknown metabolites of the plant, the chloroform extract from its aerial parts has been analyzed. The extract submitted to the fractionation procedure using different chromatographic techniques yielded, in addition to the formerly known metabolites of the plant, a new humulene derivative (compound **1**). Based on the results of spectroscopic analysis the compound was identified as 13-acetyl-2,6(12),10-humulatrien-7-ol-1-one, a structural analog of 2,6(12),10-humulatrien-7**β**-ol-1-one and demersone B, isolated earlier from *Buddleja davidii* Franch. [1] and *Ceratophyllum demersum* Linn. [2], respectively. To the best of our knowledge, **1** is the first compound of this structural type isolated from *P. dysenterica*. Moreover, several known caryophyllene derivatives and polymethoxylated flavonols of moderate cytotoxic activity [3] were found in the analyzed plant material.

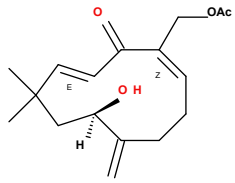

Figure 1: Chemical structure of compound **1**

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P-47

THE HERBAL FORMULATION ADAPT-232 PROMOTES LONGEVITY THROUGH MITOPHAGY-MEDIATED MITOCHONDRIAL PROTECTION

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Mitochondrial dysfunction is recognized as an antagonistic hallmark of ageing and a major driver of age-related disorders, including cardiometabolic diseases [1]. Maintaining mitochondrial function is therefore essential for promoting longevity and healthspan. The chemically defined herbal formulation ADAPT-232-S comprises standardized extracts of the adaptogenic plants *Eleutherococcus senticosus* (Rupr. et Maxim.) Maxim., *Rhodiola rosea* L., and *Schisandra chinensis* (Turcz.) Baill., with well-documented stress-protective effects; however, its role in longevity-related pathways remains insufficiently explored [2]. This study aimed to evaluate the effects of ADAPT-232 on mitochondrial dysfunction and impaired metabolism in *Caenorhabditis elegans*, with a focus on evolutionary conserved molecular pathways involved in mitochondrial quality control, autophagy, and lipid metabolism.

Mitochondrial dysfunction was induced by glucose (2%) supplementation in the nematode culture medium. Mitochondrial integrity and lipid accumulation were analysed using fluorescence imaging and lipid staining. Key regulators of mitophagy, autophagy, lipid metabolism, and stress response were analysed at both transcriptional and protein levels by RT-qPCR and GFP-reporter strains [3]. Under glucose stress, ADAPT-232 significantly restored mitochondrial morphology and reduced lipid accumulation. The formulation upregulated *pink-1*, *dct-1*, and *lgg-2*, indicating activation of mitophagy and autophagy. Increased expression of *nhr-49*, *atgl-1*, and *lipl-4* suggested improved lipid metabolism, while activation of *daf-16* and *skn-1* reflected enhanced stress-response signalling [3]. Collectively, the standardized formula ADAPT-232 alleviates mitochondrial dysfunction by activating mitophagy-related pathways and improving metabolic homeostasis, supporting its potential as a plant-based intervention for promoting longevity.

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P-48

NATURAL BIOACTIVE POTENTIAL OF EXTRACTS FROM BALKAN ENDEMIC SPECIES ACHILLEA GRANDIFOLIA

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Achillea plant species are known for their healing properties since ancient times, and extensive literature highlights their pharmacological potential due to the presence of bioactive compounds. The present study investigates the phytochemical content of both essential oil and hydroalcoholic extracts obtained from the inflorescences and leaves of *A. grandifolia* Friv (Mt. Menoikio). The chemical composition of the essential oils revealed that the major constituents in inflorescences include sabinene, α-terpinene, 1,8-cineole, *cis*- and *trans*-thujone, camphor, ascaridole, and jasmone, while in leaves the predominant compounds were 1,8-cineole, *cis*- and *trans*-thujone, camphor, borneol, and α-terpineol. The phytochemical profile of the hydroalcoholic extracts was evaluated using NMR spectroscopy and LC-MS analysis. The results were consistent with the spectrophotometric determination of total phenolic and total flavonoid content, indicating a rich presence of bioactive constituents. Furthermore, the antioxidant activity of the extracts was assessed using DPPH and ABTS radical scavenging assays, along with ferrous ion chelating ability and reducing power tests, demonstrating moderate antioxidant activity compared to the reference compound BHT. In addition, antibacterial activity against key foodborne pathogens showed moderate effects. Finally, the examination of the non-volatile content of leaves and flowers afforded the isolation of sixteen secondary metabolites. On the basis of NMR spectra, the identified compounds include ten sesquiterpene lactones; five flavonoids and an iridoid.

