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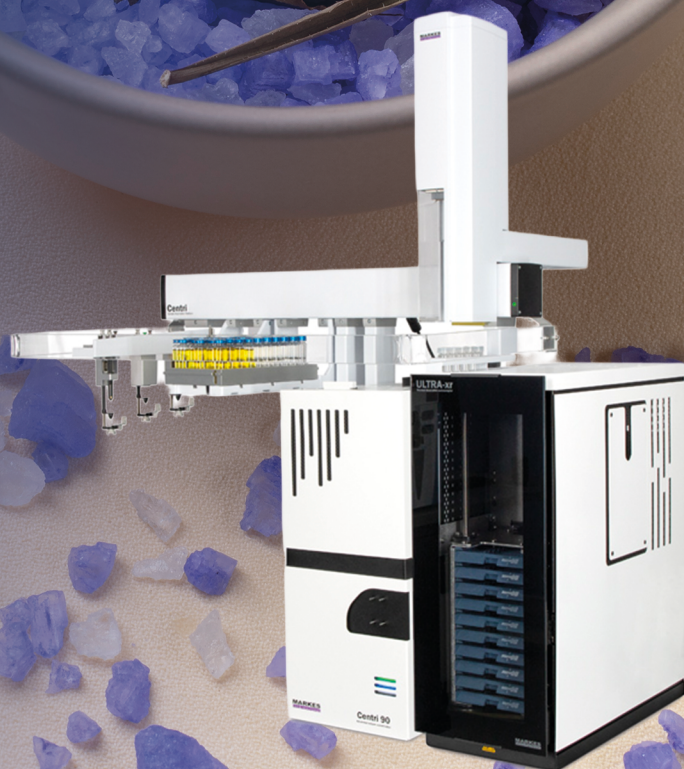


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ISEO MEDAL OF HONOR PRESENTATIONS



MP1. Essential Oils from Colombia's Biodiversity: A journey from Plants to Molecules and Sustainable Applications

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Keywords: aromatic chemodiversity, integrated plant utilisation, biodiversity-based value chains

More than thirty-five years ago, we began exploring essential oils in Colombia, a country of extraordinary biodiversity where the chemical potential of aromatic plants was still largely unknown. A seemingly simple question — what molecules do these plants contain? — gradually grew into a much broader scientific endeavour, linking botanical exploration, chemistry, biological activity, cultivation, innovation, and rural development. Field expeditions across contrasting Colombian ecosystems have yielded more than 1,200 plant accessions from around 30 botanical families, and over 1,000 essential oils have been distilled and characterised by GC–FID/MS and GC×GC–TOFMS. Their chemical diversity has been investigated in relation to antimicrobial, antioxidant, cytotoxic, photoprotective, and quorum-sensing-modulating properties, as well as repellent, larvicidal and biopesticidal effects. Floral emissions and plant–insect chemical interactions have provided another fascinating dimension to this research. This work has always been a collective endeavour, bringing together chemists, botanists, microbiologists, and engineers, and training generations of students. Laboratory findings were progressively translated into pilot cultivation and distillation projects, technological developments and patents, and into natural ingredients and products for phytotherapeutic, cosmetic, household-care, and veterinary applications. Equally important was taking knowledge beyond the laboratory. Working with farmers and rural communities, we introduced aromatic plants as new crops and shared practical knowledge on their cultivation, harvesting, distillation, essential-oil quality and potential uses, helping to build new production and value chains. More recently, our attention has extended to what remains after distillation. LC–MS/MS has revealed less-volatile and non-volatile secondary metabolites in post-distillation biomass, opening new possibilities for recovering valuable compounds, whilst hydrolates and residual plant material can find further uses, including biological crop protection and composting. What began with essential oils has thus evolved towards the integral utilisation of aromatic plants. After more than 35 years, the Colombian experience reminds us that molecules acquire their greatest significance when scientific knowledge reaches people, creates value and contributes to a more sustainable use of biodiversity.

MP2. Essential oils, related extracts and organic chemistry

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Keywords: essential oils, solvent extracts, analysis, organic chemistry

I am a chemist by training, and I became somewhat fortuitously involved in the chemistry of essential oils some sixty years ago. In particular and more deeply through the ISEO, which I have been actively attending since 1985, I have had the privilege of witnessing the remarkable evolution of scientific advances in this field, driven by the advent of increasingly powerful analytical techniques. However, over the years, I have also observed that analytical science in this field has gradually moved away from organic chemistry. I believe that, today more than ever, it is important to reconnect advanced analytical methods with a sound understanding of organic (bio)chemistry, in order to fully interpret the complexity of essential oils, their production and their possible applications. Consequently, I would like to share a concern regarding ISEO's scientific direction or coverage. It seems to me that the intimate knowledge of the composition and chemistry of essential oils is gradually giving way to an increasing number of studies of variable quality, including those making poorly substantiated claims about their potential preventive or therapeutic effects on human health. This concern was already raised at the 50th ISEO in Vienna by two of us who are here today. At that time, however, it did not seem to lead to any significant adjustment in the scientific orientation and frequency of the symposium. I believe this deserves renewed consideration, particularly given the reputation ISEO has historically built for scientific rigor and for advancing our fundamental understanding of essential oils, in relation with basic organic chemistry.

PLENARY LECTURES



PL1. Paradigm shift of structure analysis of aroma constituents in natural products applying crystalline sponge method

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The molecular structures of aroma constituents, such as terpenoids, fatty acid metabolites, and phenylpropanoids commonly found in essential oils and other natural products, are closely associated with their olfactory properties and thresholds. Consequently, structural investigation of novel trace aroma constituents including their absolute stereochemistry has become increasingly important in fragrance and flavor research. However, low-threshold odor-active compounds present in natural products typically occur at extremely low concentrations.

As a result, conventional nuclear magnetic resonance (NMR) spectroscopy and single-crystal crystallographic analysis, are often impractical for their structure investigation due to the requirement of large amounts of sample, multi-step purification, and substantial time consumption. NMR-based structure elucidation requires synthetic confirmation, while X-ray crystallographic analysis need the preparation of suitable single-crystals. In particular, acyclic terpenoids and fatty acid metabolites found in natural products often possess unrigid or flexible long-chain moieties and tend to exist as oils or amorphous solids, making single-crystal formation difficult.

In contrast, the crystalline sponge (CS) method is an innovative analytical technique that eliminates the need for single-crystal preparation of the target molecule. The CS method enables X-ray structure analysis by simply soaking trace amounts of an analyte, on the order of a few micrograms, into a porous host crystal. Moreover, it allows determination of absolute configuration, thereby dramatically streamlining structure determination by significantly reducing the required sample amount, number of analytical steps, solvent usage, and overall analysis time, contributing Green chemistry.

In our research, we applied the CS method to three types of applications: (1) odor active constituents that contribute to the aroma of essential oils important in flavor and fragrance research, (2) unknown aroma compounds present in citrus juices and peels, and (3) components obtained via noninvasive headspace sampling of natural products. The results were as follows: (1) structural information on principle odor-active constituents with complex molecule architectures was rapidly obtained from minimal sample quantities. (2) trace-level unknown components detected by gas chromatography–olfactometry analysis were isolated using various chromatographic techniques including preparative gas chromatography, enabling their structural investigation. Notably, the structure of an unidentified woody odor-active constituent in citrus was successfully determined using only a few micrograms of sample. (3) volatile constituents were directly collected from living blooming flowers and other precious samples with limited availability without damaging the natural products, and the trace components within these collected sample were successfully subjected to structural analysis.

These results demonstrate that the CS method is a significantly powerful, useful, practical, and highly efficient approach representing a paradigm shift in the structural analysis. It is anticipated the CS method will become widely adopted as a standard and a fundamental technology, for future analyses of essential oil research.

PL2. Unlocking Nature's Secrets: GC×GC-MS driving a new era of compositional and regulatory understanding of fragrance ingredients

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Keywords: GC×GC-MS, allergens, natural complex substances, fragrances

Natural ingredients are key differentiators in the fragrance industry, bringing the richness and complexity of nature to creations that inspire unique emotions. However, their chemical diversity and variability make Natural Complex Substances (NCS) particularly challenging to characterize. While the non-volatile fraction often remains only partially understood, most scientific knowledge relies on the volatile fraction, for which GC-MS is a cornerstone analytical technique. However, its limited peak capacity and the risk of co-elutions can restrict a deep and reliable understanding of the most complex natural matrices.

Comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC-MS) has progressively become a reference analytical tool thanks to its unsurpassed separation performance. Over the past decade [1], it has transformed the quantification of the 57 fragrance allergens into a robust and routine analytical workflow delivering high-confidence data. This technology enables highly selective and reliable quantification across a wide concentration range within a limited number of acquisitions while reducing the risk of overestimation due to coelutions. Initially driven by EU allergen labelling requirements [2], the same analytical method can also easily integrate additional regulated substances to address emerging regulatory challenges related to CMR substances.

Beyond targeted analysis, GC×GC-MS provides a breakthrough approach for compositional investigation, supporting raw material understanding for safety assessment and registration activities. A GC×GC-MS/FID configuration based on a Paradigm flow modulator enables simultaneous qualitative and semi-quantitative data acquisition within a single run, providing deeper insight into the composition of natural ingredients.

GC×GC-MS not only delivers robust and reliable scientific data but also reveals the extraordinary molecular diversity of essential oils, absolutes, resinoids, and other extracts that give natural ingredients their unique character in fragrance compositions.

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PL3. Biotechnological Approaches for Innovation in the Fragrance Industry

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Keywords: Biotechnology; Terpenes; Enzyme discovery; Microbial production; Fragrance ingredients

Terpenes represent one of the largest and most diverse families of natural products and constitute a major source of ingredients used throughout the fragrance industry. However, access to many valuable molecules remains constrained by the limitations of traditional sourcing, including agricultural variability, supply volatility, long cultivation cycles, and increasing sustainability challenges. Biotechnology offers complementary approaches that can help address these limitations while expanding access to new areas of chemical diversity.

This presentation will provide an overview of how modern biotechnological tools can support fragrance ingredient innovation, from the discovery of biosynthetic enzymes to the production and evaluation of novel terpene molecules. Advances in sequencing technologies, bioinformatics, synthetic biology, microbial engineering, and analytical sciences have dramatically accelerated the exploration of natural biodiversity and enabled the rapid translation of genetic information into tangible molecules. These integrated workflows allow researchers to identify promising biocatalysts, investigate previously inaccessible chemical space, and generate new opportunities for ingredient discovery.

Beyond enabling sustainable production routes for known molecules, biotechnology also provides a powerful platform for uncovering new structures with potential olfactive value. The presentation will highlight the key stages of this discovery process, from enzyme identification and experimental screening to product characterization and the evaluation of molecules as potential fragrance ingredients.

Finally, two well-known examples from the fragrance industry, Clearwood® and Dreamwood®, will illustrate how biological insights can be translated into commercially successful ingredients inspired by iconic essential oils. Together, these examples demonstrate how biotechnology can complement traditional sourcing approaches while expanding the toolbox available for future fragrance innovation.

PL4. From biological assets to legal assets- intellectual property as a strategic lever

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Innovating with Essential Oils: Keys to an Effective Intellectual Property Strategy

Innovation is a key driver in the development of essential oil-based products, encompassing novel formulations and combination, new extraction and purification processes, as well as specific therapeutic and cosmetic applications. Protecting these innovations through intellectual property (IP) is therefore a strategic challenge for companies and researchers in the sector. This is particularly relevant for natural products, which are often mistakenly regarded as offering limited opportunities for IP protection, despite the availability of robust legal mechanisms to secure and enhance innovation.

This presentation will provide an overview of the main intellectual property tools available to innovators, such as patents, trademarks, design and models, contracts, and trade secret.

A particular focus will be made on patents. We will discuss the key patentability requirements — including novelty, inventive step, and the demonstration of a technical effect — in light of the specific challenges associated with essential oils and natural products. Practical examples drawn from the examination practice and case law of the European Patent Office will illustrate the types of innovations that can be successfully protected.

The presentation will also address practical considerations that are critical for innovation-driven projects, including the preservation of confidentiality during product development, the use of appropriate contractual arrangements to secure collaborative research and development, and the assessment of freedom to operate prior to commercialization.

KEY NOTES LECTURES



KL1. ARUMA: Database of oils, extracts and aromas from the Amazon

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Keywords: Amazonian aromatic plants; Volatile organic compounds; Chemical database; Natural products; Pharmacological activity.

There is a growing demand for integrated databases that systematize botanical, chemical, and genetic information on these species, which are still poorly represented in existing databases [1]. Thus, the ARUMA database aims to gather information on aromatic plants collected in the Amazon and their products (VOCs and extracts). Volatile and non-volatile chemical compositions were analyzed by GC-FID/GC-MS and HPLC-DAD/UHPLC-MS/MS, respectively. Essential oils (EOs) and hydroethanolic extracts (HEEx) were subjected to in vivo and in vitro pharmacological tests, as well as DNA barcode analyses. The system was developed in Typescript with Next.js 14 and Supabase as the database. The database has information on 37 aromatic species and edible fruits distributed across 25 genera and 13 botanical families, with Myrtaceae (20 species) being the most prominent. The most collected species were *Protium heptaphyllum* (18 samples) and *Myrcia sylvatica* (14). Chemical analyses revealed a predominance of (*E*)-caryophyllene (107 samples), 1,8-cineole (51), 9-*epi*-(*E*)-caryophyllene (49), and (*E*)-nerolidol (42). Pharmacological tests demonstrated antioxidant (10 samples), cytotoxic (9), and anti-inflammatory (7) activities. Aruma database is a tool for integrating and systematizing chemical, botanical, and pharmacological data on Amazonian aromatic species, favoring studies in metabolomics, prospecting for bioactive compounds, and conservation of regional biodiversity.

Acknowledgements: CNPq (Edital 19/2024, Centros Avançados em Áreas Estratégicas para o Desenvolvimento Sustentável da Região Amazônica - Pro-Amazônia, process 443973/2024-5).

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KL2-KL7 : EFEO IFEAT Scientific Program Session



From Natural Complexity to Regulatory Confidence: Scientific Challenges in the Safety Assessment of Essential Oils and Natural Complex Substances

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Keywords: essential oils, natural complex substances, MOCS, REACH, CLP, regulatory assessment, matrix effects, safety evaluation.

The EFEO–IFEAT Scientific Program was established to document independent, high-quality scientific evidence to address these challenges. Bringing together academic experts, industry scientists and regulatory specialists, the Program investigates essential oils through three complementary pillars: analytical profiling, human health, and environmental fate. Current activities include improved characterisation of natural extracts, comparison of whole essential oils with individual constituents or synthetic mixtures, investigation of potential protective or modifying matrix effects on genotoxicity, evaluation of reproductive toxicity data, and investigation of biodegradation pathways.

At ISEO 2026, these questions will be explored through a series of complementary regulatory and scientific contributions. Following an overview of the evolving EU chemicals regulatory framework, the session will address analytical identification and quantification challenges, biodegradation of whole essential oils versus their constituents, the olfactory properties of natural oils compared with reconstructed mixtures, and mechanistic approaches to potential matrix-mediated modulation of genotoxicity. The session will conclude by integrating these findings, identifying remaining evidence gaps and considering the scientific priorities towards 2029.

By combining multidisciplinary scientific approaches, the Program aims to provide a robust evidence base to support scientifically sound regulatory decisions. Understanding the specific properties of natural complex substances is essential to determine whether existing classification approaches adequately reflect their behaviour and to ensure that future regulatory frameworks appropriately consider the unique characteristics of essential oils and plant-derived extracts.

KL2. Recent Developments in EU Chemicals Legislation: Simplification, CLP and the Regulation of Natural Complex Substances

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Keywords: Cosmetic Regulation, CLP regulation, derogation for plant extracts, simplification

Abstract

The amended CLP framework reinforces the assessment of substances with more than one constituent or complex substances to be assessed and classified using information on both their known constituents and the substance as a whole. For certain health hazards, such as carcinogenicity and environmental fate properties, the CLP revision from 2024 provides that a complex substance should only be classified on the basis of the classification of its hazardous constituents, unless whole substance data confirms the classification derived from the constituent. A derogation was introduced for non-chemically modified plant extracts because scientific data suggested that some isolated constituents may show hazards that are not expressed by the extract as a whole. Alternatively, ad hoc derogations can be granted on a case-by-case basis, after scientific assessment. This derogation for plant extracts is subject to a scientific review, which should allow further scientific assessment of whether the general constituent-based rules are suitable for such plant-derived substances, while still allowing operators to apply those rules if no relevant substance-level information exists. The Commission must review the available information and submit a scientific report to the European Parliament and the Council by December 2029, possibly followed by a legislative proposal if warranted. In this presentation, the objectives of the Commission report, the methodology to be followed and the key questions to be answered will be explained.

In addition, a short description of the chemical omnibus (Omnibus VI) which is part of the European Commission's broader Action Plan for the Chemical Sector aiming to streamline and modernise EU chemicals legislation to support competitiveness while maintaining safety standards, will be given focussing on the main simplifications relevant for plant extract products.

KL3. Reliable Identification and Quantification of Natural Constituents in Complex Matrices

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Keywords: method development, *Citrus* essential oils, quantitative analysis, regulatory

Identification and quantification of natural constituents in complex matrices represent a major challenge in academic and industrial research. Among natural products, *Citrus* essential oils represent highly complex mixtures containing numerous volatile and non-volatile compounds whose composition may vary depending on several factors such as botanical source, geographical origin, processing, and storage conditions.

This study, performed under the EFEO-IFEAT scientific program, aims to ensure analytical reliability, authenticity evaluation, and to establish a routine quality control for analytical investigations within the *Citrus* essential oil industry.

Different analytical approaches for the characterization and quantification of natural constituents in different *Citrus* essential oils using advanced chromatographic and spectrometric techniques will be discussed, and comparison will be presented. The aim is to achieve high selectivity, sensitivity, and reproducibility while minimizing matrix interferences. Emphasis will be laid on method accuracy through careful evaluation of different quantification methods.

Acknowledgements: The research was founded by the International Federation of Essential Oils and Aroma Trades (IFEAT) and European Federation of Essential Oils (EFEO) consortium.

KL4. New Insights into the Biodegradability of Essential Oils and Their Constituents

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Keywords: Ready Biodegradability; constituents and mixtures, Lavender oil (*Lavandula angustifolia*), Cade oil (*Juniperus Oxycedrus*), Clary sage (*Salvia sclarea*).

Further to the amendment of the CLP Regulation (Regulation (EU) 2024/2865 amending Regulation (EC) No 1272/2008), the European Commission (EC) is mandated to review the scientific basis for the exemption of plant extracts from the MOCS rule. The CIHEF research project aims to support the EC mandate by providing relevant data and expertise. After obtaining funding from France Agrimer, three research entities with expertise in biodegradation were contracted to examine the following aspects:

Phase 1: Biodegradation of

- 1) **individual substances** (selected constituents of essential oils)
 - 2) **Binary mixtures** (selected combined constituents)
 - 3) **essential oils**
- using OECD 301F in GLP (LPL) and non-GLP studies (Nantes Univ)

Phase 2 : Metabolomics

Identify and characterize bioproducts (Nantes Univ)

Phase 3: Metagenomics: Investigate microbial community diversity using metagenomic and functional gene analysis

Phase 4a:

Upgrade current biodegradation model for single substances
(focus on terpenes)

Phase 4b:

Biodegradation model for UVCBs, notably natural complex substances

A total of 6 oils are implicated but only 3 (Lavender oil (*Lavandula angustifolia*), Cade oil (*Juniperus Oxycedrus*), Clary sage (*Salvia sclarea*)) will be fully tested due to time and budget constraints. The project started in June 2025 and will continue until November 2027. The early stage of the project have yielded biodegradation results on some of the constituents and binary mixtures and these will be presented as will the remaining steps in the project.

KL5. Analytical Quantification Challenges for Regulated Molecules in Essential Oils and Other Natural Ingredients

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Keywords: MOSH/MOAH analysis, trace level quantification, unavoidable traces.

The increasing complexity and stringency of regulatory frameworks governing essential oils and other natural ingredients are placing unprecedented demands on analytical science. In particular, the reliable quantification of regulated molecules at trace levels has become a critical requirement. However, significant gaps remain between regulatory expectations and the capabilities of current analytical techniques. This contribution addresses three key challenges related to contaminants, analytical practices, and the interpretation of trace-level compounds in complex natural matrices.

A first major challenge concerns contaminants such as mineral oil hydrocarbons (MOSH/MOAH). Analytically inadequate limits are being proposed by regulators, despite the absence of fully validated and reproducible quantitative methods suitable for complex natural matrices. Current analytical approaches remain sensitive to matrix effects, are poorly harmonized, and often lead to significant inter-laboratory variability. As a result, the quantification of these regulated contaminants lacks robustness, raising concerns about the reliability and comparability of the data used to support regulatory decision-making.

A second challenge relates to the reliance on third-party laboratories for compliance testing. Driven by commercial constraints, these laboratories often use single-method approaches optimized for efficiency and cost. While suitable for routine screening, such strategies are not fit-for-purpose for the accurate quantification of regulated compounds in complex matrices. The lack of orthogonal validation and complementary techniques reduces robustness and reproducibility, and limits comparability of results across laboratories, thereby questioning the suitability of outsourced data for regulatory compliance.

The third challenge arises from the increasing sensitivity of modern analytical techniques, which enables the systematic detection and quantification of trace-level compounds. In essential oils and other natural complex substances, such traces are often unavoidable and may originate from environmental background, processing, or analytical artefacts. However, these findings are frequently interpreted without sufficient consideration of their origin or relevance, leading to potential over-interpretation and unrealistic regulatory expectations.

Overall, these challenges highlight a growing misalignment between regulatory demands for precise quantification and the current capabilities and limitations of analytical methodologies. Addressing this gap requires more robust, multi-technique analytical strategies, improved validation practices, and a clearer understanding of concepts such as unavoidable traces. Enhanced dialogue between scientists, industry, and regulators will be essential to ensure that future regulations remain scientifically sound and practically applicable.

KL6. To What Extent Are the Olfactory Properties of Essential Oils the Sum of the Individual Properties of Their Constituents?

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Keywords: NCS, olfactory properties, chemical complexity.

The odor that humans perceive from a given essential oil (EO) is the result of a complex interplay between a range of volatile molecules and the olfactory receptors (ORs) among the approximately 400 ORs identified in humans to date.

As natural complex substances, EOs can contain hundreds of individual compounds, each exhibiting specific olfactory notes and perception thresholds. Using gas chromatography with a sufficiently sensitive detector, it is possible to determine the chemical composition of most EOs with sufficient accuracy.

In this presentation, we will describe our approach to assess the extent to which it is possible to reproduce the odor of an EO through several formulation assays that progressively increase in fidelity to the original oil. For this purpose, we have worked with *Eucalyptus globulus* and *Citrus bergamia* EOs, analyzed their composition using conventional methods, and are now preparing samples of increasing complexity. These samples will undergo sensory evaluation to determine the minimum level of complexity required to mimic an authentic EO, if such a reproduction is achievable.

KL7. Mechanisms of Protection Against Genotoxicity: Deconstructing Matrix-Derived Combination Effects of Alkenylbenzenes

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Keywords: genotoxicity, mutagenicity, essential oils, matrix modulation

Compounds such as allylalkoxybenzenes, including estragole, methyl eugenol, and safrole, and structurally related propenylbenzenes, such as α -asarone, β -asarone, and anethole, are naturally occurring secondary plant metabolites found in numerous botanical sources, including basil, fennel, tarragon, nutmeg, star anise, and calamus root¹. The toxicological concern associated with some of these constituents arises primarily not from the intrinsic reactivity of the parent compounds, but from electrophilic metabolites generated through enzyme-mediated bioactivation, predominantly in the liver². Consequently, their genotoxic potential is determined by the balance between phase I oxidation reactions and phase II conjugation pathways, which both may either detoxify reactive intermediates or generate metabolites exerting genotoxic effects. Constituents naturally present in essential oils have been reported to modulate enzymes involved in the bioactivation of potentially genotoxic compounds³. Consequently, the essential oil matrix may alter the formation and elimination of reactive metabolites, thereby enhancing or attenuating the genotoxic effects observed for an isolated constituent.

In this presentation, we will describe our mechanistic experimental approach to determine how the complex chemical matrix of essential oils modulates the mutagenic and genotoxic effects of naturally occurring constituents of concern by comparing their activity as isolated compounds, and within the essential oil-based matrices.

Acknowledgements: The research was supported by the International Federation of Essential Oils and Aroma Trades (IFEAT) and European Federation of Essential Oils (EFEO) consortium.

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ORAL PRESENTATIONS



OP1. Authentication of the origin of natural ingredients in cosmetics and fragrance

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Keywords: Authentication; Origin traceability, Natural raw materials, Adulteration

The use of raw materials of natural origin is growing rapidly in the cosmetics industry. This greenification of the raw material portfolio is accompanied by new control challenges to ensure the origin and renewability of these natural ingredients. This traceability is essential to verify product authenticity in a market where fraud is common and sometimes extremely sophisticated. Some suppliers resort to adulteration to reduce production costs, improve the quality of their ingredients, or artificially increase production volumes by adding lower cost products, including cheaper natural materials, molecules of petrochemical origin or solvent dilution.

To control the presence of adulteration, appropriate authentication methods are required to control the naturalness and purity of samples. Current approaches for authentication of natural raw materials often involve a disparate application of analytical techniques, which may lack a systematic decision-making framework. While individual analytical methods like GC-MS, radiocarbon assessment, chirality approaches or stable isotopic studies are known and widely used in analytical chemistry for authentication, the prior art lacks a systematic process for the selection of the appropriate analytical tool. A global and systematic approach could adapt to various types of adulteration and origin authentication.

The primary objective of this study is to provide a structured and efficient process for selecting appropriate analytical tools to authenticate natural raw materials in cosmetic applications. More specifically, the objectives include to ensure the genuine origin of raw materials, distinguishing them from synthetic counterparts or from other origins (animal, botanical, geographical, process); to improve the detection of fraud and adulteration in natural cosmetic ingredients; and to optimize the use of advanced analytical techniques by providing a clear decision-making pathway based on specific chemical characteristics of the material and the type of traceability of interest.

By systematically applying this process, it will lead to enhance capability to detect sophisticated adulteration, improve traceability and origin authentication of natural raw materials, support naturalness claim on cosmetic finished products and optimize the analytical strategy and the selection of suitable analytical tools from a selected pool of techniques. This process was applied to authenticate the genuineness of the mandarin *Citrus reticulata* essential oil.

OP2. Comparative Pathways to Sustainability in Essential Oil Production: Two Case Studies: Agarwood and Frankincense

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Keywords: Sustainability, Pathways, Case Studies, Cultivation, Wild Harvesting, *Aquilaria Crassna* (Oudh), *Boswellia Sacra* (Frankincense).

Sustainability has become a central objective of the global essential oil industry(1). Practical pathways toward production systems that are simultaneously environmentally sound, economically viable, and socially equitable remain poorly documented.

This presentation draws on more than three decades of field experience in Africa, Asia, and North America to examine two contrasting essential oil supply systems: *Aquilaria Crassna* (Oudh), (2) and *Boswellia Sacra* (Frankincense) (3). Each represents a distinct ecological, cultural, and commercial context. Both have faced periods of instability driven by overexploitation, market pressures, and structural weaknesses in production systems.

We analyse the status quo preceding project intervention, the strategies implemented to address unsustainable supply, and the outcomes observed during and after implementation. The presentation highlights both successes and failures, emphasising lessons learned rather than idealized outcomes. Key issues examined include the pros and cons of cultivation versus wild harvesting and its impact on aroma and chemistry.

Much of the material presented derives from unpublished practical experience and long-term industry involvement, offering insights seldom accessible in the scientific literature

These case studies demonstrate that sustainability is not a fixed endpoint but an adaptive process shaped by ecological realities, economics, and human systems. The lessons presented aim to inform future initiatives striving for resilient and sustainable essential oil supply chains.

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OP3. Evaluation of a simplified configuration for GC×GC MS/FID for essential oil quality control

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Keywords: GC×GC, Parallel FID/Single Quadrupole MS, Reversed flow modulation

The analysis of essential oils and fragrances has been a spearhead application of capillary gas chromatography (GC) since more than 50 years [1]. Soon after their introduction, new developments in GC, such as multidimensional techniques, were applied in quality control and in target analysis of constituents such as fragrance allergens. In parallel, also developments in mass spectrometry hyphenated to GC (GC-MS) were successfully used. Ten years ago, we presented an overview of analytical GC-MS methods that can be applied for target and non-target analysis of essential oils and fragrances. The compared techniques included heart-cut GC (GC-GC), comprehensive GC (GC×GC), single quadrupole MS, triple quadrupole MS and high resolution time-of-flight MS [2]. Since then, additional technologies such as GC and GC×GC in combination with (very) high resolution MS have been evaluated [3-5]. Without doubt, all these techniques have great potential to unravel the complexity of essential oils and fragrance mixtures and determine specific constituents. However, as stated during discussion sessions in various ISEO meetings, due to high investment and operational costs, some of these techniques are only accessible for high-end laboratories. Robust and low cost configurations, such as single quadrupole MS systems (for identification) and flame ionization detection (for quantification) are therefore still valuable for routine tests.

In our laboratory, a new configuration of reversed flow modulation, combined with single quadrupole MS and flame ionization detection (FID) was evaluated for qualitative and quantitative analysis. The system configuration will be described, and the obtained results will be discussed in terms of peak capacity gain, MS identification capabilities (ie sample composition) and FID area percent.

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OP4. Discovery of Synthetic Markers for Essential Oil Authentication

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Keywords: synthetic markers; linalool oxide acetate furanoids; *Lavandula angustifolia*; *Salvia sclarea*; *Citrus bergamia*; *Mentha citrata*; GC-MS authentication

Essential oil adulteration remains difficult to control because modern adulterants are increasingly made from natural precursors and can mimic chromatographic, isotopic and chiral signatures of genuine oils. Market surveys show that adulteration remains widespread [1,2]. GC-MS/FID may pass oils when major constituents fall within ISO or AFNOR limits, while chiral GC, ¹³C-IRMS, ²H-SNIF-NMR and ¹⁴C/pMC may fail when synthetic aromachemicals are derived from modern biogenic carbon. This gap requires route-specific synthetic markers rather than broader compositional limits [3,4].

Authenticated lavender oils from France, Bulgaria, China, Australia, Russia, India and the USA were compared with commercial materials by systematic GC-MS screening. Two diagnostic peaks at RI 1245 and RI 1262 on a ZB-5 column, with identical mass spectra, were absent from all authenticated lavender oils but present in suspicious samples. HRMS, ¹H NMR, ¹³C NMR and ¹H-¹H NOESY identified them as cis- and trans-linalool oxide acetate furanoids, designated LOACF and LOATF.

Possible non-adulteration origins were tested. The markers were absent across authenticated oils from multiple origins, cultivars, harvest years and chemotypes. Natural ageing and open-air exposure produced hydroperoxy, hydroxy and epoxy derivatives of linalool or linalyl acetate, but not LOACF or LOATF. Forced oxidation using mCPBA, H₂O₂ and KMnO₄ also failed to generate these furanoid acetates. Co-elution with NMR-characterized synthetic references, supported by matching HRMS and NMR data, indicates process-related impurities formed during industrial acetylation of fractionated Ho-wood or Ho-leaf linalool. Marker-positive samples can then be confirmed by isolating linalyl acetate and applying ¹⁴C/pMC testing.

Because LOACF and LOATF indicate a synthesis route rather than a botanical origin, their application extends beyond lavender to linalyl-acetate-rich oils such as clary sage, bergamot, bergamot mint, lavandin and thyme chemotypes. Based on the market frequency of linalyl-acetate adulteration and related precursor-derived synthetic additions, these markers could help decipher approximately 20% of EO adulteration cases unresolved by conventional testing. Systematic synthetic-marker discovery is therefore critical for stronger authentication, producer protection and consumer trust.

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OP5. Reconstitution of “Ranjatai” agarwood scent by olfactometric and analytical approach, a historic Japanese national treasure

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Keywords: Ranjatai, Agarwood, Incense wood, Mon-kō, Kōdō

“Ranjatai”, a historic piece of agarwood (length: 1.560 m; weight: 11.6 kg), is regarded as one of the most iconic and highly valued incense woods in Japanese history. It is preserved in the Shōsōin repository of Tōdai-ji Temple, constructed in the 8th century. Historical records indicate that a famous shogun cut a portion from this precious wood in the 16th century. Although Ranjatai has long been esteemed as one of the most celebrated scents in Japan, its olfactory characteristics had not been scientifically investigated prior to this study.

In 2024, the Imperial Household Agency commissioned a project to analyze and replicate the scent of Ranjatai to ensure its preservation for future generations. We conducted chemical and aroma component analyses using gas chromatography–mass spectrometry (GC–MS) and gas chromatography–olfactometry (GC–O), complemented by a headspace analytical approach (AROMASCOPE®) applied directly to the large wood sample. GC–MS analysis revealed high levels of 3-phenylpropionic acid, suggesting its importance as a characteristic odorant of Ranjatai.

In addition, we performed *Mon-kō* (聞香), a traditional smelling practice ceremony within *Kōdō* (香道), the Japanese art of incense appreciation. A small fragment of Ranjatai was gently heated to an appropriate temperature and evaluated the scent. This procedure was considered to approximate the scent experience perceived historically and was therefore defined as the target profile for reconstitution. Surprisingly, despite having aged for over 1,000 years, this wood was found to have preserved significant and recognizable aroma character. The scent was characterized as sweet, spicy, woody, and ambery, reminiscent of labdanum, with anisic nuances and gourmand tonalities suggestive of tonka and vanilla.

Based on the integrated analytical data and the sensory insights obtained through *Mon-kō*, we successfully reconstituted the scent profile of Ranjatai. This study represents the first scientific investigation and reconstitution of the scent of this culturally significant incense wood, contributing to the olfactory heritage preservation.

OP6. Vacuum steam distillation: A technique to both investigate and mitigate still notes in essential oils

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Keywords: Distillation off notes, vacuum distillation, GC-olfactometry, *Humulus lupulus*

Distillation off notes, or “still notes”, are well known by plants distillers and could be described as unpleasant aromas, often reminiscent of overcooked vegetables, burnt matter and even sewage, which are not noticeable in the plant material but build up during the distillation and end up in the essential oil (EO) and/or hydrolate. This issue has been known for decades and E. Guenther even described in 1948 how to alleviate the issue by simple aeration or by bubbling hot air into the most affected EOs [1].

More recently, Coleman, Lawrence & Craven worked on off notes in freshly distilled *Mentha piperita* EO and proposed a SPME/GC-MS method to objectively classify the most impacted production batches and decide whether they should be subjected to simple aeration or to redistillation. These authors identified four compounds as being the most important contributors of *Mentha piperita* EO off notes: dimethyl sulfide, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal [2]. Besides this study, and a previous one by the same authors, published work on EOs or hydrolates off notes are scarce at best.

We recently examined the impact of vacuum steam distillation (VSD) versus atmospheric pressure steam distillation (APSD) on the compositions and organoleptic qualities of hop (*Humulus lupulus*) EOs, using a modular distillation device allowing to modify the pressure (and thus distillation temperature) while keeping other parameters constant. There were major differences in the smell and the compositions of the very volatile fraction of both EOs. The four components reported above were identified in the EO obtained by APSD, but we also identified many related components, sulfurous or not, that were only present in the APSD EO and not in the VSD EO.

The objectives of this study are therefore to update the list of compounds in EO that arise from some degradations during the distillation, link some identifications to the perception of the so-called off notes (all EOs were analyzed by GC-olfactometry) and document the interest of vacuum distillation in the production of EO with highly preserved aroma compared to the plant material.

Acknowledgements: We are grateful to Houblon Bastien (QC, Canada) for providing the hop used in this study.

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OP7. GC-FTIR coupling and Custom Spectral Libraries for the Characterization of Natural and Synthetic Fragrance Compounds

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Keywords: libraries, fragrance, natural and synthetic compounds, GC-FTIR

The identification of fragrance compounds in essential oils (EOs), natural matrices, and synthetic formulations is a complex task due to the structural similarity of many constituents and the frequent incidence of isomeric compounds. While gas chromatography-mass spectrometry (GC-MS) is the choice technique for these analysis, often complementary spectroscopic analysis is required for confident identification of compounds with similar fragmentation patterns. To this regard, gas chromatography coupled with Fourier transform infrared spectroscopy (GC-FTIR) can provide complementary information, through the observation of specific and unique vibration bands, providing molecular fingerprint.

In the present study, the use of a GC-FTIR instrument with solid deposition (*-sd*) interface (enabling high resolution FTIR spectra) was employed for the identification of natural and synthetic fragrance compounds, commonly encountered in EOs and fragrance analysis. The analytical workflow integrates GC chromatographic separation, *sd*-FTIR spectral acquisition, and spectra matching research into dedicated FTIR spectral database for flavours and fragrance compounds, including also retention index (in a similar way as MS databases). In addition, comparison to spectra acquired with other IR techniques such as vapour phase (LP) or Attenuated Total Reflection (ATR), will be discussed in terms of spectra resolution and similarity match. Lastly, an innovative integrated approach involving the use of a hyphenated instrument, consisting of both MS and FTIR detector, namely GC-MS-*sd*-FTIR, will be reported to achieve confident compound identification in complex matrices.

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OP8. Unraveling Synergistic Effects in Essential Oils: A Novel Preparative Three-Dimensional Gas Chromatographic Approach for Targeted Component Depletion and Toxicological Evaluation

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Keywords: Essential oil safety, Regulatory restrictions, 3D-Preparative gas chromatography, Component-depletion & Synergism

Recent European Union regulatory frameworks imposing strict limitations on the use of essential oils (EOs) due to the presence of regulated, potentially toxic, or hazardous constituents pose a critical threat to the entire essential oil sector and its industrial applications. EOs are highly complex natural mixtures primarily composed of volatile terpenes, which vary drastically in both qualitative and quantitative profiles. Because of this inherent complexity, the holistic biological activity of an EO is rarely a mere summation of its individual components; rather, it is dictated by intricate, yet poorly understood, synergistic or antagonistic interactions among the constituents. Consequently, assuming that the hazardous potential of a single isolated compound can be directly extrapolated to the entire essential oil matrix lacks comprehensive scientific justification. To address this challenge and provide rigorous experimental evidence, this study introduces an innovative analytical strategy utilizing a preparative three-dimensional gas chromatography (3D-Prep-GC) system. This advanced technological setup is uniquely capable of isolating complex EO fractions, as well as generating a naturally reconstituted "deprived" essential oil, completely stripped of specific targeted hazardous constituents. Crucially, this high-resolution multi-dimensional isolation process preserves the pristine chemical blueprint, ratios, and enantiomeric integrity of all the remaining native components. By providing customized, component-depleted matrices that perfectly mimic the original oil minus the target allergen or toxin, this approach opens unprecedented avenues for systematic toxicological testing. Ultimately, this methodology serves as a powerful tool to scientifically evaluate true synergistic or toxicological behaviors, offering a robust, data-driven defense for the safety evaluation of complex natural products under evolving regulatory pressure.