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NATURAL ALKALOID INHIBITORS OF A-GLUCOSIDASE FROM *HAPLOPHYLLUM TUBERCULATUM*: IN VITRO AND IN SILICO APPROACHES WITH STRUCTURE–ACTIVITY RELATIONSHIPS

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In a search for new drug-like molecules, we investigated *Haplophyllum tuberculatum* as a potential source of α-glucosidase inhibitors. Five new natural products (**1-5**), and one previously synthesized compound (**6**), isolated here as a natural product for the first time, were isolated from an ethyl acetate extract. Additionally, fifteen known compounds (**7-21**) were also characterized. The structures of all compounds were elucidated by 1D- and 2D-NMR techniques and HR-ESI-MS. All phytochemicals were evaluated for inhibitory activity against α-glucosidase enzyme. Among them, six compounds exhibited notable inhbition with IC_{50} values of 2.28 ± 0.64 to 8.94 ± 0.37 μM, seven compounds had appreciable activity with IC_{50} values ranging from 12.14 ± 0.35 to 24.60 ± 0.57 μM, whilst six compounds exhibited weak activities with IC_{50} values of 36.52 ± 1.68 and 260.53 ± 3.18 μM, respectively, compared to the standard drug acarbose (IC_{50} = 875.75 ± 1.24 μM). The α-glucosidase inhibitory activities of all compounds are reported here for the first time. A kinetic study of the most potent compounds was also performed and exhibited concentration dependent type of inhibition. Furthermore, a structure-based prediction of the compounds' binding mode suggested that these inhibitors fitted exceptionally well within the active site of the target enzyme, α-glucosidase, forming multiple hydrogen and hydrophobic interactions with its active site residues. In conclusion, compounds with potent α-glucosidase inhibitory activity are abundant in nature and can be explored and further developed for treating diabetes mellitus.

Keywords: *Haplophyllum tuberculatum* (Forssk.) A. Juss., Rutaceae, alkaloids, α-glucosidase inhibition, NMR spectroscopy, molecular docking.

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CHEMICAL COMPOSITION AND BIOACTIVITIES OF THE DRY METHANOL EXTRACTS OF *PASTINACA HIRSUTA* ROOTS, LEAVES AND FRUITS

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Pastinaca hirsuta Pančić (Apiaceae) is endemic to the central Balkan Peninsula (east Serbia, North Macedonia and south and west Bulgaria). In this work, the composition and antioxidant, antimicrobial and cytotoxic activities of the dry methanol extracts of the roots, leaves and fruits of *P. hirsuta* from Serbia were investigated. Dried plant material was extracted with methanol (after pretreatment with dichloromethane). Dry methanol extracts were subjected to qualitative LC-QTOF-MS/MS and quantitative HPLC analyses. Total polyphenols were determined using Folin-Ciocalteu reagent. Total antioxidant activity was evaluated in FRAP test, antiradical activity in DPPH assay, antimicrobial activity against 14 strains (6 Gram-positive, 4 Gram-negative bacteria and 3 *Candida* species) in microdilution method and cytotoxicity on 3 cancer cell lines in MTT test. The total of 40 compounds, including phenolic acids, flavonoids, coumarins and other benzofurane derivatives, were identified. Chlorogenic acid was the dominant in the leaf extract (58.1 mg/g), whereas both the leaf and fruit extracts were rich in quercetin and methylquercetin glycosides (103.7 and 163.9 mg/g). A hexoside of 5,8-dihydroxy psoralene was the most abundant in the root extract (6.3 mg/g). The leaf extract had the highest amounts of total phenolics (70.1 mg quercetin equivalents/g) and displayed the strongest total antioxidant (FRAP value 1.0 mmol Fe^{2+}/g) and anti-DPPH (64.6 mg quercetin equivalents/g) activities. The best antimicrobial activity was revealed for the fruit extract against *Micrococcus luteus*, followed by the root and leaf extracts against the same bacterium (MIC=0.625-1.25 mg/mL; MBC=5 mg/mL). No cytotoxicity (CC_{50}>500 µg/mL) was determined for all extracts against non-cancerous monkey kidney (VERO) cells and cancerous cells derived from stomach (AGS), hypopharynx (FaDu) and colon (RKO). This study establishes *P. hirsuta* as a source of bioactive polyphenols and coumarins and justifies further research on this plant.

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HEALTH-PROMOTING BIOACTIVES FROM INSECT LARVAE: CPC-ENABLED ISOLATION AND ENRICHMENT

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In this study, we focused on insect larvae as sources of potentially bioactive natural products that may benefit human and animal health. We evaluated the small molecule composition of a mass-reared edible insect species by using UHPLC-HRMS/MS to select a variety of potentially relevant target molecules. After harvesting, the larvae were processed, lyophilized, and milled. The resulting larval powder was extracted with methanol, and the crude extract was concentrated under reduced pressure. Further fractionation is planned by using centrifugal partition chromatography (CPC). The aim of the CPC-based workflow is to overcome challenges presented by the larval fatty-tissue matrix and to obtain fractions and pure compounds suitable for subsequent biological and pharmacological testing.

CPC represents a non-conventional chromatographic technique in which both the stationary and the mobile phases are liquids. Separation is governed by the differential partitioning of analytes between two immiscible liquid phases, while a centrifugal force field retains the stationary phase inside the rotor. This configuration enables rapid separation methods, robust hydrodynamic operation, and straightforward scale-up without the limitations associated with solid stationary phases. Consequently, CPC is considered a green, reusable, scalable, and economically attractive purification technology. To identify suitable conditions for CPC, we determined partition coefficients of our selected target compounds in the extract in a series of biphasic solvent systems and selected the most promising system for preliminary CPC experiments.

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NEW POLYPHENOLIC METABOLITES FROM *DRACOCEPHALUM FORRESTII* SHOOT CULTURES

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Dracocephalum forrestii W.W. Smith (Lamiaceae) occurs naturally in the northwestern mountainous region of Yunnan (China) and is traditionally used in Tibetan medicine as an antipyretic, astringent, and diuretic agent [1]. Its pharmacological properties are associated with phenolic acids and flavonoids. Because obtaining sufficient plant material from natural populations is difficult, *in vitro* cultures represent not only an alternative source of phytochemicals but may also produce metabolites not previously reported in the species.

The aim of this study was to isolate and identify new discovered polyphenolic compounds from *D. forrestii* shoot culture. Shoots cultivated on Murashige and Skoog agar medium with 0.2 mg/L meta-topolin and 0.2 mg/L indole-3-acetic acid [2] were used as the experimental material. Powdered shoots (40 g) were extracted by sonication with chloroform and subsequently with 80% methanol. The dry extract was suspended in water (0.25 L) and successively partitioned with diethyl ether, ethyl acetate, and n-butanol (8 × 100 mL each). The n-butanol fraction was subjected to silica gel column chromatography using methanol-water (1:1) to obtain a fraction enriched in phenolic acids and flavonoid glycosides. Further purification was performed by preparative HPLC on a Kinetex Biphenyl column (5 µm, 100 Å, 150 × 2.1 mm), and the mobile phase consisted of 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B). Four compounds were isolated: DF-1 (17 mg), DF-2 (4.1 mg), DF-3 (4.7 mg), and DF-4 (17 mg). Their purity was verified by UHPLC-DAD-MS (Kinetex Biphenyl, 1.7 µm, 150 × 2.1 mm), and structures were elucidated using ^{1}H NMR spectroscopy (500 MHz).