OP9. Terpenoid Biosynthesis Regulation and Essential Oil Quality Improvement in *Lavandula* Species

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Keywords: *Lavandula*, terpenoid biosynthesis, essential oil quality, glandular trichome

Lavandula is a globally vital aromatic plant whose terpenoid-rich essential oil is widely used in cosmetics, pharmaceuticals and daily chemicals. The three primary oil-producing species (*L. angustifolia*, *L. latifolia*, *L. × intermedia*) show distinct variations in oil composition and quality, dictating their industrial applications: *L. angustifolia* has high-quality oil with linalyl acetate and linalool, *L. latifolia* is rich in camphor and 1,8-cineole, and *L. × intermedia* features moderate quality but high yield. This study investigated terpenoid biosynthesis regulation and oil quality improvement in *Lavandula* via genomic mining, functional gene validation and metabolic regulation, achieving pivotal progress in elucidating oil biosynthesis mechanisms and enhancing oil quality and yield. High-quality chromosome-level genomes of the three species were assembled and annotated [1,2], clarifying the genetic basis of interspecific terpenoid metabolic divergence and enabling the mining of oil synthesis-related key genes. Secretory cavities in glandular trichome apex cells mediate essential oil biosynthesis, and four R2R3-MYB genes associated with trichome development were identified [3], providing a novel strategy to boost oil yield by regulating trichome density. Key genes and transcription factors governing core terpenoid biosynthesis were verified: LaMYC4 and LaMYC7 positively regulate monoterpenoid and sesquiterpenoid synthesis, with LaMYC7 upregulating both MVA and MEP pathways to promote bioactive linalool and caryophyllene production [4,5]. The LaBAHD acyltransferase gene family, critical for ester synthesis and oil quality, was genome-wide identified, and five candidate genes catalyzing linalyl acetate and lavandulyl acetate synthesis were screened from 166 members [6]; LaBAHD57 was confirmed to directly catalyze linalyl acetate formation in *L. angustifolia* oil via transient transformation and hairy root systems. Exogenous methyl jasmonate (MeJA) regulates *Lavandula* volatile terpenoid release in a dose- and time-dependent manner [7,8]; MeJA treatment disrupts oil component emission circadian rhythm, induces trichome proliferation, and activates de novo terpenoid synthesis in leaves by upregulating key pathway genes, offering an effective technical approach for artificial regulation of oil yield and composition. Based on these findings, high-yield and high-quality *Lavandula* cultivars were bred, including the national approved 'Jingxun' series (25–30% higher per-mu oil yield than mainstream cultivars, compliant with national oil standards) and tetraploid 'Luoshen' (high ester content, strong stress resistance, broad ecological adaptability). Additionally, application research showed that *Lavandula* oil inhalation aromatherapy exerts significant beneficial biological effects, increasing deep sleep duration, reducing sleep latency and lowering anxiety scores in postoperative patients [9], providing a scientific basis for its

high-value healthcare application. This study deciphers the molecular regulatory mechanisms of terpenoid biosynthesis in *Lavandula* oil, provides key functional genes and technical strategies for genetic improvement of oil quality and yield, and lays a theoretical and practical foundation for the high-quality development of the aromatic plant essential oil industry.

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OP10. Evidence Map of Clinical Effectiveness of Aromatherapy

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Keywords: aromatherapy, aromatology, clinical effectiveness, evidence map, human health, beneficial biological effects.

This report consolidates the main evidence on the health outcomes analyzed in the studies included in the Evidence Map. Developed with the support of a working group, the Brazilian Consortium for Integrative Health (CABSIN), the Latin American and Caribbean Center for Health Information (BIREME/PAHO/WHO), and Brazilian Association of Aromatherapy and Aromatology (ABRAROMA).

Aromatherapy is a therapeutic technique that primarily uses essential oils, as well as hydrosols and fatty vegetable oils. Based on an extensive literature search conducted in BVS, PUBMED, EMBASE, and CINAHL databased, 126 review studies clinical (systematic reviews with meta-analysis, systematic reviews of randomized controlled trials, systematic reviews without meta-analysis, and standalone meta-analyses), most of them published in the last five years (2019-2024). These systematic review studies bring together 885 primary studies, with a total of approximately 93,000 human participants, considering the removal of repeated primary studies between reviews. Most studies reported a positive effect, followed by positive potential, inconclusive, and for the remaining associations, no effect was reported. It should be noted that no negative or potentially negative effects were reported.

This Evidence Map provides an overview of the evidence on the effects of aromatherapy for different health outcomes: Mental Health (Mental Disorders and Indicators); Pain and Acute Conditions; Wellness, Vitality, and Quality of Life; Metabolic and Physiological Indicators; Chronic Diseases; Cancer and Palliative Care and Patient Safety. In Brazil since 2018, Aromatherapy has been one of the 29 Integrative and Complementary Practices in Health (PICS) included in the National Policy of Integrative and Complementary Practices (PNPIC) and incorporated into the scope of the Unified Health System (SUS). This expanded the trade of essential oils in Brazil.

This work was made available through a website¹, where we can select: the population that underwent the intervention, the outcomes, and the essential oils used. The website aims to expand and assist clinic and hospital managers and healthcare professionals in using aromatherapy through essential oils, hydrosols, and vegetable oils.

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OP11. Integration of a mixture of essential oils in ventilation systems of intensive rabbit farms to reduce the spread of dermatophytes responsible for veterinary and human diseases.

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Keywords: *Microsporum canis, Trichophyton mentagrophytes, Trichophyton interdigitale, zoonoses.*

The spread of such infections in animal husbandry poses an important health problem for operators because of the potential zoonotic infection that such microorganisms can cause. Specifically, dermatophytosis is an extremely relevant problem in commercial rabbit farms where these fungi can cause considerable morbidity and economic losses in terms of animals. The objective of this study is to evaluate the effectiveness of essential oil (Eo) spray for the control of fungal load in rabbit farms in order to counteract the spread of dermatophytosis in a One Health bigger aim.

The tested fungi were *Microsporum canis*, *Trichophyton mentagrophytes* and *Trichophyton interdigitale*. Minimum inhibitory and fungicide concentration values were extrapolated from the tests. The cytotoxic efficacy of the volatile components of the mix was evaluated by spraying experiments in confined environments. After treatment, the samples were then incubated in a Cytation 5 Cell Imaging Multi-Mode Reader with image acquisition every 3 hours for 60 hours. Finally, the samples were observed with scanning electron microscope at 300x, 1000x and 10000x magnification.

Preliminary data suggest that the spraying of Eo administered may be a valuable treatment for controlling fungal load in rabbit farms in order to avoid both animal and operator infections.

OP12. Microplate disc volatilization assay with sealing lid SLIM-V[®] for *in vitro* evaluation of vapor toxicity

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Keywords: Cytotoxicity, inhalation, respiratory infections, plant-derived volatiles, volatilization

Lower respiratory tract infections are frequent and life-threatening diseases contributing significantly to global morbidity and mortality rates. Inhalation therapy is an effective approaches for the treatment of respiratory infections, as it enables direct delivery of the active antibacterial agents to the site of infection in respiratory system [1]. Plant-derived pharmaceuticals containing essential oils (EOs) and their constituents have been used in the prevention and treatment of respiratory infections in humans [2]. Before the introduction of new inhalation therapies into clinical practice, data on the toxicology and safety of EOs are required, whereas *in vitro* screening is usually the first step in this process [3]. However, the availability of cost-effective and easy-to-use high-throughput systems for investigating vapor phase toxicity and developing new inhalation therapies remain limited. In this study, the vapor-phase cytotoxicity of thymoquinone, β -thujaplicin, 2,5-dimethyl-1,4-benzoquinone, 2,5-dihydroxy-1,4-benzoquinone, and EOs obtained from *Thymus vulgaris*, *Callophylum lancifolium*, *Dipterocarpus grandiflorus*, *Tristanopsis decorticata* to human bronchial epithelial cells BEAS-2B was evaluated by using novel microplate disc volatilization assay [4] combined with microplate sealing lid SLIM-V. Among the tested samples, 2,5-dimethyl-1,4-benzoquinone was found to be the most toxic with IC₅₀ value of 35.53 $\mu\text{g}/\text{cm}^3$, followed by β -thujaplicin and thymoquinone (IC₅₀ 64.03 and 54.10 $\mu\text{g}/\text{cm}^3$, respectively). The remaining tested samples did not significantly affect cell viability (IC₅₀ > 160 $\mu\text{g}/\text{cm}^3$). In summary, the recently developed microplate disc volatilization assay combined with sealing lid SLIM-V is suitable for testing vapor cytotoxicity of plant-derived volatiles. This method may potentially be used as an effective tool in research of new pharmacological preparations used for the treatment of respiratory infections.

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OP13. Synergistic Effects of Essential Oils and Tobramycin Against Multidrug-Resistant *Pseudomonas aeruginosa* and *Burkholderia* spp.: Preliminary Investigations for Aerosolized Therapeutic Strategies.

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Keywords: *Cymbopogon martinii*, *Melaleuca alternifolia*, *Thymus vulgaris*, tobramycin, *Pseudomonas aeruginosa*, *Burkholderia* spp, multidrug-resistant strains

This work investigates the feasibility and scientific rationale of combining essential oils (EOs) with tobramycin to counteract *Pseudomonas aeruginosa*, particularly multidrug-resistant (MDR) strains, and *Burkholderia* spp. which are among the major pathogens involved in pulmonary infections affecting patients with cystic fibrosis [1]. It was assessed the *in vitro* susceptibility of 50 clinical *P. aeruginosa* isolates and 4 different species of *Burkholderia* to 14 EOs derived from botanicals included in EMA monographs. Sixteen percent of isolates were MDR, and 37.5% of these exhibited resistance to tobramycin. MICs were determined using a microbroth-dilution method optimized for hydrophobic and volatile matrices, in line with EUCAST and ISO guidelines, ensuring reliable reproducibility. Based on these results, three EOs—*Cymbopogon martinii*, *Melaleuca alternifolia* and *Thymus vulgaris* CT thymol—were selected for combination studies with tobramycin. These EOs are frequently used in traditional inhalation, topical, or oral formulations and possess well-documented antibacterial and anti-inflammatory activity. Combinations were tested against: the reference strain of *P. aeruginosa* ATCC 27853, three MDR clinical isolates and 4 clinical isolates of different species of *Burkholderia*. Fractional inhibitory concentration index (FICI) analysis revealed strong synergy ($FICI \leq 0.5$) for all combinations against MDR strains, with the *Thymus vulgaris*–tobramycin combination showing the strongest effect ($FICI = 0.148$). Against the susceptible reference strain, additive or indifferent interactions were observed, highlighting the relevance of EO–tobramycin synergy for resistant pathogens. To evaluate EO activity in the volatile phase, we performed nebulization experiments using passive micro-atmosphere and active Venturi devices (~10 μ m droplets). All tested EOs significantly reduced microbial load of standard strains (*P. aeruginosa*, *S. aureus*, *E. coli*, *E. hirae*) according to EN 13697:2001, confirming that volatile components retain antimicrobial efficacy when aerosolized. Together, these datasets—EO susceptibility, EO–tobramycin synergy, EO nebulization efficacy,—provide a coherent foundation for developing EO–tobramycin inhalation strategies, optimizing dose-sparing regimens, and improving therapeutic outcomes for MDR *P. aeruginosa* and *Burkholderia* spp. infections in cystic fibrosis.

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OP14. Microbiota-sparing essential oils: A promising strategy against vaginal pathogens

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Keywords: Vaginal infections, vaginal microbiota, biofilm, essential oil combinations, *Lactobacilli* spp, antimicrobial.

Three out of four women will experience a vaginal infection during their lifetime. Rising antimicrobial resistance, coupled with disruption of protective *Lactobacilli* by conventional therapies identifies the need for microbiota-preserving alternatives. Essential oils, known for antimicrobial efficacy presents a potential solution.

This study evaluated 46 essential oils and 602 combinations for antimicrobial efficacy (minimum inhibitory concentrations) against vaginal pathogens. In addition, the toxicity (brine shrimp lethality assay), effects on beneficial microbiota and biofilm inhibition were examined. *Citrus aurantifolia* L. and *Origanum vulgare* L. exhibited broad-spectrum antimicrobial activity, low toxicity, and minimal inhibition of vaginal microbiota. Among the combinations tested, 101 showed synergistic antibacterial activity and 48 demonstrated antifungal synergy; 69% were non-toxic, and 35% of essentials had no effect on the vaginal biome test organisms. The most promising combination was *Ocimum sanctum* L. with *Salvia officinalis* L., displaying strong synergy (Σ FIC 0.07–0.44) against all tested pathogens except *Candida krusei* (ATCC 2159). Notably, *Cinnamomum camphora* J. Presl combined with *Melaleuca cajuputi* Banks, Maton & Sm. ex R.Powell inhibited *Candida albicans* (ATCC 10231) and *Candida glabrata* (ATCC 90030) biofilms by 82% and 72%, respectively, outperforming individual oils.

These findings demonstrate that selected essential oils and combinations possess *in vitro* antimicrobial and antibiofilm activity, have microbiota-sparing properties and display non-toxicity.

OP15. *Thuja plicata* monoterpenoates as diversified anti-inflammatory building blocks

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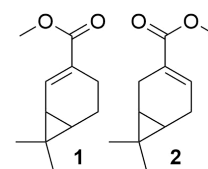
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Keywords: *Thuja plicata*, anti-inflammatory activity, hemisynthesis, methyl monoterpenoates

Western red cedar (*Thuja plicata* Donn. ex D. Don) provides decay-resistant and fragrant reddish wood whose essential oil is commercially available. Its main monoterpenoid methyl thujate (~50%) [1] was described as mold-inhibiting [2], with the whole oil being antifungal [3,4] and anti-inflammatory [1]. In 1973, some homologous thujate esters and amides were prepared and tested for insect repellency [5]. The oil further features methyl myrtenate and other monoterpenic methyl esters absent in MS databases, representing ~15% of the GC-FID profile. The project objectives were to characterize some of these esters to improve the phytochemical knowledge of *T. plicata*; and to tap into its structural diversity to examine the structure-activity relationship of uncommon monoterpenic ester derivatives for anti-mold, anti-inflammatory and olfactory properties. Fractionation over silica, C18 reverse-phase and AgNO₃-impregnated silica led to the NMR identification of methyl isochaminate (**1**, 2.3% of GC) and methyl chamate (**2**, 1.9%), carane esters reported for the first time in nature, among others. From the saponified acids of *T. plicata* oil, a series of 15 homologous alkyl and aromatic mixed esters were prepared via Steglich esterification. Benzyl (IC₅₀ 113 ± 5 µM) and propyl (IC₅₀ 178 ± 13 µM) esters inhibited NO generation in stressed RAW264.7 macrophages, inhibiting >90% at 400 µM without associated toxicity (*L*-NAME achieved 77% inhibition at 1000 µM), while methyl esters were inactive (IC₅₀ > 400 µM). Mold-inhibiting assays are ongoing.



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OP16. Anti-tumor effect of volatile components in essential oils for breast cancer

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Keywords: Lemon Myrtle (*Backhousia citriodora*), Nioi Kobushi (*Magnolia salicifolia*), Breast cancer

Objective Cancer therapy using plant-derived essential oils is attracting attention as one of the promising methods. We have been studying the antitumor effects of aroma oil volatiles on breast cancer cells and have identified two promising essential oils and volatiles with antitumor activity.

Methods Breast cancer cells were cultured in a 96-well plate, and essential oil was added dropwise only to the central well. By incubating the plate at 37 degrees for 48 hours, the effect of the volatile components of the essential oil on the surrounding breast cancer cell growth ability was examined using the MTT assay and fluorescence microscope. In addition, using gas chromatography, we investigated the concentration of transpiration components that may affect cancer cells. We also estimated the anti-tumor effects by using of in vivo-mouse models.

Results Forty kinds of essential oils were tested, and the essential oils of Lemon Myrtle (LM) [1] and *Magnolia salicifolia* (MS) were found to have strong antitumor effects against mouse breast cancer cells (4T1) and in vivo mouse model. Even when these essential oils were diluted more than 300-fold, they could inhibit the growth of breast cancer cells around the essential oils. The antitumor effect presents in the evaporated components of LM and MS essential oil decreased in a distance-dependent manner. LM and MS contain a large amount of low molecular weight compounds such as 1,8-Cineole, Neral, Geranial, and trans-Anethole. The anti-tumor effect of Citral (Neral + Geranial), which is the most abundant of these compounds, and was the most important antitumor factor in LM and MS essential oil. It was shown that the mechanism by which Citral induces cancer cell death is through the release of cytochrome C and activation of caspases 3 and 9 following a decrease in mitochondrial membrane potential induced by Bax.

Conclusion Volatile components of LM and MS essential oils exert strong antitumor effects primarily through citral-mediated mitochondrial apoptosis. These findings highlight the potential of inhalable phytochemicals as novel anticancer agents.

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OP17. Assessing Essential Oil Authenticity from Pure Oils to Finished Consumer Products Using GC×GC-TOFMS

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Keywords: Flow modulation, essential oils, fragrance, GC×GC-TOFMS, authenticity, chemometrics, trap-based enrichment

Essential oils are intricate natural substances commonly utilized in fragrances, flavors, aromatherapy, and consumer products. Their composition naturally varies depending on the botanical source, cultivation environment, extraction process, and storage conditions. Nonetheless, issues such as adulteration, dilution, and substitution pose significant challenges for quality assurance and for verifying product authenticity. Consequently, robust analytical methods are essential for distinguishing genuine natural variations from alterations that could affect quality, effectiveness, or consumer confidence.

Our work presented here will focus on using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) to determine the authenticity of essential oils. The improved separation capability of GC×GC is especially useful for these complex samples, which contain monoterpenes, sesquiterpenes, and oxygenated derivatives that may be difficult to fully separate with standard one-dimensional GC-MS. Automated comparison workflows can simplify interpretation by quickly identifying compounds that differ between oils, enabling faster detection of quality markers, adulteration, or compositional shifts. Using GC×GC-TOFMS data with automated comparison, the differences among essential oils of varying quality can be found within minutes while preserving mass spectral information for compound identification.

In addition to analyzing pure essential oils, we evaluate finished consumer products, particularly candles that claim to contain essential oils. Our comparison includes both premium and lower-cost candle brands to determine whether the essential oil content can be identified in the final product and to observe any significant differences across price ranges. We employed headspace sorptive extraction to collect volatile and semi-volatile fragrance compounds directly from the candle material, requiring minimal sample preparation. These workflows together offer an effective method for verifying essential oil authenticity, assessing product quality, and understanding how natural ingredients are showcased in finished consumer products.

OP18. *Pinus cembra* essential oil: Lab-Scale vs Industrial Steam Distillation

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Keywords: Stone Pine, Arolla Pine, *Pinus cembra*, Pinaceae.

Stone pine essential oil is commercially obtained by steam distillation of the twigs and needles of *Pinus cembra* L. (Pinaceae), a small- to medium-sized evergreen conifer that grows in the Alps. Production commonly takes place in small, family-owned distilleries in Tyrol, a region spanning southwestern Austria and northern Italy. In industrial practice, steam distillation of *P. cembra* is typically limited to 2 – 3 h, yielding 0.2 – 0.8% of a monoterpene-rich essential oil. The aim of this study was to compare the yield and chemical composition of *P. cembra* essential oil obtained by lab-scale (Clevenger) hydro distillation and by industrial-scale steam distillation, employing the same batch of dried plant material. To ensure maximal recovery, distillation was extended to 6 h in both cases, well beyond standard industrial conditions. The total essential oil yield was comparable, amounting to 0.50% in the laboratory and 0.46% at industrial scale. At the industrial plant, 58.7% of the total essential oil was recovered within the first hour of distillation, increasing to 87.3% after 3 h, with only minor increases during the remaining distillation period.

The chemical composition of the essential oil changed markedly over the course of distillation. Major monoterpenes (α -pinene, β -pinene, limonene and β -phellandrene) were predominantly recovered in the early fractions, resulting in a decrease in their relative abundance with prolonged distillation. After 3 h, the essential oil generally comprised the full terpene profile present in the plant material, ranging from volatile monoterpene hydrocarbons to sesquiterpenes—mainly germacrene D—and trace amounts of diterpenes (cembrenes). However, comparison of the complete essential oil obtained after 6 h revealed distinct compositional differences between laboratory and industrial-scale distillation. The lab-distilled essential oil contained lower proportions of monoterpene hydrocarbons (74.7% vs. 81.0%) but substantially higher levels of sesquiterpene hydrocarbons (10.8% vs. 6.8%) and diterpenes (0.8% vs. 0.3%) compared with the industrial product. Notably, germacrene D was significantly higher in the lab-distilled essential oil (2.4%) than in its industrial counterpart (0.9%).

These results demonstrate that, despite extended distillation times, complete recovery of higher-boiling constituents is not achievable under industrial steam distillation conditions. This limitation should be considered when defining realistic specification limits and monographs for *P. cembra* essential oil.

OP19. Oxygenated neryl diesters from *Doronicum austriacum* subsp. *giganteum* root oil: synthesis-supported GC-MS/NMR identification and retention-index rules

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Keywords: *Doronicum austriacum* subsp. *giganteum*; acyloxyneryl esters; oxygenated monoterpene diesters; retention index database; NMR structure elucidation; Asteraceae

The underground parts of *Doronicum austriacum* subsp. *giganteum* (Griseb.) Stoj. & Stef., a Balkan montane Asteraceae taxon, were investigated as a previously unexplored source of organ-specific volatile metabolites. Hydrodistillation afforded a yellow root oil (0.21% w/w) dominated by neryl isobutyrate and neryl 2-methylbutyrate, while targeted dry-flash fractionation revealed a cluster of late-eluting, oxygenated acyclic monoterpene diesters that could not be reliably assigned from EI-MS data alone. A synthesis-guided strategy was therefore developed to establish their structures. Authentic standards were prepared by SeO₂ allylic oxidation of neryl and geranyl esters, NaBH₄ reduction, and Steglich esterification with short-chain branched acids, followed by GC-MS co-injection, high-field 1D/2D NMR, NOESY analysis, and diagnostic ¹³C-¹H coupling measurements. This approach confirmed nine previously undescribed natural products, including 8- and 9-isobutyryloxyneryl isobutyrate, mixed isobutyrate/2-methylbutyrate and isobutyrate/isovalerate analogues, and the corresponding neryl 2-methylbutyrate-derived diesters. The data also showed that closely related C-8/C-9 positional isomers and neryl/geranyl geometrical isomers may exhibit very similar mass spectra, making orthogonal evidence essential for secure identification. Systematic retention-index measurements on DB-5MS and HP-Innowax columns revealed reproducible structure-retention relationships: 8-acyloxygeranyl derivatives eluted after 8-acyloxyneryl derivatives, which in turn eluted after the corresponding 9-acyloxyneryl isomers, with larger separations on the polar phase. These correlations enabled practical retention-index rules for recognizing related, not-yet-observed congeners in complex oils. The results expand the known phytochemical space of *Doronicum* and support allylic oxidation followed by acylation of nerol-derived precursors as a recurring biosynthetic theme in Asteraceae roots. More broadly, the study provides a validated GC-MS/RI and NMR reference framework for future identification of oxygenated acyclic monoterpene diesters in essential-oil research.

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OP20. Transitioning from Helium to Hydrogen in GC×GC-TOFMS/FID for High-Complexity Perfume Analysis

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Keywords: Perfume, formulation, hydrogen, GC×GC, TOFMS, FID, dual detection,

The formulation and quality control of perfumes rely on detailed chemical characterization of highly complex mixtures that may contain hundreds of volatile components spanning a wide range of chemical classes and concentrations. Even trace level compounds can play a decisive role in the overall olfactory profile, making both comprehensive separation and reliable identification essential in fragrance analysis. As a result, gas chromatography (GC) coupled with mass spectrometry (MS) and flame ionization detection (FID) has become a cornerstone technique within the perfume industry for raw material evaluation, quality assurance, and competitive benchmarking.

Conventional one-dimensional GC methods, however, often struggle to fully resolve the compositional complexity of fragrances. Comprehensive two-dimensional gas chromatography (GC×GC) provides a powerful alternative by offering enhanced peak capacity and structured separations, greatly improving the resolution of co eluting compounds. When combined with time-of-flight mass spectrometry (TOFMS), GC×GC enables advanced spectral deconvolution and improved compound identification, while parallel GC×GC-FID detection supports robust quantitative analysis.

Most GC×GC systems have historically being operated with helium (He) as the carrier gas due to its inertness and favorable chromatographic properties. However, increasing costs and supply limitations have driven interest in hydrogen as an alternative carrier gas. Hydrogen (H₂) offers higher optimal linear velocities and can significantly reduce analysis times and operational expenses. Despite these advantages, its applicability in GC×GC remains sparse due to concerns regarding the spectral quality or sensitivity.

In this context, the present work explores the feasibility and analytical performance of transferring a He-based GC×GC-TOFMS/FID method to H₂ for perfume formulation analysis. By evaluating chromatographic behavior, spectral quality, sensitivity, and efficiency, this study aims to assess whether H₂ can serve as a practical and economical carrier gas without compromising analytical performance in high complexity fragrance applications.

OP21. Elicitation as a tool in improving volatile production of selected plant species

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Keywords: *Ocimum basilicum*, *Majorana hortensis*, *Hyssopus officinalis*, *Mentha x piperita*, *Achillea collina*, methyl-jasmonate,

Recently, physical and chemical elicitation has been discussed as a promising tool to enhance the accumulation of special compounds in plants [1]. One of the most well-known natural elicitors are the jasmonates, which have a crucial role in plant defense reactions and several other physiological processes. Although elicitors have already been used more frequently *in vitro*, their *in vivo* application is still less elaborated. Therefore, a 3 year long study was conducted in Budapest with 5 popular aromatic species: basil, hyssop, marjoram, peppermint and yarrow, to establish whether methyl-jasmonate (MeJa) could be effective in elevating the volatile synthesis without changing the characteristic spectrum of the essential oil (EO). Two weeks before the harvest in flowering stage, the plants were sprayed with MeJa dissolved in water and 3% ethanol (0.1mM and 2mM). The control plants were only sprayed with water and ethanol. The treatments were repeated after 1 week. EO was extracted by Clevenger apparatus while GC-FID and GC-MS analysis were used to determine its composition as described in [2].

The higher dosage of MeJa resulted in significant increase of the EO yield of marjoram in all years and in basil in 2 of them. It was also effective in peppermint and hyssop, however, in these perennial species the age and condition of the plantation was also an important influencing factor. As the only exception, yarrow have not reacted to the treatment or - in one of the years- it demonstrated a significantly lower EO yield compared to the control.

In marjoram oil, *cis*-sabinene hydrate and linalool showed a slightly decreasing while terpinene type compounds an increasing tendency due to the higher MeJa dosage, but in most cases changes were not significant. The menthol/menthone ratio of peppermint oil decreased only in one of the years (by 10-13%). Significant changes in basil oil were detected only in the ratios of minor compounds: increase of eugenol and decrease of *iso*-bornyl acetate. In hyssop oil the ratios of *trans*-pinocamphone increased with increasing concentrations of the MeJa treatment (by 27-500%) while the chamazulene content in yarrow could not be influenced by the elicitation. Altogether, it seems, that MeJa application might help improving the quality of the raw material but the technology is strictly depending on the species and growing conditions.

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OP22. Chemotypic diversity and long-term stability of aroma profiles in *Alpinia zerumbet* and related taxa

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Keywords: *Alpinia zerumbet*; *Alpinia zerumbet* var. *excelsa*; *Alpinia formosana*; chemotype; DHS–TD–GC/MS; chemotaxonomy; aroma fingerprinting

Alpinia zerumbet (Pers.) B.L. Burtt & R.M. Sm. is an aromatic perennial plant widely distributed across tropical and subtropical regions and traditionally used in Okinawa as a medicinal and aromatic plant. Its leaves and essential oils exhibit characteristic fragrances; however, their volatile profiles vary considerably among individuals and related taxa. Herein, we summarize and integrate our multiyear chemotaxonomic and aroma fingerprinting studies on *A. zerumbet* and related taxa collected from the Ryukyu Islands and Bolivia. Aroma profiles were analyzed using dynamic headspace–thermal desorption–gas chromatography–mass spectrometry (DHS–TD–GC/MS), principal component analysis, hierarchical cluster analysis, and heatmap-based fingerprinting. These studies demonstrated that the aroma characteristics of *A. zerumbet* cannot be explained solely by geographic origin but are strongly influenced by individual plants, lineages, and chemotypes. Across the examined *Alpinia* species, recurring chemotype groups were observed across distant islands, indicating that genetically governed lineage effects can dominate local environmental influences in shaping the volatile profiles. Comparative analysis of *A. zerumbet* (AZ), *A. zerumbet* var. *excelsa* (AZe), and *A. formosana* (AF) demonstrated that several volatile compounds, including sabinene, γ -terpinene, terpinen-4-ol, and β -pinene, are useful chemotaxonomic markers for distinguishing related taxa and detecting possible contamination or misidentification in commercial essential oil materials. Recently, we extended this approach to AZ cultivated in Colonia Okinawa, Bolivia, a settlement founded by Okinawan immigrants in the mid-20th century [1]. Among three Bolivian accessions, two showed sabinene/ γ -terpinene/terpinen-4-ol-rich profiles corresponding to the AZe-like chemotype, whereas one clustered with the Okinawan AZ-type profile. These findings suggest that certain chemotypes, including the sabinene-rich AZe-type, can be maintained not only within the Ryukyu Islands but also after long-term cultivation in a non-native environment. This integrated dataset provides a basis for chemotype-based selection, species authentication, quality control of *Alpinia* essential oils, and development of aromatic crop resources.

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OP23. Quantum biology of essential oil production

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Keywords: *Mentha piperita*, *Mentha longifolia*, *Ocimum basilicum*, terpenoid gene expression, non-photochemical quenching, quantum coherence, time-resolved Schrödinger equation.

Plant biology traditionally explains stress responses through classical physics and biochemistry. However, growing evidence indicates that quantum phenomena, typically associated with the microscopic realm, may significantly influence biological processes [1]. This emerging discipline, termed quantum biology (QB), investigates the potential role of quantum mechanics (QM) in vital plant functions, including photosynthesis, light perception, and terpenoid production [2]. We recently reported that a reduced geomagnetic field (GMF) modulates photosynthetic electron flow, menthol yield, flavonoid photoprotection and ROS homeostasis in peppermint (*Mentha × piperita* L.) [3]. The work has been extended to *Mentha spicata* and *Ocimum basilicum*, showing the effects on terpenoid gene expression and flavonoid modulation depending on QB events.

In this presentation I will show how QM calculation demonstrates that essential oil production is tightly dependent on photosynthetic efficiency, which in turn is affected by QB events occurring at the electron transfer chain around Photosystem II. This is the first evidence that QB is used to predict essential oil fluctuations in industrial essential oil crops in response to GMF variations. Our research demonstrates that the GMF is an active environmental regulator rather than a passive background condition. Its reduction modulates plant physiology and metabolism, triggering a cascade of systemic changes in essential oil plants. In peppermint and basil, the absence of a normal GMF modulates electron transfer chain activity, leading to a metabolic trade-off where resources were reallocated toward maintaining redox balance at the expense of productivity. These findings establish GMF as a critical, fine-tuning factor regulating essential oil crops metabolic homeostasis, providing potential new insights for optimising crop quality. By bridging these disciplines, our work demonstrates that the complex macro-biochemistry of essential oils is fundamentally intertwined with, and governed by, subatomic quantum biological events

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OP24. Cocrystal-based essential oil components for preventive conservation of cultural heritage

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Keywords: cocrystals, terpenes, controlled release, antimicrobial activity, biodeterioration, cultural heritage.

Essential oils and their main constituents, derived from aromatic plants, are widely recognized for their antimicrobial properties and their potential application in the conservation of cultural heritage materials [1]. However, their high volatility, low physicochemical stability, and rapid degradation significantly limit their practical use in the protection of paper-based heritage against biodeterioration [2, 3]. In this work, cocrystallization is employed as a strategy to stabilize volatile bioactive molecules, with a particular focus on correlating antimicrobial activity with release trend. Cocrystals were prepared using isonicotinamide – a commonly employed coformer for the preparation of pharmaceutical cocrystals - and selected essential oil components, including thymol, carvacrol and eugenol. Biological assays were performed against biodeteriogenic fungi using a vapor-phase exposure approach, in order to mimick realistic conditions. The release behaviour of the volatile molecules was monitored, allowing the evaluation of mass loss profiles and the presence of different crystalline phases, analyzed through powder X-ray diffraction. The results confirmed the antimicrobial activity and controlled release over time. Cocrystals significantly influence both the stability and release rate of the volatile components, enabling a more sustained delivery compared to the pure substances, while preserving their antimicrobial effectiveness under the tested conditions.

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YOUNG SCIENTISTS ORAL PRESENTATIONS



HEAT

YS1. Quantitative analyses of volatile organic compounds emitted in fields: lavandin application

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Keywords: TD-GC-MS, *Lavandula x Intermedia*, quantification, *in-situ* sampling, breakthrough volume

Plants emit a wide range of volatile organic compounds (VOCs). They are involved in defense mechanisms, inter-plant communication or pollinator attraction [1]. Qualitative analyses are well studied in various fields, but there remains a lack of quantitative studies [2]. As an emblematic plant of Southern France, lavandin was selected for this study. The aim of this study is to develop and optimize a field-based VOCs emission quantification method. VOCs can be sampled using either passive or dynamic methods. For a robust quantification, dynamic sampling is preferred. For this type of sampling, adsorbents like Tenax TA are commonly used to capture a large range of VOCs. This is followed by thermal desorption and GC-MS analysis [3].

Field sampling of VOCs represents a significant challenge due to environmental parameters. Indeed, with preliminary studies, the breakthrough phenomenon was identified as an issue during dynamic sampling. This parameter required a special focus to ensure robust quantitative results. This phenomenon was highlighted especially with temperatures above 20°C. To limit this phenomenon, a new transportable and innovative temperature-controlled set up was developed for the collection of VOCs. Validation was first performed under controlled laboratory conditions [4]. This optimized sampling protocol was implemented in the fields to ensure collection without the breakthrough phenomenon. Following validation of the new set up, quantification of targeted molecules emitted by lavandin was carried out. Emission levels ranged from 10 to 100 µg/m³ during the blooming season.

The new optimized protocol provides a robust tool for reliable field quantification of plant VOC emissions under variable environmental conditions and opens perspectives for other studies.

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YS2. Enantioselective multidimensional GC coupled to isotopic ratio mass spectrometry to unravel *Citrus* essential oils composition

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Keywords: *Citrus* EOs; volatile compositional profiling; enantio-selective multidimensional gas chromatography; compound-specific isotope analysis; enantiomeric distribution; authenticity markers

Citrus essential oils (EOs) are complex natural products of high commercial value, whose authenticity assessment is challenged by natural variability, possible adulteration, and the occurrence of co-eluting volatile compounds. In this work, an integrated analytical approach based on *heart-cut* enantio-selective multidimensional gas chromatography coupled in parallel to isotope ratio mass spectrometry and single quadrupole mass spectrometry (Es-MDGC-C-IRMS/qMS) is proposed for their comprehensive characterization. The method enables the combined evaluation of qualitative, quantitative, chiral, and carbon isotopic profiles within a single analytical system. The first chromatographic dimension provides the volatile fingerprint, while selected fractions are transferred to a second enantioselective dimension for the separation of target chiral compounds. The parallel detection setup provides complementary information by combining mass spectral identification with compound-specific $\delta^{13}\text{C}$ determination, including isotopic information on individual enantiomers when adequate chromatographic resolution is achieved. The approach was applied to *Citrus* essential oil samples from different origins and production seasons, showing its potential to highlight compositional variability and support authenticity assessment. The integration of enantiomeric and isotopic markers provided complementary information useful for sample discrimination and traceability. Compared with conventional workflows based on separated analyses, this multidimensional configuration reduces analysis time, sample consumption, and instrumental workload, while improving data reliability by limiting co-elution issues. The proposed methodology represents a powerful tool for the advanced characterization of *Citrus* EOs and related natural products, with promising applications in quality control, authenticity studies, and analytical traceability.

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YS3. Beyond Terpenes: Characterization of Trace Key Odorants in Orange Essence Oil

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Keywords: *Citrus sinensis* (L.) Osbeck, orange processing byproducts, essence oil, key odorant compounds, carbonyl compounds extraction, GC-O, prep-GC.

The contemporary challenge for *Citrus* industry is based on extending commodities shelf-life without compromising their organoleptic quality. Although fresh orange juice is esteemed for its distinctive aroma, its inherent chemical instability necessitates stabilization treatments causing the degradation or volatilization of key aroma-active compounds, which must subsequently be recovered or restored to uphold sensory quality. The primary objective of this study was to evaluate the feasibility of reclaiming these volatile molecules from industrial processing byproducts, specifically focusing on the orange essence oil generated by the company Agrumaria Reggina during the juice concentration phase.

The preliminary volatile characterization based on conventional GC-FID and GC-MS lacked the sensitivity to identify trace-level constituents responsible for the characteristic orange aroma. To overcome this, a fractionation protocol was optimized to remove terpene hydrocarbons which, despite comprising 90% of the oil, possess negligible aromatic significance due to high odor thresholds. Acidic and phenolic components were subsequently isolated from the oxygenated matrix, revealing previously unreported straight-chain and fatty acids. Aldehydes and ketones were selectively extracted from the remaining neutral fraction to enrich odor-active carbonyl compounds. All fractions underwent olfactometric analyses (GC-O) to pinpoint unidentified odorants. To address the reproducibility limitations of AEDA method, this study prioritized a detection frequency approach (NIF/SNIF), offering superior reliability and quantitative precision [1,2]. Finally, unknown molecules perceived by the panelists via GC-O were isolated through preparative gas chromatography (prep-GC) and characterized via nuclear magnetic resonance (NMR) spectroscopy for full structural elucidation.

Acknowledgments: The authors thank Agrumaria Reggina S.r.l. for providing the samples and Shimadzu Corporation and Merck KGaA for their continuous support.

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YS4. The Curious Case of Isosenecioates in Essential Oils

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Keywords: *Sambucus nigra* L., 3-methylbut-3-enoic acid, isosenecioic acid, isosenecioates, Steglich esterification, mass spectrum, retention index, spectral library

During the course of our previous work on the essential oil of the dried flowers of *Sambucus nigra* L. (Adoxaceae), we encountered a moderately abundant unknown constituent, which proved to be, upon isolation, the benzyl ester of 3-methylbut-3-enoic acid, i.e. benzyl isosenecioate (**1**, Figure 1) [1]. Further investigations into this essential oil revealed the presence of two more esters of this carboxylic acid, namely, those of phenethyl alcohol (**2**) and (Z)-hex-3-en-1-ol (**3**). To the best of our knowledge, the occurrence of these esters in the plant kingdom was not previously reported. As isolating minor constituents of essential oils is often not straightforward, we sought to improve the capacity of essential-oil researchers to identify this class of compounds by gathering more relevant spectral and GC retention data, which we report on this occasion. While isosenecioates were already found to form by deconjugation side reactions during the course of esterifications in the presence of 4-dimethylaminopyridine (DMAP) [2], synthesis of a small library of isosenecioates proved easiest starting from pure isosenecioic acid, available from the one-step oxidation of 3-methylbut-3-en-1-ol. We found that primary and most secondary alcohols easily form isosenecioates under the conditions of the Steglich esterification. When comparing isomeric isosenecioates, senecioates, tiglates, and angelates, isosenecioates were found to consistently elute first on poly(5% diphenyl/95% dimethyl)siloxane columns.

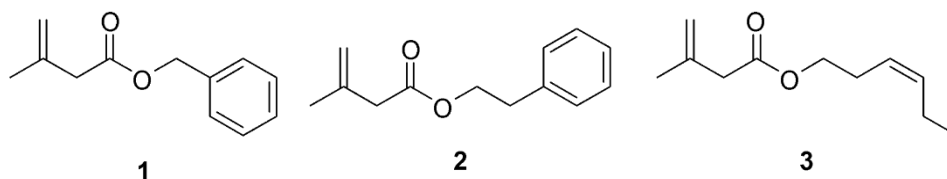


Figure 1: Isosenecioates (**1–3**) found in the essential oil of the dried flowers of *S. nigra*.