The isolated metabolites were identified as two acacetin derivatives (DF-1, 962 g/mol; DF-2, 958 g/mol) and two salvianolic acids (DF-3 and DF-4, 718 g/mol). These compounds have not previously been reported in *Dracocephalum* genus. The results demonstrate that *D. forrestii* shoot cultures can represent a valuable *in vitro* system for the discovery and production of bioactive polyphenols.

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LED-INDUCED MODULATION OF ROSMARINIC ACID BIOSYNTHESIS IN *DRACOCEPHALUM RUYSCHIANA* SHOOT CULTURES

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Dracocephalum ruyschiana L. (Lamiaceae) is a perennial plant used in the traditional medicine of Eastern Europe and Central Asia. Its aerial parts are employed to treat rheumatoid arthritis, laryngitis, respiratory infections, and gastric ulcers [1]. The biological activity of this species is mainly associated with polyphenolic constituents and essential oils [2]. The aim of the present study was to investigate the influence of LED light of different wavelengths, acting as an abiotic stress factor, on polyphenolic metabolism and rosmarinic acid (RA) biosynthesis in *D. ruyschiana* shoot cultures.

Shoots were cultivated for three weeks in liquid Murashige and Skoog medium [3] with 0.5 mg/L 6-benzylaminopurine, 0.2 mg/L indole-3-acetic acid, and 0.5 mM putrescine in a RITA bioreactor. Cultures were exposed to four LED light regimes: blue (430 nm), red (670 nm), red : blue (70:30), and white light (430–670 nm).

For phytochemical analysis, lyophilized plant material was extracted with methanol-water (8:2, *v/v*). Qualitative profiling of polyphenolic constituents was performed by UPLC-DAD/ESI-MS, which allowed the identification of phenolic acids: rosmarinic acid, methyl rosmarinate, chlorogenic acid, dicaffeoylquinic acid, and flavonoid derivatives of acacetin and apigenin. Quantitative determination of RA, the predominant metabolite in the analyzed cultures, was carried out using an Agilent Technologies 1290 Infinity HPLC system with a diode-array detector (DAD) and an Eclipse XDB-C18 column (150 × 4.6 mm, 5 µm). RA content was calculated from a calibration curve based on peak area versus concentration of the reference standard and expressed as mg/g dry weight (DW).

LED wavelength significantly modified RA accumulation in *D. ruyschiana* culture. The highest RA content (12.3 mg/g DW) was detected in shoots grown under blue LEDs. This corresponded to a production level of 60 mg/L, over six-fold higher than in the control, confirming that blue light effectively stimulates RA biosynthesis in *D. ruyschiana* shoot cultures.

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CORRELATION BETWEEN CHEMICAL PROFILE OF GEORGIAN PROPOLIS EXTRACTS AND THEIR ACTIVITY AGAINST *HELICOBACTER PYLORI*