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YS5. γ -Terpinene as a Key Antioxidant in Citrus Essential Oils: Lipidomics Insights into the Modulation of Lipid Peroxidation Pathways

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Keywords: Citrus essential oils; antioxidant mechanisms; γ -terpinene; lipid peroxidation; lipidomics; phosphatidylcholine

Essential oils (EOs) are widely applied in food systems as natural antioxidants [1]; however, the key constituents responsible for lipid protection and their mechanistic roles remain incompletely understood [2]. In this study, we investigated the key components responsible for antioxidant activity in Citrus EOs by integrating compositional profiling, lipid oxidation assays, and lipidomics-based mechanistic analysis. Gas chromatography–mass spectrometry (GC–MS) analysis of six Citrus EOs revealed that antioxidant activity in a micellar lipid oxidation system was strongly associated with γ -terpinene content, whereas limonene exhibited negligible antioxidant activity under the same conditions. Building on our previous findings that γ -terpinene can convert highly reactive lipophilic radicals into more hydrophilic species that diffuse into the aqueous phase [3], we further explored its mechanism in a phosphatidylcholine (PC) liposome model using both water-phase and lipid-phase radical initiators. Lipidomics analysis demonstrated that γ -terpinene markedly reshaped oxidation-induced lipid profiles and effectively protected unsaturated acyl chains from oxidative modification. In particular, it suppressed the formation of hydroxylated and oxo derivatives of major PC species, including PC (16:0_18:1) and PC (16:0_18:2). Notably, γ -terpinene significantly inhibited the generation of the truncated product 9-oxononanoyl-PC induced by lipid-phase radicals, a species widely associated with membrane damage and signaling during lipid peroxidation. Overall, these findings highlight γ -terpinene as a key functional component in Citrus EOs and provide mechanistic insight into its role in modulating lipid peroxidation pathways. This work offers a molecular basis for the targeted application of essential oils and their constituents in food and biological systems.

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YS6. Determination of Essential Oil Components of *Salvia nemorosa* and *Salvia virgata* and Investigation of Antimicrobial Activities

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Keywords: *Salvia nemorosa*, *Salvia virgata*, Lamiaceae, GC-MS, α -pinene, MIC.

Within the scope of this study, the essential oil compositions of the flowers and aerial parts of *Salvia nemorosa* and *S. virgata* species, distributed in the Eastern Anatolia Region of Türkiye, were investigated alongside the antimicrobial potentials of extracts obtained from different plant parts. Essential oil analyses were performed via GC-MS. The SPME technique was utilized for the analysis of flower samples. Identification was carried out through computer-aided matching using RRI calculations and Wiley [1], MassFinder [2], and Başer libraries. Results indicated that α -pinene (26.5%) and sabinene (23.5%) were the main components in the flower oil (SPME) of *S. nemorosa*, while sabinene (27.7%) and α -pinene (24.7%) were predominant in *S. virgata* flower oil (SPME). Additionally, high levels of caryophyllene oxide were detected in the aerial parts of both species (27.1% and 20.7%, respectively); however, no analyzable essential oil yield was observed in the root parts.

Antimicrobial studies were conducted on methanolic and other extracts obtained from flowers, aerial parts, and roots using the standard microdilution method. Tested microorganisms included *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida* species. Findings revealed that the extracts exhibited high antifungal activity, particularly against *C. tropicalis*; the methanolic flower extract of *S. nemorosa* provided the strongest inhibition with a MIC value of 156.25 μ g/mL, while the *S. virgata* methanolic flower extract reached 312.5 μ g/mL. These results demonstrate that while both *Salvia* species show similarities in essential oil composition, their extracts carry significant pharmacological potential, especially as candidate antifungal agents.

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YS7. Volatile profile and quality assessment of basil cultivars for pesto: role of genotype and growing conditions

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Keywords: *Ocimum Basilicum* L., industrial pesto production, HS-SPME-GC-MS, estragole

Sweet basil (*Ocimum basilicum* L.) has experienced a marked increase in global demand, driven by the popularity of pesto, leading to cultivation expansion beyond traditional geographical regions such as Liguria, where Protected Designation of Origin (PDO) basil is produced [1,2]. To meet industrial needs, production has intensified through large-scale open-field systems and multiple harvest cycles per season to maximize the yield [2]. However, in recent years, basil has become increasingly affected by numerous pathogens, notably downy mildew, and the recent withdrawal of key crop protection products by the European Commission due to toxicological concerns has further complicated disease management [2,3]. This scenario is promoting the continuous development of new disease-resistant cultivars, requiring ongoing chemical investigation. The resulting variability in growing conditions and genetic background poses major challenges in chemotype characterization and requires monitoring of compounds such as estragole, a potentially genotoxic compound, absent in the PDO “Genovese” basil [4]. Therefore, this study focuses on the volatile fraction of basil leaves, assessing the effects of cultivar, cultivation area, and harvest period on the aromatic profile. It aims to identify crops most suitable for pesto production that align with the “Genovese” quality standard while meeting key industrial and safety criteria, ultimately supporting product standardization and ensuring consistent quality. In this context, the present work demonstrates the applicability of an analytical workflow based on HS-SPME technique coupled with GC-MS analytical platform to assess the quality of basil leaves used for pesto production. Samples from different agronomic conditions were analyzed using a sampling strategy designed to capture the full range of variability. According to our results cultivar selection, together with cultivation area and harvest time, strongly influences the chemical composition of the volatilome of basil cultivars. Moreover, both traditional and newly developed disease-resistant cultivars selected for pesto production are characterized by a predominance of linalool and low estragole levels.

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YS8. Chemical characterization, evaluation of antimicrobial potential and cytotoxic activity of *Thuja occidentalis* L. and *Myrtus communis* L. essential oils for topical applications

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Keywords: Essential oils (EOs), *Thuja occidentalis* L., *Myrtus communis* L., antimicrobial activity, antibiofilm activity, cytotoxic activity

Objective: *Myrtus communis* L. (myrtle), a member of the Myrtaceae family, is an oil plant renowned for its essential oil, which has been used in traditional medicine for many centuries, is now used in pharmacology and the food and cosmetic industry, as well as in various medicinal and cosmetic applications due to its emollient properties [1]. *Thuja occidentalis* L. (thuja), part of the Cupressaceae family, is a plant native to North America, known for its medicinal properties and also used as an ornamental plant in many parts of the world [2]. *T. occidentalis* has been demonstrated to exhibit antioxidant, antiviral, antispasmodic, anti-inflammatory, antitumoral, antibacterial, antifungal, antidiabetic, hypolipidemic and atheroprotective, gastroprotective, antiviral, and immune-stimulatory activities [3].

In this study, we investigated the chemical composition, antimicrobial and antibiofilm activities, and cytotoxicity of *Thuja occidentalis* L. (thuja) and *Myrtus communis* L. (myrtle) EOs to evaluate their potential as natural alternatives for topical applications.

Methods: Thuja EOs were extracted from fresh and dried leaves and cones, while myrtle EO was obtained from fresh leaves. The chemical composition of these EOs was characterized by gas chromatography coupled with mass spectrometry (GC-MS). Finally, we evaluate their antimicrobial and antibiofilm activity and cytotoxic activity to assess their potential as natural alternatives to conventional antibiotics.

Results: GC-MS analysis revealed that myrtle oil was rich in oxygenated monoterpenes (58.2%), predominantly eucalyptol (41.86%) and α -pinene (20.98%). In contrast, thuja oils were dominated by monoterpene hydrocarbons (55–82%), with α -pinene as the major component (29–66%), and their composition varied markedly with plant organ and drying process. Myrtle EO displayed the most potent and broad-spectrum antimicrobial activity, with MIC values as low as 3.096 μ L/mL against *Staphylococcus aureus* and *Escherichia coli*, and effectively inhibited microbial adherence. Thuja EOs showed selective activity, particularly against Gram-positive bacteria and *Candida parapsilosis*, with fresh leaves and cones exhibiting the lowest MICs (1.95–7.8 μ L/mL). All EOs were non-cytotoxic to human keratinocytes at concentrations $\leq$ 125 μ g/mL and, when diluted to 0.05%, demonstrated excellent skin compatibility in human patch tests.

Conclusion: This study demonstrates that myrtle and thuja EOs, particularly myrtle, are promising and safe natural antimicrobial agents for dermatological and cosmetic formulations.

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YS9. Anti-adipogenic potential of *Curcuma amada* rhizome essential oil: Mechanistic insights via PPAR γ modulation using integrated *in-vitro* and *in-silico* approaches

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Keywords: Anti-adipogenesis, *Curcuma amada* essential oil, GCxGC-TOF/MS, Molecular docking, Molecular dynamics simulation, PPAR γ

Curcuma amada Roxb. is an aromatic rhizomatous herb, traditionally used to treat gastrointestinal diseases, inflammation and manage post-partum uterine recovery [1]. It has also exhibited potent hepatoprotective and lipid-modulating effects, indicating its influence on metabolic disorders, particularly obesity [2]. However, scientific evidence regarding its bioactive constituents and their molecular mechanisms in regulating adipogenesis remains limited. This study characterized the chemical composition and evaluated the anti-adipogenic potential of *Curcuma amada* rhizome essential oil (CAREO) using integrated analytical, *in vitro*, and *in-silico* approaches. Volatile profiling of CAREO using GCxGC-TOF/MS enabled the identification of 110 novel and 31 co-eluted constituents. The composition was predominantly characterized by monoterpene hydrocarbons, with β -myrcene, p-camphorene, m-camphorene as major constituents (>1%). In 3T3-L1 preadipocytes, CAREO significantly inhibited adipocyte differentiation and lipid accumulation, accompanied by reduced triglyceride uptake and downregulation of key adipogenic genes, notably PPAR γ . Molecular docking revealed favorable binding of p-camphorene with key PPAR γ residues (Phe282, Cys285, Arg288, His323, Leu330, Ile341), showing binding energy (-8.1 kcal/mol) comparable to rosiglitazone (-8.2 kcal/mol). Molecular dynamics simulations and MM/PBSA analysis over 100 ns confirmed the stability and thermodynamic favorability of the complexes. Collectively, these findings establish the CAREO's mechanistic relevance to be used as a natural therapeutic agent in obesity management.

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YS10. Essential Oils from *Protium* Oleoresins: Chemical Variability and Inhibition of SARS-CoV-2 Spike-Mediated Entry

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Keywords: volatiles, terpenes, Burseraceae, antiviral activity, chemometrics.

Despite advances in vaccination, SARS-CoV-2 remains a challenge due to the emergence of variants and persistent infections, reinforcing the search for therapeutic strategies targeting viral proteases or the Spike-ACE2 interaction [1]. Natural products, especially essential oils (EOs) rich in terpenes from *Protium* oleoresins (Burseraceae), exhibit anti-inflammatory, analgesic, antimicrobial, and anxiolytic properties, in addition to a history of traditional use in the Amazon region [2,3]. However, integrated evaluations of these EOs against viral targets are still limited [4]. This study analyzed the chemical composition of EOs from *Protium* oleoresins and their activity against SARS-CoV-2 entry mediated by the Spike protein. Seventeen samples were characterized by GC-MS and GC-FID, followed by multivariate statistical analysis using the MetaboAnalyst platform. Biological assays included MTT cell viability testing in Vero E6/ACE2 cells and inhibition of vesicular stomatitis virus (VSV) pseudotyped with the SARS-CoV-2 Spike protein from the wild-type Wuhan strain and the Omicron variant. Chemical analysis did not reveal a single (EO) profile, with samples dominated by *p*-cymene, limonene, or α -phellandrene, while others showed distinct signatures, including β -ocimenes and high levels of α -terpinene or 1,8-cineole. Multivariate analysis (PCA and PLS-DA) suggested the clustering of EO samples according to their terpene composition, supporting the chemical variability observed among oleoresins. After cell viability tests, a concentration of 25 μ g/mL was defined as safe. Regarding viral entry inhibition, one sample stood out as the most promising, reducing Omicron strain entry into cells by $97.0 \pm 1.0\%$ and wild-type strain entry by $95\% \pm 2.2\%$. The wide variation in neutralizing activity, from 1.6% to 97% for the Omicron strain and from 0.6% to 98.9% for the original strain, suggests that antiviral activity may be related to the chemical composition and origin of each (EO). These partial results identify *Protium* EO samples capable of inhibiting SARS-CoV-2 Spike-mediated cell entry, highlighting Brazilian biodiversity as a source of potential antivirals.

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POSTERS

SESSION 1: Monday 7th Sept.



PP1-1. Development of quality standards of Taif Rose oil using non-targeted metabolomics approach

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Keywords: Rose oil, *Rosa damascena*, multivariate, quality standards.

Rose Oil is a highly economically valuable product in many countries. Due to its high cost it is cheated with cheaper low quality volatile oils, unsaturated oils or petroleum products. although these additives are safe but some of them are harmful to the health. For the sake of maintain the integrity of the industry and prevent the negative impact on the rose oil economics and cultivation there has to be a precise sensitive method of quality measures for Rose oil. All the available quality control methods rely on monovariables which makes its cheating nondetectable, however, multivariate analysis may make cheating rose oil very difficult due to the complexity of the rose oil components.

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PP1-2. Chiral Properties and Biological Activity of Pungent Sesqui- and Diterpenoids from Liverworts and Ferns

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Bryophytes (mosses, Liverworts, hornworts) and pteridophytes (ferns, lycopodium) grow everywhere except ocean and there are 23000 and 12000 species were known in the world respectively. We have been interested in tasty (pungent, bitter and sweet) constituents obtained from liverworts and ferns and their chirality and biological activity. [1-5] One of the most significant chemical profile is that mono-, sesqui- and diterpenoids found in liverworts are enantiomers to those isolated from the higher plants except germacranes. It is enzymatically interesting that the different species belonging to the same genus produce normal sesquiterpene lactone and its enantiomer, as *Frullania tamarisci* and *F. dilatata*. Thus the chemical constituents of liverworts have been focused by organic chemists and many chiral terpenoids were synthesized. Among liverworts, *Porella vernicosa* complex produces potent pungent sesquiterpene dial, polygodial (**1**) and warburganal (**2**), the former of which was found in two ferns, *Blechnum fluviatile* and *Thelypteris hispidula*. Their chirality is the same as that isolated from higher plant, *Polygonum hydropiper* and *Warburgia ugandensis*. The following pungent sesquiterpenoids, tulipinolide (**3**), diplophyllolide (**4**) and diplophyllin (**5**), 1 α -hydroperoxy-4 α ,5 β -epoxygermacra-10(14),11(13)-dien-12,8 α -olide (**6**) and its 1 β -isomer (**7**) were obtained from liverworts. *Wiesnerella denudata*, *Diplophyllum albicans* and *Porella acutifolia* subsp. *tosana*. The liverworts, *Plagiochila* are chemically distinct from the other liverworts, since they biosynthesize *ent*-aromadendrane (**8**) and many *ent*-2,3-secoaromadendrane sesquiterpenoids such as plagiochilines A (**8**) and I (**9**) possessing potent pungency and their related products (**10-19**). *Pellia endiviifolia* belonging to the Metzgeriales elaborate pungent sacculatal (**20**) and 1 β -hydroxysacculatal (**21**). Both compounds possess the same chiral property as that of polygodial (**1**). Polygodial (**1**) shows potent piscicidal and insect antifeedant activity. Plagiochiline series indicate strong cytotoxicity against human cancer cell lines, piscicidal, nematocidal, insect antifeedant and growth reduction, and antitrypanosomal and germination inhibitory activity of rice seeds. Sacculatal (**20**) also shows the similar biological activity as that of polygodial.

Acknowledgements : The authors thank Prof Dr. Alicia Bardon (Tucuman National University, Argentina) for her collection of Argentinian liverworts and ferns and valuable discussion. Thanks are also due to Dr. Danko Bukvicki (University of Belgrade, Serbia) for her antimicrobial test of sacculatal..

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PP1-3. Exploring Brazilian Biodiversity through the Essential Oils of *Piper multinodum* C. DC.

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Keywords: *Piper multinodum*; Piperaceae; Seasonality; Circadian rhythm; Chemophenetics; *Candida glabrata*.

Brazilian biodiversity represents a strategic reservoir of chemically diverse natural products, especially among underexplored native species with ecological and pharmacological relevance [1]. This study investigated the leaf essential oils of *Piper multinodum* C. DC., focusing on seasonal and circadian variation, environmental and edaphic drivers, chemophenetic patterns, and antifungal potential. Fresh leaves were collected at different periods, and the essential oils were obtained by hydrodistillation. The volatile profiles were characterized by GC-MS and GC-FID, allowing qualitative and quantitative assessment of the oil constituents. In the seasonal survey, 62 volatile compounds were identified, and essential oil yields ranged from 0.09 to 0.68%. The oils were dominated by non-oxygenated monoterpenes, mainly α -pinene, myrcene, and β -pinene, indicating a terpene-rich chemophenetic profile. Higher yields were recorded during the dry season, especially from June to August, coinciding with reproductive phenophases. Pearson correlation analysis showed significant negative associations between oil yield and humidity, precipitation, and temperature, whereas soil copper and iron levels were positively related to yield. Principal component analysis, hierarchical clustering, and canonical correspondence analysis confirmed seasonal chemical differentiation and demonstrated the contribution of abiotic and edaphic variables to the observed metabolic patterns. Non-oxygenated monoterpenes were favored under drier conditions, while sesquiterpenes became more expressive during wetter periods. The circadian evaluation revealed daily modulation of both yield and composition. During the rainy season, the highest yield occurred at 12:00 h, whereas in the dry season the chemical profiles were more homogeneous, with higher yields between 00:00 and 06:00 h. Antifungal activity against fluconazole-resistant *Candida glabrata* showed that the November oil was the most active (IC₅₀: 139 μ g/mL), associated with δ -3-carene, β -longipinene, and γ -elemene. These findings support optimized harvesting strategies for pharmacological and biotechnological exploration of *P. multinodum* as a source of bioactive essential oils.

Acknowledgements: UFBA, CNPQ, FAPESB e FAPERJ.

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PP1-4. Revisiting irone distribution as a marker of botanical origin in orris raw material: combined chemical and genetic insights

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Keywords: Orris, *Iris pallida*, *Iris germanica*, Irone enantiomers, Genetic distance, Authentication

Iris pallida (Italy, France) and *Iris germanica* (mainly Morocco, China) are the main botanical sources of orris used in perfumery. These species are commonly described as exhibiting distinct and specific irone profiles, with *I. pallida* associated with higher levels of cis γ irone and *I. germanica* with cis α irone predominance. (1) However, this view remains insufficient to reliably discriminate botanical or geographical origin. (2)

To further investigate these differences, a comparative analytical study and a genetic distance analysis were conducted on 74 varieties of *I. germanica* rhizomes and 14 *I. pallida* cultivars. Among *I. germanica* samples, 95% showed a higher relative abundance of cis γ irone compared to cis α irone, challenging the commonly accepted cis α predominance. These results highlight the high variability of irone isomeric distribution and demonstrate that the cis α /cis γ ratio is not a robust species-specific marker.

Genetic distance analyses revealed that samples referred to as “Moroccan *I. germanica*” likely correspond to hybrid populations between *Iris pumila* and *I. germanica*, suggesting a complex botanical origin. In addition, enantiomeric profiling of irones proved to be a powerful tool for confirming botanical origin and deserves a deeper investigation.

Differentiating iris species requires a multi-disciplinary approach, combining both genetic and analytical fingerprinting of irones enantiomers and isomers. (3)

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PP1-5. Volatile Profile and chemotypic characterization of essential oil from *Artemisia annua* L.

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Keywords: *Artemisia* ketone, Borneol-rich chemotype, Oxygenated monoterpenes, Sabinene, Sweet wormwood.

Artemisia annua L. is an annual medicinal plant from the family Asteraceae widely recognized as the primary natural source of artemisinin, a sesquiterpene lactone extensively used in antimalarial therapy and investigated for its anticancer potential [1]. In addition to artemisinin, the plant is known for its volatile metabolites, whose composition may vary depending on geographical origin, developmental stage, environmental conditions, and genetic background. Such variability may significantly influence the biological and aromatic properties of the plant [2].

In the present study, essential oil obtained from dried leaves of *A. annua* was isolated by hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia method 2.8.12, and its volatile composition was analyzed using an optimized GC/MS method.

GC/MS analyses resulted in the identification of 21 compounds, representing 95.8% of the total composition. The analyzed sample was predominantly composed of oxygenated monoterpenes, with borneol (17.92%), sabinene (15.65%), 1,8-cineole (10.74%), α -pinene (9.98%), and camphor (9.05%) identified as the major constituents. *Artemisia* ketone (6.15%), a characteristic compound commonly detected in *Artemisia* species, was also present in a notable proportion. Furthermore, several sesquiterpenes, including caryophyllene oxide (5.34%), E-caryophyllene (2.52%), germacrene D (1.26%), and spathulenol (1.21%), were detected in lower amounts.

The predominance of oxygenated monoterpenes together with the presence of sesquiterpene derivatives indicates notable pharmacological and industrial potential of the investigated essential oil. The detected volatile profile suggests possible antimicrobial, antioxidant, anti-inflammatory, and aromatic properties, supporting its potential application in pharmaceutical, cosmetic, and food-related products.

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PP1-6. GC/MS Profiling of Lemon Verbena (*Aloysia citrodora*) Essential Oil Revealing an Oxygenated Sesquiterpenes Rich Chemotype

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Keywords: *Aloysia triphylla*, *Verbena triphylla*, *Lippia citriodora*, terpenes, volatile compound

The growing interest in natural products as sources of biologically active compounds has increased scientific attention toward medicinal and aromatic plants. In this context, the essential oil of Lemon verbena was selected for phytochemical investigation due to its reported antioxidant, anti-inflammatory, and antimicrobial properties associated with its volatile constituents [1,2].

The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus according to the European Pharmacopoeia method 2.8.12. An optimized GC/MS method was applied for characterization of the volatile profile of the essential oil and identification of its major volatile constituents.

GC/MS analysis resulted in the identification of nine compounds, representing the total composition (100%) of the analyzed essential oil. The predominant constituents were spathulenol (34.17%) and caryophyllene oxide (30.97%), indicating the predominance of oxygenated sesquiterpenes in the investigated sample. Other identified compounds included geranial (9.28%), nerolidol (7.06%), neral (6.07%), *ar*-curcumene (6.01%), *E*-caryophyllene (2.56%), limonene (2.07%), and γ -cadinene (1.83%). The obtained results exhibited a chemically distinct volatile profile compared to the commonly reported citral-rich chemotype of *Aloysia citrodora* being characterized by the predominance of oxygenated monoterpenes. [3].

The predominance of oxygenated sesquiterpenes highlights the natural variability in essential oil composition and suggests the potential biological and pharmacological relevance of the investigated essential oil for further pharmaceutical, cosmetic, and aromatherapeutic applications.

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PP1-7. Respiratory Infection Risk in Parkinson's Disease: Potential Local Antibacterial Relevance of Sweet Orange Essential Oil

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Keywords: *Citrus sinensis* (L.) Osbeck, (+)-limonene, GC-MS, MRSA, *P. aeruginosa*

In Parkinson's disease (PD), dysphagia, reduced mucociliary clearance, and aspiration increase the clinical significance of nasal and upper respiratory tract pathogens. In such cases, colonizing bacteria may easily enter the lower respiratory tract through aspiration. This may ultimately lead to aspiration pneumonia, which is considered one of the leading causes of mortality in patients with PD [1]. Sweet orange essential oil (SEO) and its major component, limonene, possess multifactorial bioactive properties. Our research group has previously demonstrated their *in vitro* antioxidant, anti-inflammatory, and anti-apoptotic activities, and modulatory effects [2]. Based on these findings, we are currently developing a nasally applicable solution that may be relevant not only for neuroprotective purposes, but also for its local antibacterial and antibiofilm effects. Methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* can regularly cause respiratory tract problems [3]. The quality of the SEO is a key aspect of this study. The exact composition was determined using an Agilent 7890B gas chromatograph coupled to an Agilent 7010B triple-quadrupole mass spectrometer. Chiral analysis was carried out on the same GC-MS, applying a Supelco β -DEXTM 120 column (Merck Kft., Hungary). The main components of the SEO were (+)-limonene (91%), myrcene (3.23%), and α -pinene (1.82%). The minimum inhibitory concentration (MIC) of SEO and limonene will be determined spectrophotometrically at 600 nm (results will be expressed in mg/mL). Planned biofilm degradation assays will be performed using samples at MIC/2 concentration. Following crystal violet staining, absorbance will be measured spectrophotometrically at 595 nm (results will be expressed in % of inhibition rates). In the case of significant antibacterial or antibiofilm activity, further studies may support the development of an essential oil-containing formulation, which may contribute to reducing the risk of respiratory infections in PD.

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PP1-8. Development of sustainable alternatives to plant protection products

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Keywords: *Achillea ligustica*, *Lippia citriodora*, *Eucalyptus polybractea*, *Myrtus communis*, secondary metabolites, allelopathy, GC-FID, GC-MS

The future of agriculture, particularly in the plant sector, is moving toward smart and sustainable production systems capable of achieving high performance without relying on synthetic pesticides. Within this context, aromatic plants native of the Mediterranean basin represent a promising source of bioactive compounds. Ligurian yarrow (*Achillea ligustica*), lemon verbena (*Lippia citriodora*), cryptone eucalyptus (*Eucalyptus polybractea*), and Corsican myrtle (*Myrtus communis*) are especially notable for their richness in secondary metabolites with biological activity.

This study explores the potential of these species, all present in Corsica, for crop protection through the development of innovative, plant-based biostimulants. Essential oils were chemically characterized using hyphenated techniques (GC-FID, GC-MS). Major terpenoid compounds identified included p-cymene, 1,8-cineole and spathulenol (*E. polybractea*), santolina alcohol and camphor (*A. ligustica*), α -thujene and 1,8-cineole (*M. communis*), geranial, limonene, and neral (*L. citriodora*).

Allelopathic potential was evaluated through germination and growth bioassays. The results revealed strong phytotoxic effects, with growth inhibition ranging from –100% to –30.27% in leek and radish. In contrast, *M. communis* essential oil exhibited a stimulatory effect on radish root growth, reaching an increase of 28.49%.

Overall, these results highlight the ecological significance of these Corsican species and open up new possibilities in the development of natural, environmentally friendly bioherbicides.

PP1-9. Comprehensive GC×GC–TOFMS for the Quantitative Determination of 57+ Regulated Fragrance Allergens

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Keywords: Contact allergens, GCxGC, TOFMS, labelling requirements

Fragrances are widely used in perfumes and cosmetic products to impart characteristic and appealing scents. However, many formulations contain fragrance allergens that may provoke contact allergies and skin irritation. The adoption of Commission Regulation (EU) 2023/1545 (26 July 2023), amending Regulation (EC) No 1223/2009, significantly expands mandatory labelling requirements by increasing the number of regulated fragrance allergens to 57+ compounds. This regulatory update imposes substantial analytical demands on flavour and fragrance laboratories, particularly with respect to sensitive, selective, and high-throughput quantification in complex matrices.

Conventional methodologies for allergen determination are predominantly based on dual one-dimensional gas chromatography mass spectrometry (GC-MS) workflows employing orthogonal stationary phases (apolar × mid-polar). While effective in mitigating coelution phenomena, this approach necessitates multiple injections and extensive post-acquisition data reconciliation, limiting throughput and increasing analytical uncertainty. The addition of additional analytes and more structural isomers further aggravates chromatographic co-elution and impairs accurate quantification.

To overcome these limitations, a comprehensive two-dimensional gas chromatography approach coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) was developed. This technique provides enhanced peak capacity through orthogonal separations, increasing peak capacity and enabling superior resolution within a single analytical run. Method implementation was carried out using the LECO Pegasus BTX4D platform, offering high spectral acquisition rates, and advanced deconvolution algorithms. The system demonstrates high sensitivity, robust repeatability, and an extended linear dynamic range suitable for trace-level quantification in complex cosmetic matrices.

Importantly, the system complies with International Fragrance Association (IFRA) recommendations by employing orthogonal stationary phases. Overall, the GC×GC–TOFMS workflow significantly improves analytical efficiency, reduces sample handling, and enhances confidence in quantification of the new expanded contact allergen list, providing a future-proof solution for regulatory compliance.

PP1-10. New advances in the chemical characterization of the Corsican liverwort *Radula complanata*

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Keywords: *Radula complanata*; bibenzyls; allelopathic activity.

Bryophytes, particularly liverworts, are known to produce a wide diversity of secondary metabolites, including terpenoids and aromatic compounds [1]. However, their chemical composition remains largely underexplored [2]. Among them, the genus *Radula* exhibits a distinctive chemical profile mainly characterized by bibenzyl derivatives and related phenolic compounds.

In the present study, the chemical composition of the essential oil of *Radula complanata*, a liverwort species collected in Corsica, was investigated for the first time. The identification of components was carried out using a combination of retention indices and mass spectra obtained on both polar and apolar columns, compared against in-house and commercial libraries. Compounds matched with authenticated standards from the in-house library were considered unequivocally identified, while those identified solely by commercial library database required further confirmation. To achieve unambiguous identification and complete structural elucidation, selected fraction obtained by column chromatography were subjected to comprehensive NMR analyses.

A total of 57 compounds were identified, accounting for 83.2–86.2% of the total oil composition. The main constituents were 3-methoxybibenzyl, δ -cuparenol, and several cuparane-type derivatives, such as 1,2-cuparenediol and 2,4-cuparenediol. Notably, this work reports for the first time, the ¹³C NMR data of 2,4-cuparenediol as a natural product.

In addition, the biological potential of the extracts was evaluated through allelopathic assays, which revealed significant phytotoxic activity. These findings suggest that bibenzyl derivatives may play a key ecological role, although other terpenoid and phenolic compounds likely contribute to the observed effects.

Overall, this study provides new insights into the chemical diversity of *R. complanata* and highlights liverworts as promising sources of structurally diverse and biologically active secondary metabolites.

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PP1-11. Decoding Pink Pepper Complexity Through a Multidisciplinary Analytical Strategy

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Keywords: Pink pepper, HPTLC, GC-IRMS, chirality, orthogonal methods.

Pink pepper, derived from the berries of *Schinus terebinthifolius* Raddi and *Schinus molle* L. (Anacardiaceae), has gained increasing attention as a high-value natural ingredient in the flavor and fragrance industry. Being botanically distinct from green, black, and white pepper (all from *Piper nigrum*), pink pepper has a higher chemical complexity [1]. Its compositional variability is strongly influenced by geographical origin, with some sources perceived as premium due to their distinctive sensory and characteristic metabolomic profiles. Therefore, the discrimination of both geographical origin and extraction process is critical for accurate quality assessment, compositional characterization, and product valorization.

To address this challenge, the present study aimed to establish a robust and comprehensive analytical strategy for the differentiation of 40 pink pepper samples from Brazil, Madagascar, Réunion, South Africa, and Mauritius based on their geographical provenance and extraction history. To this end, a multidisciplinary approach combining chromatographic, isotopic, and spectroscopic techniques was implemented:

- Gas Chromatography-Mass Spectrometry (GC-MS) fingerprinting, enabling the capture of global volatile profiles and highlighting major compositional variability and anomalies.
- Gas Chromatography-Isotope Ratio Mass Spectrometry (GC-IRMS) was employed to investigate stable isotope signatures ($\delta^{13}\text{C}$, $\delta^2\text{H}$), exploring potential insights into geographical origin, extraction processes, and atypical profiles.
- Enantioselective-Gas Chromatography (Es-GC) was applied to assess the enantiomeric distribution of selected terpenes, as these ratios are tightly controlled in natural products and may deviate in the presence of non-natural inputs.
- High-Performance Thin-Layer Chromatography (HPTLC) and Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS), targeting non-volatile constituents, provided a rapid assessment of sample variability and enabled differentiation between extraction processes using characteristic marker identification.

Overall, this study highlights the importance of a multidisciplinary analytical workflow for the characterization of complex natural ingredients. The integration of complementary analytical techniques enables more comprehensive sample characterization, underscoring the value of orthogonal methodologies in overcoming the limitations of single-technique approaches and addressing the intrinsic complexity of natural products.

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PP1-12. Development of plant-based alternatives to synthetic pesticides for controlling *Tapinoma magnum*

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Keywords: *Tapinoma magnum*, Corsican flora, plant-based insecticide, biocontrol.

The control of insect pests, particularly the invasive ant *Tapinoma magnum*, which poses a significant threat to Corsican agriculture, represents a major challenge in the context of developing sustainable and environmentally friendly farming systems (1). In response to the environmental and health issues associated with the widespread use of synthetic insecticides, the development of natural alternatives has become a priority (2). In this context, we propose to develop an insecticidal formulation based on plant extracts from insular flora.

An extract library comprising more than 200 samples -including essential oils, hydrosols and hydroalcoholic extracts from 71 plant species- was established and subjected to rigorous biological screening. A metabolomics approach, combining GC-MS chemical profiling and multivariate analysis, was used to correlate chemical composition with biological activity. Bioassay data were ranked using a scoring system to identify the most effective extracts, with results currently under intellectual property protection. Bio-guided chromatographic fractionation will enable the isolation and identification of active compounds.

The most promising extracts will be formulated for physicochemical stability and tested under controlled and field conditions. This interdisciplinary project aims to deliver a stable and effective insecticidal formulation prototype, with potential application against other invasive species.

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PP1-13. Variability of thujones content in the essential oil of cultivated accessions of *Achillea erba-rotta* subsp. *moschata*

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Keywords: Asteraceae, thujones, toxicity, camphor, caryophyllene derivatives.

Achillea erba-rotta subsp. *moschata* is a perennial species of the Asteraceae family, endemic of the Central Alps, occurring on siliceous substrates in alpine and subalpine habitats. Traditionally used in human and veterinary folk medicine and as an aromatic plant, it is nowadays mainly employed as an ingredient of several commercial liqueurs.

Since no cultivated varieties occur, following a first study on natural populations of the species [1], an open field cultivation trial comparing different accessions was established. High variability in the essential oil composition resulted among the investigated accessions. Among oxygenated monoterpenes, the main class of detected compounds, camphor (24% on average) and *cis*- and *trans*- thujones were the most abundant compounds, these last ones varying from 0% to 71% and 67%, respectively, followed by 1,8-cineol (6%). Among sesquiterpenes, the group of caryophyllenes, including caryophyllene and its oxygenate derivatives, were quoted up to 30% of the total oil.

Due to the well-known toxicity of thujones for human consumption [2], the results obtained in this study assume a particular importance with the aim to select plants with a lower content of thujones, in the perspective of starting a breeding program for the obtainment of an envisaged cultivar.

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PP1-14. Aroma Profiling and Molecular Characterization of Ester-Rich Volatiles in the Improved Red Pepper Variety “Kogarashi”

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Keywords: Kogarashi, pepper, *Capsicum annuum* CH-19 sweet, branched-chain esters

“Kogarashi” is a commercial red pepper variety developed in Japan by crossing the non-pungent *Capsicum annuum* CH-19 sweet [1] with either Bhut Jolokia or Habanero. Although it has almost no spiciness, it possesses a strong and “exotic” aroma that is expected to enhance the flavor of various dishes. In this study, we analyzed the aroma of “Kogarashi” and characterized its volatile composition.

Four commercially available types of “Kogarashi” peppers were used for aroma analysis. Freeze-ground fruit peels were suspended in saturated sodium chloride solution, and 1-ethylcyclohexanol was added as an internal standard. Aroma compounds were extracted using headspace solid-phase microextraction (HS-SPME) and analyzed by GC–MS. The GC–MS data obtained from each sample were subjected to comprehensive and comparative analysis. To identify aroma-contributing components, “Kogarashi” from the orange Habanero variety was soaked in diethyl ether, and the aroma compounds were extracted. The extract was then subjected to solvent-assisted flavor evaporation (SAFE) to isolate the volatile fraction. After distillation, the obtained aroma constituents were analyzed by GC–MS/olfactometry.

GC–MS chromatograms from all varieties revealed that esters were the most abundant group of compounds, accounting for approximately 70–80% of the total peak area. Furthermore, branched-chain esters were the predominant ester subgroup. Based on these results, we concluded that branched-chain esters contribute substantially to the aroma of “Kogarashi”. Principal component analysis and volcano plots indicated that “Kogarashi” from the orange Habanero and red Bhut Jolokia varieties contained significantly higher levels of volatile compounds compared with the other varieties. Therefore, these two varieties were selected for GC–MS/Olfactometry. Aroma extract dilution analysis (AEDA) showed that several branched-chain esters were the most potent aroma-active compounds.

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PP1-15. Identification of Key Aroma Compounds in Essential Oils Produced from Bitter Orange Flowers

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Keywords: *Citrus aurantium*, Orange flower absolute, Neroli oil

Bitter orange (*Citrus aurantium*) flowers are the source of two commercially important essential oils — Orange flower absolute and Neroli oil — both widely used in fragrance and flavor applications. Despite sharing major constituents such as linalool and oxygenated monoterpenes [1], the two oils possess distinctly different odor profiles. The compounds responsible for these differences have not been systematically identified. This study aimed to elucidate the key odor-active compounds of both oils, which were then compared with those of fresh bitter orange flowers.

Orange flower absolute and Neroli oil (both of North African origin) were analyzed by GC-MS/FID and AEDA (aroma extract dilution analysis). Fresh bitter orange flowers cultivated at the R&D Center of T. HASEGAWA in Kawasaki, Japan, were analyzed by dynamic headspace sampling followed by AEDA.

GC-MS/FID analysis of both oils confirmed linalool as the dominant component, but revealed markedly different monoterpene profiles: Orange flower absolute contained 68% oxygenated monoterpenes and 4% monoterpene hydrocarbons, while Neroli oil contained 46% and 43%, respectively. AEDA identified linalool, geraniol, methyl anthranilate, 2-phenylethanol, geranyl nitrile, and 2-isobutyl-3-methoxypyrazine as high FD (flavor dilution) factor components common to both oils. The first four were also prominent in fresh flowers, establishing them as the backbone of bitter orange flower aroma. Compounds with elevated FD factors unique to Orange flower absolute, included 5-vinylguaiaicol, guaiaicol, phenylacetic acid, vanillin, and raspberry ketone, collectively imparting a rich, sweet character. Neroli oil was distinguished instead by geranyl acetate, (*E*)-ocimene, myrcene, α -pinene, and (1*E*,3*E*)-4-methyl-1-(prop-1-en-2-yloxy)hexa-1,3,5-triene [2], contributing a fresher, more citrus-green note. These findings clarify the compounds responsible for the characteristic aroma profiles of Orange flower absolute, Neroli oil, and fresh bitter orange flowers.

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PP1-16. Noninvasive and High-Throughput Structure Determination of Trace Natural Products in Plants by Combining the Crystalline Sponge Method with Headspace Sampling

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Keywords: Crystalline sponge, Single-crystal X-ray diffraction, Absolute configuration, enantiomer, Headspace, Structure determination

The crystalline sponge (CS) method [1] is an innovative structure-elucidation technique because it has expanded the applicability of single-crystal X-ray diffraction (SCXRD) to liquid samples without requiring crystallization. In this method, porous metal–organic framework (MOF) single crystals are used to accommodate target analytes within their periodically arranged pores via host–guest interactions. As a result, both the MOF and the guest molecules become periodically ordered, enabling the structural analysis of the guest molecules by SCXRD. Another important feature of the CS method is that only a few micrograms is required for structure investigation. This suggests that the CS method coupled with noninvasive headspace sampling (HS-CS) enables structure determination of natural products from previously inaccessible samples of limited availability, rare and precious, and even endangered species.

In this report, we applied the HS-CS method to analyze the structure of trace components in living natural flowers and trees. For the collection of volatile components, a film bag was first gently placed over the sample and adjusted to avoid mechanical stress. Subsequently, a MonoTrap RCC (GL Sciences) sorbent, suspended with wire, was positioned near the sample inside the film bag for at least 24 h to adsorb volatiles. Volatiles adsorbed on the sorbent were transferred to a preparative gas chromatography (GC) using a thermal desorption system for isolation of the target components. The isolated compounds were soaked into the CS at 26–50 °C for at least three days to allow the guest molecules to be accommodated within the CS.