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Helicobacter pylori (*H. pylori*) is considered the most common bacterial pathogen colonizing stomach mucosa of almost half the world's population and is associated with various gastrointestinal diseases (from digestive problems and ulcers to gastric cancer). A lack of new drugs and a growing number of *H. pylori* antibiotic-resistant strains is a serious therapeutic problem.As a mixture of natural compounds, propolis has antimicrobial activity based on high concentrations of bioactive polyphenols (mainly flavonoids and phenolic acid derivates). The chemical composition of tested Georgian propolis is characterized by the presence of flavonoids aglycones, and phenolic acid monoesters, e.g., pinobanksin-5-methyl ether, pinobanksin, chrysin, pinocembrin, galangin, pinobanksin-3-*O*-acetate, pinostrobin and pinobanksin-3-*O*-butanoate, or isobutanoate and methoxycinnamic acid cinnamyl ester. The anti-*H. pylori* activity of 70% ethanol water extracts of 10 Georgian propolis samples was evaluated in vitro by MIC (minimal inhibitory concentration) against the reference strain (*H. pylori* ATCC 43504) and 10 clinical strains with different antibiotic-resistance patterns. The strongest anti-*Helicobacter* activity (MIC and MBC = 31.3 µg/mL) was observed for propolis from Orgora, Ota, and Vardzia and two from Khaheti. Lower levels of activity (MIC = 62.5 µg/mL) were found in propolis obtained from Qvakhreli and Pasanauri, while the lowest effect was observed for Norio and Mestia (MIC = 125.0 µg/mL). However, despite differences in MIC, all evaluated samples exhibited bactericidal activity. We selected the most active propolis samples for assessment of urease inhibition property. Enzyme activity was inhibited by propolis extracts, with IC_{50} ranging from 4.01 to 1484.8 µg/mL. Principal component analysis (PCA) and hierarchical fuzzy clustering (dendrograms) coupled with matrix correlation analysis exhibited that the strongest anti-*Helicobacter* activity was connected with black poplar origin and high flavonoid content of propolis. Samples with lower activity contained higher presence of aspen markers and/or dominance of non-flavonoid polyphenols over flavonoids. In summary, Georgian propolis can be regarded as a source bioactive compounds that can be used as adjuvant in therapy of *H. pylori* infection.

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8-METHOXYPEUCEDANIN: EVALUATION OF ANXIOLYTIC EFFECTS AND MODULATION OF NEURONAL ACTIVITY RELATED GENES IN A ZEBRAFISH ANXIETY MODEL

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For thousands of years, medicinal plants and their constituents have been used, mostly empirically/ethnopharmacologically, to cure patients with central nervous system (CNS) disorders. Anxiolytics derived from natural products (NPs) often share similar mechanisms of action to synthetic ones (e.g., benzodiazepines, BDZs). Although typically as effective as synthetic anxiolytics, NPs are considered to be devoid of the serious side effects linked to the use of BDZs. 8-Methoxypeucedanin (8-MP) is a rare furanocoumarin present in the fruits of *Peucedanum luxurians* Tamamsch. (Apiaceae). The primary objective of the presented study was to assess the anxiolytic activity of 8-MP using a zebrafish (*Danio rerio*) model of anxiety. *Danio rerio* larvae at 5 days post-fertilization (dpf) were used, with reversed thigmotaxis considered as an index of the anxiolytic activity. In addition to the behavioral study, qPCR analyses were performed to assess the role of 8-MP in modulating the expression of *c-fos* and *bdnf*, two key genes involved in neural activity. As evidenced by the behavioral study, 8-MP (1.5–15 μM) exhibited a significant influence on anxiety, with a U-shape dose–response effect. Moreover, the expression of *c-fos* and *bdnf* genes was significantly downregulated, providing novel insights into the mechanisms of action of the tested furanocoumarin.

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A NOVEL LC–MS-BASED POST-COLUMN ENZYMATIC SCREENING METHOD FOR ACETYLCHOLINESTERASE INHIBITORS

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A novel screening method based on high-performance liquid chromatography coupled with mass spectrometry was developed for the evaluation of inhibitory activity of natural compounds against acetylcholinesterase. The system consists of a post-column enzymatic reactor, in which the analyte stream is mixed with the enzyme and substrate, enabling real-time monitoring of enzymatic reaction products using mass spectrometric detection.

The method allows indirect assessment of enzyme inhibition by measuring the decrease in product signal intensity. A calibration curve was constructed using galantamine as a reference inhibitor, and the results were expressed as galantamine equivalent activity, enabling semi-quantitative comparison of inhibitory potency of tested samples.

The analytical performance of the method was evaluated in terms of linearity, precision (intra- and inter-day), sensitivity, and robustness. The method demonstrated good repeatability with relative standard deviation values below 10% and satisfactory inter-day precision. The influence of solvent composition on the analytical response was investigated and did not significantly affect the reproducibility of the measurements.

The developed approach provides a rapid and reliable screening tool for the identification of acetylcholinesterase inhibitors in complex matrices, with potential application in natural product research and drug discovery.