Headspace volatiles from *Chamaecyparis lawsoniana* ‘Silver star’ were collected, and ca. 6 µg of the major component was isolated via a single preparative GC run. The molecular structure of 5,5,9,13-tetramethyltetracyclo[11.2.1.0^{1,10}.0^{4,9}]hexadec-14-ene (hibaene) was subsequently determined by the CS method. Furthermore, the Flack parameter was sufficiently small, allowing unambiguous assignment of the absolute configuration of hibaene as the (1*S*,4*S*,9*S*,10*R*,13*R*)-form. Structure determination of volatile compounds from other plant species was also performed using this workflow and will be presented.

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PP1-17. Chemical Composition of Essential Oils of *Heracleum* Species

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Keywords: *Heracleum argaeum*, *Heracleum platytaenium*, *Heracleum sphondylium* ssp. *Ternatum*

Objective: The genus *Heracleum* L. (Apiaceae) is taxonomically significant due to its high species diversity and broad geographical distribution. It comprises approximately 125 taxa, mainly concentrated in Asia. In Türkiye, 23 *Heracleum* taxa have been documented, nine endemics, corresponding to a noteworthy endemism rate of 41%. Several *Heracleum* species have long been utilized in traditional medicine and folk practices and are commonly known by regional names such as Tavşancıl Otu and Herkül Otu [1,2].

Methods: In this study, the aerial parts of *Heracleum argaeum* Boiss. & Balansa, *H. platytaenium* Boiss., and *H. sphondylium* L. subsp. *ternatum* (Velen.) Brummitt were collected from Kayseri (for the first two taxa) and Konya (for the third), Türkiye. The voucher specimens were deposited under the herbarium numbers S. DOĞU 3037, 3038, and 3036, respectively. Essential oils were extracted by hydrodistillation using a Clevenger-type apparatus for 3 hours and subsequently analysed by gas chromatography with FID and MS.

Results: A total of 44, 55, and 60 volatile constituents were identified in the essential oils of *H. sphondylium* subsp. *ternatum*, *H. platytaenium*, and *H. argaeum*, respectively. The oil of *H. sphondylium* subsp. *ternatum* was dominated by octyl acetate (53.6%), octyl butyrate (18.5%), and myristicin (7.9%). In *H. platytaenium*, hexyl butyrate (40.8%), and octyl acetate (13.7%) were the major constituents. The essential oil of *H. argaeum* was characterized by a more diverse profile, with α -pinene (15.0%), β -pinene (9.0%), limonene (8.9%), hexyl butyrate (8.3%), bornyl acetate (7.8%), and (*E*)- β -ocimene (6.1%) as the predominant compounds.

Conclusions: The chemical compositions of the studied *Heracleum* species showed significant interspecific variability. Aliphatic esters predominated in *H. sphondylium* subsp. *ternatum* and *H. platytaenium*, while *H. argaeum* exhibited a distinct dominance of monoterpene hydrocarbons. These chemotypes' distinctions reinforce the taxonomic value of volatile metabolite analysis within the genus.

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PP1-18. Aroma Profiling of Okinawan Citrus Fruits Based on Structurally Related Volatile Compounds

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Keywords: Okinawan citrus fruits, aroma characteristics, structural similarity, complex odor

Okinawa harbors diverse citrus fruits, including local and traditional varieties with distinctive aromatic characteristics. In this study, five Okinawan citrus fruits, Shiikuwasa (*Citrus depressa*), Tarogayo (*C. tarogayo*), Kabuchi (*C. keraji* var. *kabuchii*), Tanibuta (*C. ryukyuensis*), and Otoh (*C. oto*) were examined to clarify the relationship between volatile composition and aroma characteristics.

The aroma profile of Shikuwasa differs from that of Okinawan lemons (*C. limon*). To interpret these differences, the volatile compounds were classified into structurally related groups, and the compositional features of each group were compared. Acyclic monoterpene aldehydes are characteristic of lemons, whereas acyclic monoterpene esters were mainly detected in Shikuwasa. Marked differences were also observed between the groups, such as monocyclic aromatic compounds and coumarins. These results indicate that the aroma characteristics of citrus fruits are determined not only by major constituents, such as limonene, but also by combinations of minor volatile compounds within structurally related groups.

Comparative profiling was conducted for Tarogayo, Kabuchi, and other citrus fruits, including Sudachi (*C. sudachi*), lemon (*C. limon*), and Unshu mandarin (*C. unshiu*). Although limonene was the predominant compound in many samples, variations in minor constituents appeared to contribute substantially to their characteristic aromas. In addition, Tanibuta (*C. ryukyuensis*), a wild Ryukyuan citrus species considered ancestral to several traditional Ryukyuan and Japanese mandarin-type citrus fruits [1], was compared with related species, Shikuwasa and Tachibana (*C. tachibana*), as well as Otoh, whose lineage remains uncertain. Eight volatile compounds were detected in Tanibuta, Shikuwasa, and Tachibana. Aromatic monoterpenes, particularly thymol-related components, have been suggested as useful indicators of relatedness to Tanibuta. Otoh contains all eight common components, indicating a potential relationship with Tanibuta. Comparisons with ten additional citrus fruits further suggested that Sudachi may share volatile characteristics with Tanibuta. These findings demonstrate that structure-based volatile profiling is useful for understanding aroma diversity and potential chemotaxonomic relationships among Okinawan citrus fruits.

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PP1-19. Influence of storage duration on essential oil composition in ground and unground lemon thyme

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Keywords: *Thymus* × *citriodorus*, raw material grinding, oxygenated monoterpenes, geraniol, nerol, geranial, neral, germacren-D-4-ol

Lemon thyme (Lamiaceae, *Thymus*), which produces an essential oil with a pleasant lemon scent and taste, is often used to make medicinal or nutritional teas. In the production of teas in disposable bags, the plant raw material is ground. However, grinding can increase the volatility of the compounds that make up the essential oils, especially when the ground raw material is stored for a longer period. Therefore, the aim of this work was to evaluate the effect of the storage duration of ground and unground lemon thyme raw material on the amount and chemical composition of the essential oil.

Lemon thyme was collected at the bloom phase, dried at room temperature, and divided into two groups: one was ground, while the other remained unground and served as the control. The raw materials thus prepared were stored under the same conditions at room temperature. To evaluate changes in essential oil composition during storage, essential oils were extracted by hydrodistillation from ground and unground samples at 7 times every 4 weeks, and after 24 months, and analysed by GC-MS.

The results showed that the essential oil percentage in both groups of raw materials decreased over time. While the grounding of the raw material did not have a statistically significant effect on the percentage of lemon thyme essential oil, its content throughout the study was higher in the ground raw material: the essential oil percentage in the ground lemon thyme was on average $0.58 \pm 0.03\%$, and in the unground one, $0.64 \pm 0.03\%$. The percentage of the most abundant group of compounds – oxygenated monoterpenes – was higher in the essential oil of unground lemon thyme ($71.73 \pm 1.04\%$) throughout the experiment and significantly different from the percentage in the essential oil of ground lemon thyme ($68.46 \pm 0.45\%$). With the increase in storage, the percentage of oxygenated sesquiterpenes in both types of raw materials decreased, while the percentage of oxygenated monoterpenes increased. The main compounds of the essential oils of ground and unground lemon thyme were monoterpene alcohols geraniol and nerol, aldehydes geranial and neral, and sesquiterpene alcohol germacren-D-4-ol. Only geranial percentage differed significantly between the two types of raw materials: the average percentage of geranial in the essential oil of unground raw material was 1.42% higher than in ground one. In both types of raw materials, the isomers geraniol and nerol showed an increasing trend with time, whereas germacren-D-4-ol showed a decreasing trend.

PP1-20. Diversity of Aroma Profiles in Kuromoji (*Lindera umbellata*) Leaves from Oguni, Yamagata, Japan

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Keywords: *Lindera umbellata*; Kuromoji; intra-regional variation; volatile profile; DHS-TD-GC/MS; chemotype; linalool/1,8-cineole ratio

Lindera umbellata Thunb., commonly known as Kuromoji, is an aromatic shrub native to Japan and valued for its characteristic woody, floral, and spicy aromas. Previous chemotaxonomic studies have shown marked geographical variations in the leaf terpene composition of Kuromoji and related *Lindera* taxa, classifying them into linalool-, carvone-, caryophyllene-, and cineole-rich types [1]. More recently, individual-level variations in major terpenoids, including linalool and 1,8-cineole, in native *L. umbellata* populations from the same region have been reported [2]. In Oguni, Yamagata Prefecture, Japan, Kuromoji is locally harvested for essential oil production; however, producers have recognized that the aroma quality differs substantially among collection areas within the town. In this study, we investigated the intra-regional diversity of Kuromoji aroma profiles using essential oil compositional analysis and dynamic headspace–thermal desorption–gas chromatography/mass spectrometry (DHS-TD-GC/MS) of leaves collected from different districts of Oguni.

The volatile profiles consisted mainly of monoterpenes and oxygenated monoterpenes, including linalool, 1,8-cineole, limonene, α -pinene, terpinen-4-ol, α -terpineol, and carvone-related compounds. Hierarchical clustering analysis revealed that the Oguni samples did not form a single homogeneous group but were separated into several clusters. Samples exhibiting a high linalool/1,8-cineole ratio formed a group, whereas others were positioned near the carvone-rich reference type or formed distinct clusters with different compositional balances. Principal component analysis supported this separation, indicating that the relative proportions of linalool-, 1,8-cineole-, and carvone-related components were the major factors contributing to the observed diversity. To clarify the chemical positioning of the Oguni samples, they were compared with representative chemotype profiles reported by Hayashi and Komae and with external Kuromoji samples from Iwaizumi, Iwate Prefecture.

The results indicate that Kuromoji from Oguni is not chemically uniform and comprises multiple aroma profile types on a local scale. The combination of essential oil analysis, DHS-TD-GC/MS, and multivariate analysis is useful for evaluating raw material quality, selecting collection areas, and developing region-specific Kuromoji fragrance products.

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PP1-21. Chemical Composition of the Essential Oil and Volatile Aroma Profile of *Campomanesia phaea* (O. Berg) Landrum Fruits

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Keywords: *Campomanesia phaea*, Myrtaceae, fruit volatiles, sesquiterpenes, aroma profile

The fruits of *Campomanesia phaea* (cambuci) are prized for their aroma and use in artisanal drinks. While past research has mostly addressed leaf essential oils or fruit nutrition [1,2], little is known about the fruit's own essential oil and volatile compounds. This study provides a first detailed analysis of the chemical profile and VOCs released by cambuci fruit. Essential oil obtained by hydrodistillation of ripe fruits yielded 0.2% (m/m) and was analyzed by GC–MS [3], revealing at least 48 constituents. Sesquiterpenes dominated the oil ($\approx 85\%$), comprising 57% hydrocarbons and 28% oxygenated derivatives. Major components included viridiflorene (10.5%), 8-cadinene (7.0%), γ -muurolene (5.7%), α -cadinol (5.5%), β -caryophyllene (5.0%), (E)- β -ocimene (3.8%), α -humulene (3.9%) and trans-cadina-1(6),4-diene (3.6%). Volatile emissions from fruits at early and full maturity were collected by SPME and analyzed by GC–MS. Forty-five compounds were detected in early-stage fruits and 29 in fully ripe fruits. Sesquiterpene hydrocarbons predominated in both stages (77% and 67%, respectively), with viridiflorene as the major early-stage compound (14.3%) and β -caryophyllene as the major ripe-fruit constituent (12.5%). Monoterpene contribution increased markedly during ripening (from $\sim 2\%$ to $\sim 18\%$), while fatty acid esters remained minor components, with hexyl acetate as the main ester (2.8–5.6%). These findings demonstrate that the fruit aroma is strongly shaped by sesquiterpene hydrocarbons and that ripening significantly alters the volatile profile. Differences between essential oil composition and emitted aroma are primarily attributed to esters not recovered by hydrodistillation.

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PP1-22. Chemical composition and allelopathic activities of Apiaceae and Asteraceae Essential oils from Corsica

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Keywords: Allelopathy, Plant-based herbicide, Apiaceae, Asteraceae

The reduction of chemical inputs is a major challenge for 21st-century agriculture. Pesticides and phytochemical substances presented negative impact on environment and human health, that stimulated for alternative sustainable and eco-friendly alternatives [1]. In this context, we investigated the chemical composition and allelopathic activity of twelve essential oils obtained from on nine Apiaceae and three Asteraceae species native to Corsica. The aim was to investigate their potential as plant-based alternatives.

GC and GC/MS analyses revealed diverse chemical profiles. Essential oils from the aerial parts of *Daucus carota* and *Foeniculum vulgare* were characterized by high levels of phenylpropanoids (46% and 40%, respectively), whereas *Crithmum maritimum* and *Ammoides pusilla* were rich in thymol derivatives (12% and 25%, respectively). *Bupleurum fruticosum* and *Laserpitium halleri* ssp. *cynapiifolium* were dominated by monoterpene hydrocarbons (92% and 62%, respectively). In contrast, *Peucedanum paniculatum*, *Achillea ageratum*, *A. ligustica*, and *A. maritima* were characterized by a predominance of irregular monoterpenes (56% ; 40% ; 22%, 58% respectively). Notably, the essential oils of Corsican *A. pusilla* and *L. halleri* are described here for the first time.

Antigerminative assays were performed on leeks and radishes. Leeks were more sensitive to the treatments, as all essential oils exhibited inhibitory effects (77.3-98.3%). In contrast, radishes showed a more heterogeneous response. While most essential oils showed significant inhibitory effects (34.8–96.3% inhibition), *Bupleurum fruticosum* oils promoted plant growth with +14.75% relative to the control.

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PP1-23. Indication of immunomodulatory effect of a phytogenic feed additive *in vitro*

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Keywords: immunomodulation, phytogenics, vanillin, cinnamaldehyde, IPEC-J2

The intestinal epithelial barrier represents the first line of defense against threats to animal health, and a balanced immune response is crucial. Phytogenic feed additives (PFAs) have shown the potential to modulate the intestinal epithelial immune response under inflammatory conditions [1]. The PFA Digestarom® (BIOMIN GmbH, which is part of dsm-firmenich) is a blend of essential oils and other phytogenics with vanillin as the main bioactive component and was studied *in vitro* using a model of intestinal epithelial immune response.

Porcine intestinal epithelial cells (IPEC-J2) were pre-incubated for 24 h with medium alone (untreated control) or the extracted feed additive. Cells were stimulated with TNF- α (10 ng/mL, 5h). Cell viability and cytokine production were determined using commercial kits (WST-1, Merck kGaA, Darmstadt; Porcine Luminex® Discovery Assay, R&D systems, Minneapolis, USA). Experiments were performed in triplicate and evaluated statistically (Prism 10, GraphPad, San Diego, CA, USA).

The immunostimulation induced IL-6 (interleukin-6) and GM-CSF (granulocyte-macrophage colony-stimulating factor) levels significantly ($p < 0.05$). The PFA additionally stimulated the release of IL-6 and GM-CSF ($p < 0.05$). In absence of the challenge, cell viability and baseline epithelial immune signaling were not influenced by the PFA as indicated by unchanged WST-1-signal and interleukin-8 levels ($p > 0.05$), respectively.

This suggests that the PFA Digestarom® might not interfere with basal epithelial inflammatory signaling but induces a targeted modulation of the epithelial immune response under inflammatory conditions. The observed cytokine response may be considered favorable in the context of supporting early intestinal immune activation.

To our knowledge, this is the first study reporting GM-CSF secretion by the IPEC-J2 cell line. Further studies, especially on the complex immune system in live animals are required to fully illuminate the immunomodulatory potential of this feed additive.

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PP1-24. Diversity of floral scent components in wild *Rosa* species

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Keywords: genus *Rosa*, species roses

Genus *Rosa* is distributed in the Northern Hemisphere, and there are approximately 150 wild *Rosa* species. Genus *Rosa* is divided into four subgenera, and subgenus *Rosa* is further divided into ten sections. In this study, we analyzed the scent components emitted by 40 wild taxa of subgenus *Rosa* (sect. *Pimpinellifoliae* (6 taxa), sect. *Gallicanae* (3), sect. *Caninae* (3), sect. *Carolinae* (2), sect. *Rosa* (*Cinnamomeae*) (13), sect. *Synstylae* (9), sect. *Chinenses* (2), and sect. *Bracteatae* (2)) and 2 wild taxa of subgenus *Platyrhodon*.

The scents of wild *Rosa* species have been categorized into several types: a typical rose-like scent (Damask rose), an unpleasant sweet and dry scent reminiscent of *Houttuynia cordata* with a citrusy undertone, a floral scent with green notes, a sweet honey-like scent, a medicinal scent without sweetness, a plum blossom-like scent, a sweet spicy scent, a sweet tea-like scent, and a weak sweet scent.

Recently, we have described three new scent components from genus *Rosa*.

2,4-decadienal (2,4-DA) and 2,4-decadienol (2,4-DO) in two species of sect. *Pimpinellifoliae* (including *R. foetida*) and four species of sect. *Rosa*. 2,4-DA has a strong, fried-chicken-like fatty odor, and 2,4-DO has a strong, oily fatty odor; when combined with sweet scent components such as geranyl acetate, they produce an unpleasant odor [1].

Methyl 2,4,6-decatrienoate (2,4,6-DT) in one species of sect. *Rosa*, two species of sect. *Synstylae*, and two species of subgenus *Platyrhodon*. 2,4,6-DT is one of stink bug pheromones and has an unusual odor like an old wooden closet. Trace amount of 2,4,6-DT was contained in *R. multiflora* (sect. *Synstylae*), however, its flowers did not have unpleasant odor. On the other hand, ‘Anne-Marie de Montravel’, a descendant of *R. multiflora*, contained a high proportion of 2,4,6-DT, and had an old wooden closet-like odor [2].

In this presentation, we will not only report on these unusual odors of wild *Rosa* species but also provide detailed information on other pleasant fragrances and show the diversity of fragrances in wild *Rosa* species.

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PP1-25. From Anamur to Alanya: Volatile Signatures of *Salvia viridis* L. from Southern Türkiye

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Keywords: *Salvia viridis*; GC-MS; volatile compounds; chemotaxonomy.

Salvia viridis L. is the only annual *Salvia* species naturally distributed in Türkiye, and its flowering shoots have been traditionally used in Anatolian folk medicine. [1] This study aimed to determine the essential oil composition of *S. viridis* collected from two different localities in southern Türkiye. The samples were collected from Mersin, Anamur, Ermenek road, 10 km, at 298 m altitude on 17 April 2026 (ÖÇ 8279 TE), and from Antalya, Alanya, Bektaş village, at 498 m altitude on 14 May 2026 (ÖÇ 8285). The air-dried aerial parts were hydrodistilled for 3 h using a Clevenger-type apparatus, and the essential oils were analyzed by GC-MS and GC-FID. [2,3]

The identified compounds constituted 98.5% of the Anamur oil and 93.7% of the Alanya oil. The major constituents of the Anamur sample were β -phellandrene (19.2%), β -caryophyllene (15.0%), β -pinene (14.0%), germacrene D (9.0%), limonene (7.3%), caryophyllene oxide (6.2%), and α -humulene (4.9%). In the Alanya sample, β -pinene (17.9%), germacrene D (17.9%), β -caryophyllene (9.1%), caryophyllene oxide (6.2%), δ -cadinene (3.5%), and γ -muurolene (3.0%) were the main compounds. The comparison of the two samples demonstrated marked quantitative differences in their volatile compound profiles. In particular, β -phellandrene and limonene were found at higher levels in the Anamur sample, whereas β -pinene and germacrene D were detected at higher levels in the Alanya sample. These differences suggest that geographical origin, altitude, and collection period may influence the essential oil composition of *S. viridis*. The obtained results contribute to the phytochemical characterization of *Salvia* species distributed in Türkiye and provide useful data for future chemotaxonomic studies.

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PP1-26. Essential oil of *Tagetes lucida* Cav. from a wild population of Guatemala

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Keywords: GC/MS, Phenylpropanoids, *Tagetes lucida*.