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SAFETY AND NEUROACTIVITY OF *LYSIMACHIA PUNCTATA* EXTRACT IN THE *DANIO RERIO* MODEL

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Plants belonging to the Primulaceae family are characterized by a high content of bioactive components, such as benzoquinones, triterpene saponins, coumarins, and polyphenols [1-3]. The genus *Lysimachia* is one of the most important within the family due to its high abundance. It is considered cosmopolitan, widespread across the world, with five native species in Poland and several cultivated ones used as ornamentals. Representatives of the genus have been used for centuries in traditional medicine, and their biological activity is currently being verified in scientific studies [3].

The aim of this study was to evaluate the safety profile and neuroactivity of a characterized ethanolic fraction of an ethyl-acetate extract derived from the roots of *Lysimachia punctata*, using the *Danio rerio* model.

For this purpose, the characterization of the plant extract was conducted using an HPLC-MS/MS procedure. The safety profile was assessed using the fish embryo acute toxicity (FET) test, and neuroactivity was evaluated using the light-dark locomotor activity test.

The analysis revealed a high content of benzoquinones (embelin and its homologues), as well as phenolic compounds such as flavonoids (quercetin and kaempferol derivatives) and phenolic acids. Additionally, triterpenoid derivatives were identified. Concentrations ranging from 12.5-100 µg/mL were selected for behavioural assessment. The lowest tested doses induced a slight stimulation of larval activity in the dark phase and increased the difference between light and dark phases compared to the control group.

This study provides the first preliminary profiling of the chemical composition of the *L. punctata* extract and its *in vivo* biological activity in the *Danio rerio* model. The observed behavioural effects suggest potential neuroactive properties. Nevertheless, further studies are required to determine their exact nature (e.g. anxiogenic or related to increased psychoreactivity).

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UNTARGETED METABOLOMICS PREDICTS THE MEDICINAL PLANT GENOTYPE OF HIGHER PHARMACEUTICAL POTENTIAL

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The plant family Lycopodiaceae produce unique bioactive alkaloids called *Lycopodium* alkaloids, and medicinal plants containing *Lycopodium* alkaloids have been used for enhancement of memory and treatment of rheumatism [1]. The alkaloid huperzine A (hupA) is a one of the most potent natural acetylcholinesterase inhibitors with high pharmaceutical potential, which was first isolated from Chinese *Huperzia selago* [2]. Previous studies have shown large variations of hupA among Icelandic *Huperzia* specimens [3], and it is interesting to investigate the alkaloid profiles in relation to plant genotypes. The current study included 96 *H. selago* specimens collected all around Iceland and aimed to characterize the overall variation of their hupA contents and alkaloid profiles using high performance liquid chromatography-photodiode array detection (HPLC-PDA) and ultrahigh performance liquid chromatography-mass spectrometry (UPLC-MS) methods. Genotypes were recognized using chloroplast DNA barcoding, and polyploidy levels were determined by flow cytometry. Our results reveal genotype-specific patterns of alkaloids [4] as well as quantitative variations of hupA in Icelandic *H. selago*, which tends to be related to polyploidy levels - higher polyploidy level in proportional to higher hupA contents. The study emphasizes the need for genotype-level phytochemical knowledge and has broad implications to plant chemotaxonomy and natural product discovery and exploitation. It highlights the utility of integrating plant barcoding and metabolomics in selecting taxa of high pharmaceutical interest.

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IMPACT OF *GAILLARDIA MEGAPOTAMICA (SPRENG.) BAKER* ETHANOL EXTRACT ON AMINO ACIDS PROFILE IN *MYCOBACTERIUM TUBERCULOSIS* AND *MYCOBACTERIUM MARINUM*.

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of the leading causes of death from a single infectious agent worldwide. According to the Global Tuberculosis Report 2025 published by the World Health Organization, an estimated 10.7 million new TB cases were reported globally in 2025, accompanied by approximately 1.23 million deaths. Despite ongoing control efforts, the global burden of TB remains high[1].

A major challenge in TB management is the increasing prevalence of drug-resistant strains, particularly multidrug-resistant and extensively drug-resistant forms of TB. This has intensified the search for novel therapeutic strategies, including the identification of plant-derived compounds as adjuvants to antibiotic regimens. Natural products offer a diverse collection of bioactive molecules that may enhance antimicrobial efficacy, reduce resistance development, or modulate bacterial metabolism.

In this context, *Gaillardia megapotamica (Spreng.) Baker* has attracted attention due to the antimycobacterial activity demonstrated by the ethanol extract of this plant, with a minimum inhibitory concentration (MIC) against *M. tuberculosis H37Ra* of 64 µg/ml, confirming its potential as a source of biologically active compounds.

Comparative investigations were conducted using *M. tuberculosis H37Ra* and *M. marinum*, a closely related species and established model organism for studying tuberculosis pathogenesis. *Danio rerio* is widely employed for *in vivo* studies of *M. marinum* infection, providing a relevant system for translational research. Parallel analysis of these two species allows for the assessment of similarities and discrepancies in the response of these bacteria to active plant extracts.

An ethanol extract of *Gaillardia megapotamica (Spreng.) Baker* was evaluated for its effects on metabolic responses in *M. tuberculosis H37Ra* and *M. marinum*. Bacterial cultures were exposed to the plant extract under controlled conditions, followed by isolation of amino acid fractions from both strains. The resulting amino acid profiles were characterized and comparatively analyzed. Quantitative analysis of amino acid extracts was performed using liquid chromatography–mass spectrometry (LC–MS). The MIC of the plant extract was determined using a resazurin-based microdilution assay, employing a standard two-fold serial dilution method.

This study demonstrates that the tested plant extract, comprising a mixture of multiple active compounds, induces significant alterations in bacterial metabolites, with a particular emphasis on changes in amino acid profiles in both *M. tuberculosis* and *M. marinum*. Changes in the levels of specific amino acids may suggest an effect of the extract on specific metabolic pathways in both Mycobacterium species.

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ISOLATION OF 4-O-METHYLHONOKIOL FROM *MAGNOLIA GRANDIFLORA* L. SEEDS OIL

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4-O-Methylhonokiol (MHNK), a methylated derivative of honokiol naturally occurring in *Magnolia* species, has attracted considerable attention due to its broad spectrum of biological activities, including antidiabetic [1], neuroprotective [2], anti-inflammatory [3], and anticancer [4] effects. However, its relatively low abundance in plant matrices and the lack of efficient isolation methods limit its wider application in pharmacological research and standardization. The present study addresses this limitation by developing a rapid and efficient method for the isolation of MHNK from fatty oil obtained from seeds of *Magnolia grandiflora* L., using centrifugal partition chromatography (CPC).

The method comprises three stages. First, fatty oil is obtained from *M. grandiflora* seeds by cold pressing. In the second stage, the oil is subjected to *n*-hexane extraction via maceration (30 min, 10:1 *m*/*v*), followed by cooling (4 °C, 120 min), filtration, and solvent evaporation to yield a concentrated oil extract enriched in MHNK. In the final stage, the extract is purified using CPC with a biphasic solvent system composed of *n*-hexane, ethyl acetate, methanol, and water (10/2/10/2 to 10/5/10/5, *v*/*v*/*v*/*v*; optimal 10/3/10/3). The separation is conducted in ascending mode with the lower phase as the stationary phase, at 26 °C, with a flow rate of 10 mL/min and a rotation speed of 1700 rpm. Fractions are collected under UV detection at 290 nm.

The developed method enables the isolation of MHNK with a purity exceeding 99.5% within 30 minutes, achieving a process yield of 6.03% relative to the mass of the concentrated oil extract. Compared to previously reported CPC-based approaches, this method significantly improves both efficiency and yield, while eliminating the need for multi-step purification procedures.

In conclusion, the presented CPC-based protocol constitutes a novel, rapid, and scalable approach for obtaining high-purity MHNK from a previously underexplored natural source, namely *M. grandiflora* seed oil. This method provides a valuable tool for further biological studies, supports the development of MHNK-based therapeutics, and enables its use as a reference standard in phytochemical analyses.

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