The genus *Tagetes* (Asteraceae) comprises a group of herbaceous plants, annual or perennial, native to the Americas, with a primary center of diversification in Mexico and Central America [1]. This genus includes approximately 55 species, seven of which are found in Guatemala, including *Tagetes lucida* Cav., known as “pericón” in Guatemala and México [2]. *T. lucida* is used in the treatment of illnesses of the nervous central system and gastrointestinal diseases, also as in insecticidal applications [3]. The purpose of the study was to determine the composition of the essential oil of *T. lucida* from a wild population of Guatemala to evaluate their potential for oil production regarding its composition and yield. Aerial parts of several individuals of *T. lucida* were collected in October 2024 from a population of the species located in pine-oak forests in Chiché, province of Quiché at the west of Guatemala, at an altitude above 2000 m. The vegetal material was then dried and extracted by hydrodistillation yielding 0.6% of essential oil (EO). The EO was then analyzed by gas chromatography coupled to mass spectrometry (GC-MS) using a HP5 (5% phenylmethylsilicone) column. The EO compounds were identified by their retention indexes compared with those published in the literature. Sixteen compounds were identified, the main components being the phenylpropanoids, estragole (methyl chavicol) (28.3%), anethole (27.3%) and methyleugenol (31.8%), which amounts to almost 90% of the EO composition. The results are consistent with results obtained in other countries, where the same compounds have been reported as major components in the EO of *T. lucida* [4], and similar to the EO of *T. filifolia* from Guatemala, in which trans-anethole and methyl chavicol were the main compounds [5]. The three main compounds found in *T. lucida* EO in this study have applications in the food industry, so it is suggested that further research be carried out on the possible uses of the oil and its biological activity.

PP1-27. Chemotypic variability in terpene and cannabidiol profiles of wild *Cannabis sativa* L. from Bulgaria

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Keywords: *Cannabis sativa*, Wild Hemp, Spontaneous Hemp, Feral Hemp

Wild *C. sativa* populations, shaped by long-term ecological adaptation, represent a potentially valuable reservoir of previously uncharacterized phytochemical diversity. The present study aimed to characterize the terpene and cannabinoid composition of 19 wild Bulgarian *C. sativa* populations. Essential oils (EOs) were isolated by hydrodistillation using a Clevenger-type apparatus, and their chemical profiles were analyzed by gas chromatography–mass spectrometry (GC–MS).

A total of 61 EO constituents were identified, with sesquiterpenes representing the predominant fraction (70.72–95.62%). β -Caryophyllene was the major compound in most samples, ranging from 12.65% to 57.54%. Other sesquiterpenes, including α -humulene, α -selinene, and β -bisabolene, exhibited moderate to high variability, indicating substantial chemotypic differentiation among populations. Monoterpene content showed pronounced heterogeneity, with α -pinene varying from non-detectable levels to 13.98%, suggesting differences in terpene biosynthesis and ecological adaptation. Oxygenated sesquiterpenes, such as caryophyllene oxide and α -eudesmol, occurred at comparatively lower but variable concentrations. CBD levels also varied considerably (0–7.69%), with several samples displaying minimal or undetectable amounts. The observed variation in terpene and cannabinoid composition underscores the extensive phytochemical diversity present within wild Bulgarian *C. sativa* germplasm. In conclusion, these findings demonstrate that wild populations of this species constitute a valuable genetic and biochemical resource with potential applications in breeding, chemotype development, and future studies on the ecological and functional significance of terpene–cannabinoid interactions.

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PP1-28. Authentication of Commercial Copaiba Samples (*Copaifera* spp.) through GC-MS Chemical Fingerprinting of Essential Oils, Physical Characterization, and Chemometric Analysis.

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Keywords: *Copaifera* spp., β -caryophyllene, sesquiterpenes, chemical adulteration.

Copaiba oleoresin is widely recognized as one of the most important non-timber forest products of the Amazon and has attracted substantial interest from the pharmaceutical, cosmetic, and personal care sectors due to its bioactive properties and characteristic aroma [1]. Due to its economic value, the commercial samples of copaiba oleoresin can show adulteration problems. In this sense, 28 samples of oleoresin were purchased in open-air markets and compared to an authentic sample collected in Santarém (Pará, Brazil). The EO chemical composition analyzed by GC-MS identified β -caryophyllene as the most abundant and frequent constituent, ranging from 19.35% to 59.08%. However, some samples contain other sesquiterpenes, specifically α -*trans*-bergamotene, β -bisabolene, α -copaene, and germacrene D as major compounds. Based on Hierarchical Cluster Analysis (HCA), the samples were classified into six groups. Regarding physical properties, the standard sample exhibited a density of 0.96595 g/cm³, a refractive index of 1.51261, and a specific rotation of -0.766° . In contrast, most commercial samples displayed lower densities (0.87522–0.94814 g/cm³) and samples 10, 15, and 16 exhibited higher densities (>0.98 g/cm³). A similar trend was observed for the refractive index, with lower values (≈ 1.47 – 1.48) predominating in several samples. Samples 15 and 16 showed elevated indices (>1.515). The specific rotation displayed the greatest variability (-3.757° to $+0.949^\circ$), highlighting significant differences in the enantiomeric composition of the oils. In general, the total color difference (ΔE^*) between the commercial samples and the authentic standard was classified as distinguishable (4 samples), easily distinguishable (6 samples), or very large (18 samples) according to the classification of the DIN 6174 standard (2007) [2], showing a significant colorimetric variation between the commercial samples and the authentic oleoresin. Overall, the results provide strong evidence supporting the occurrence of adulteration in the commercial copaiba samples.

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PP1-29. Characterization of *Tessaria fastigiata* essential oil from Bolivia

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Keywords: *Tessaria fastigiata*, GC-FID/MS investigation, GC-olfactometry, chiral GC

Bolivia is home to a wide diversity of plants adapted to the diversified geographic characteristics of the country. The Asteraceae shrub *Tessaria fastigiata* (Griseb.) Cabrera, formerly known as *Pluchea fastigiata* Griseb., is part of this flora. We investigated the essential oil from this species as part of an ongoing aromatic survey in Bolivia [1]. Three 1.5-2 kg accessions of the aerial parts were collected in October 2025 near Apote, Villavicencio Avenue and Tolavi River in the Cochabamba valley, and hydrodistilled to yield 0.08-0.11% m/m of essential oils. Methodical examination of their composition by GC-FID and GC-MS with retention indices on two capillary columns, led to the identification of over 230 compounds. The enantiomeric composition of key monoterpenes was assessed using a MEGA-DEX DET Beta chiral column. (*S*)-(-)- β -pinene (12-29% of chromatograms) and sabinene (4-10% of the chromatograms, 17-24% (*R*)-(+)) and 76-83% (*S*)-(-)) dominated the early profile. The main sesquiterpene β -caryophyllene (9-18%) was accompanied chiefly by caryophyllene oxide (6.5-9.5%) but also rarer derivatives such as caryophylladienols I and II (1.3-2.5% total), caryophylla-2(12),6-dien-5-one (0.1-0.3%) and caryophylla-2(12),6(13)-dien-5-one (0.5-1.0%). The mass spectrum of the latter was improperly registered within the Wiley mass spectral database with a prominent *m/z* 135 peak, whereas the original publication reported *m/z* 134 instead [2]. GC-olfactometric examination of subfractions of the essential oils further allowed to notice trace amounts of β -damascenone which majorly contributed to the peary-fruity character of the oil despite going unnoticed on the raw chromatograms.

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PP1-30. Automated solvent-assisted flavor evaporation 2.0 – the new analytical standard for the artifact-avoiding isolation of volatiles from natural products

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Keywords: aSAFE 2.0, volatile isolation, high vacuum, gas chromatography–olfactometry (GC–O).

To characterize the volatiles in natural products using gas chromatography, the prior removal of all non-volatile compounds is an essential prerequisite. Numerous methods that were widely used in the past, such as steam distillation and solid phase microextraction techniques, have since been phased out of high-end labs due to the high risk of thermal artifact formation. Artifacts are a particular problem when the volatiles are to be screened for odorants by gas chromatography–olfactometry: given the enormous range of the odor threshold concentrations for common volatiles, even the formation of trace amounts of artifacts may be sufficient to substantially falsify the outcome [1, 2].

A non-thermal and artifact-avoiding alternative for volatile isolation based on a high-vacuum transfer was first proposed in 1970 by Weurman and his team [3]. The method was further developed into the solvent-assisted flavor evaporation (SAFE) [4], which became a global standard for more than 20 years. In 2022, we automated the SAFE [5], and recently an improved system, the aSAFE 2.0, was launched. The aSAFE 2.0 combines a dropping funnel equipped with an automated valve and a level sensor, a high vacuum transfer unit, and a liquid nitrogen-cooled trap. Solvent extracts of up to 200 mL obtained from natural products can be automatically separated into volatiles and non-volatiles without the need of monitoring. Once the separation is complete, the systems remains in a stable state. Customized settings for the valve open/closed times enable improved yields compared to the manual SAFE, particularly for volatiles with high boiling points from high-fat extracts.

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PP1-31. Diversity and Distribution of Aroma Volatiles in Rosaceae Fruit: A Focus on Japanese Cultivars

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Keywords: *Malus domestica*, *Prunus persica*, *Pyrus pyrifolia*, *Fragaria × ananassa*, comparative aroma profiling, linalool, 4-decanolide, genomic evolution

Aroma is a key determinant of fruit quality and plays a central role in cultivar differentiation. To better understand aroma diversity, we conducted a comparative analysis of multiple cultivars across four Rosaceae crops—apple, peach, pear, and strawberry. Our results demonstrate that variation among cultivars is greater than within species, highlighting the importance of aroma profiles in defining fruit distinctiveness.

Both shared and species-specific volatile compounds were identified. C6 alcohols and their acetate esters were common across all crops, whereas phenylpropene compounds were characteristic of apples and lactones of peaches. Notably, γ -decalactone, which imparts peach- and coconut-like aromas, was also detected in certain pear and strawberry cultivars. In contrast, nicotinic acid esters were frequently found in pear and strawberry cultivars but were absent in apples and peaches.

These findings suggest that the diversity and distribution of aroma compounds within Rosaceae species may have arisen through repeated genetic mutations during evolutionary divergence from a common ancestor. However, a systematic and large-scale integration of aroma profiles with genetic variation has yet to be achieved.

Here, we present an overview of aroma diversity in Rosaceae fruits, with a particular focus on Japanese cultivars. This work provides a foundation for future studies integrating volatile analysis with genomic and transcriptomic data, ultimately contributing to the breeding of fruits with improved flavor quality.

PP1-32. Multidisciplinary evaluation of basil cultivars for pesto industry applications

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Keywords: *Ocimum Basilicum* L., industrial pesto production, HS-SPME-GC-MS

Appreciated for its intense aroma and vibrant green colour, pesto sauce is the second most popular pasta sauce worldwide after tomato sauce [1]. Its growing industrial relevance has led to widespread production of ready-to-use products internationally, requiring strict control of both raw material quality and processing conditions. Increasing demand for basil, the key ingredient, has promoted intensive cultivation practices, including the use of multiple cultivars, expansion of growing areas, and repeated harvest cycles to maximize yield, with potential impacts on raw material quality [2]. At the same time, processing strongly affects final quality, as chlorophylls and bioactive compounds are highly sensitive to heat and low pH, which induce chlorophyll degradation into brown pheophytins, reducing colour quality and affecting consumer acceptability [1]. Despite increasing research on *Ocimum basilicum* L., current knowledge remains fragmented and rarely integrates chemical, sensory, and technological aspects. In this context, basil essential oil compounds such as linalool and eugenol, which exhibit antimicrobial activity, could be carefully monitored and strategically exploited to support preservation typically achieved through acidification and pasteurization, potentially enabling milder processing conditions and reducing losses in colour and aroma quality [3]. Accordingly, detailed chemical characterization of raw materials and its correlation with organoleptic properties is essential to guide cultivar selection and minimize processing-induced degradation. Therefore, this study aimed to correlate sensory and visual properties with the chemical composition of *Ocimum basilicum* L. cultivars grown in different Italian regions across the production season, to achieve an objective quality characterization. Volatile profiles were analysed by HS-SPME-GC-MS, while colour was monitored by spectrophotometry during harvesting, processing, and storage. Agronomic parameters (harvest time, origin, number of cuts, plant morphology, and physiological status) were also recorded. Finally, chemometrics was applied to integrate chemical, sensory, and agronomic data, enabling identification of high-quality raw materials, discrimination of cultivar-specific molecular patterns, and linking of composition to sensory traits for quality and shelf-life assessment.

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PP1-33. Re-examination of the major constituent of Okinawan *Bidens pilosa* L. var. *radiata* essential oil by GC/MS and NMR

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Keywords: *Bidens pilosa* L. var. *radiata*, phenylheptatriyne, polyacetylene

Bidens pilosa L. var. *radiata* is a medicinal plant of increasing interest because *B. pilosa* has been recognized for diverse biological activities, including antioxidant and anti-inflammatory potential [1]. In this study, we analyzed the essential oil of Okinawan *B. pilosa* var. *radiata* and found that a highly retained constituent on a DB-WAX column accounted for more than 50% of the total peak area. Based on earlier knowledge and GC/MS library-based interpretation, this major constituent had initially been assigned as 9-diazo-9*H*-fluorene, in line with the report by Singh et al. [2]. Because this assignment appeared chemically unusual, we re-examined the identity of the principal component by combining analyses on columns of different polarity, isolation of the major constituent, and ¹H NMR measurements.

On DB-5ms, the dominant peak showed a retention index of approximately 1720, whereas on DB-WAX it appeared at a much higher retention index of approximately 2580. After washing the essential oil with cold hexane and cooling the hexane-soluble fraction to –30 °C, yellowish crystals were obtained. GC/MS analysis of the isolated material gave a single dominant peak with the same chromatographic behavior as the major peak in the original essential oil. Re-analysis of the isolated NMR sample on DB-WAX/GCMS again gave a retention index of 2580.

The ¹H NMR spectrum showed an aromatic pattern integrating to approximately five protons together with a methyl signal, supporting a monosubstituted phenyl-containing structure rather than 9-diazo-9*H*-fluorene, which would be expected to show eight aromatic protons. Quantitative GC/MS analysis based on an external calibration curve prepared from the isolated compound indicated that phenylheptatriyne accounted for 67.5 w/w% of the essential oil. Taken together, these results strongly suggest that phenylheptatriyne is the primary component of Okinawan *B. pilosa* var. *radiata* essential oil. This finding is noteworthy because the high abundance of phenylheptatriyne appears unusual.

Acknowledgements: We gratefully acknowledge Ms. Kimie Yogi and Mr. Seiya Yogi of Sashikusa-ya Co., Ltd., Nanjo, Okinawa, for providing *Bidens pilosa* var. *radiata* leaves that were used in this study.

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PP1-34. Use of by-products and sidestreams as source for essential oils

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Keywords: organic side streams, ginger-pulp, orange peels, leek foliage, pre-processing, valorisation

Agriculture and food processing activities produce side-streams that are usually of limited value or that present a cost for the producer. In order to increase the sustainability of these products, their valorization in new applications is important. The RETRO-project (1 October 2025 – 30 September 2027) aims to extract essential oils from a selection of plant-based waste/side-streams that are produced from agricultural activities or from the food processing industry. Based on availability, volume and potential applications, currently 10 side-streams were identified as a potential source of essential oils or other interesting components. These side-streams comprise: ginger-pulp, apple-pulp, orange-peels, leek foliage, tomato foliage, strawberry foliage, spent coffee grounds, Lion's mane substrate, Dahlia cuttings and bramble prunings. These side-streams were collected and either frozen at -20°C or dried by freeze-drying, oven drying (65°C), and fluid bed drying at 30°C. Essential oil and hydrolates were produced using a Clevenger hydrodistillation approach as well as using a sCO₂-extraction approach. Also ethanol extracts were produced. The effect of sonication on essential oil yield is investigated as well in the project. The obtained essential oils, hydrolates, ethanol and sCO₂ extracts were characterized by GC-MS. In this paper we will introduce the RETRO-project and will present the results on yields and composition of essential oils and extracts that have been obtained, with a focus on results obtained for ginger-pulp, orange peels and leek foliage as most attention is being given to these side-streams.

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PP1-35. From Floral Scents to Honeybee Communication: Shared Volatile Compounds and Their Ecological Functions

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Keywords: floral volatiles, honeybee, *Apis mellifera*, plant–pollinator interactions, chemical ecology

Plants emit a wide range of volatile organic compounds (VOCs) that play important ecological roles in defence, stress responses and interactions with other organisms. Floral volatiles are particularly important because they mediate plant–pollinator interactions. As key pollinators, bees rely heavily on olfactory cues during foraging. Numerous studies have shown that honeybees (*Apis mellifera*) are able to recognise and discriminate characteristic floral scent profiles.

Several widespread floral volatiles, including geraniol, ocimene and related monoterpenes, occur not only in floral scent profiles but also in communication systems within honeybee colonies. Geraniol, for example, is a major component of the Nasonov pheromone, which is involved in orientation and recruitment, while β -ocimene functions as a brood pheromone that influences various colony activities and behavioural responses.

Volatile compounds shared between floral scent profiles and honeybee communication systems are examined within a broader chemical-ecological context. In plants, these compounds facilitate the attraction of pollinators and help them locate and recognise floral resources. In honeybees, the same compounds contribute to colony-level communication by transmitting information that influences orientation, recruitment and other behavioural responses. Although these compounds operate in different biological contexts, they perform comparable signalling functions. Their repeated occurrence in both plant–pollinator interactions and honeybee communication highlights the versatility and ecological importance of volatile-mediated signalling across different levels of biological organisation.

PP1-36. What Is a Hydrolate? Rethinking Terminology in Distillation Research

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Keywords: hydrolates, methodological variability, chemical composition, allergen exposure, safety evaluation

Hydrolates or hydrolats (also known as hydrosols, floral waters or aromatic waters) are receiving increasing scientific attention and are studied either as independent products or as co-products of essential oil distillation. However, the term “hydrolate” is used inconsistently in the scientific literature to describe any water fraction obtained using various distillation technologies, which leads to differences in composition. This lack of terminological clarity limits meaningful comparison between studies and complicates the interpretation of analytical, biological and safety-related findings.

Traditionally, hydrolates are defined as aqueous products obtained during the distillation of plant material, either as co-products or as primary products. They are widely used in aromatherapy, cosmetics, perfumery, food, pharmaceutical and phytopharmaceutical industries. In scientific studies, the term “hydrolate” is often applied to aqueous phases obtained under substantially different experimental conditions, such as Clevenger-type distillation with cohobation. While such products provide insight into plant volatiles and distillation processes, they do not necessarily represent hydrolates as produced and used in practice.

Differences in starting plant material and distillation methodology can substantially influence volatile composition, total volatile content and the concentrations of individual constituents, including allergens. Consequently, aqueous distillation products generated under different experimental conditions may not be directly comparable, and their biological or safety-related interpretation should be approached with caution.

In addition, hydrolate studies are commonly reported using relative composition (%) without quantification of major constituents. Absolute concentrations of volatile compounds are essential for the interpretation of biological effects, exposure assessment and safety evaluation. This limitation is particularly relevant when conclusions regarding allergen content are extrapolated to commercially produced hydrolates.

This work encourages discussion on the terminology and characterisation of aqueous distillation products in hydrolate research, particularly regarding production methodology. A rosemary (*Salvia rosmarinus* Spenn.) case study is used to illustrate the practical implications of the challenges outlined above. Improved reporting and explicit differentiation between hydrolates and other aqueous distillation products would enable more meaningful comparison and interpretation of research findings, support future safety assessment and facilitate the development of evidence-based guidance for hydrolates used in practice.

PP1-37. Essential Oil from Needles and Twigs of the European Larch (*Larix decidua* Mill.) from Tyrol

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Keywords: larch, *Larix decidua*, Pinaceae, Tyrol

The European larch (*Larix decidua*) is widely distributed in the Alpine region and represents the only deciduous conifer species occurring in this area. The tree typically reaches heights of up to 46 m and trunk diameters of up to 1.8 m [1]. It is exploited not only for timber but also for its oleoresin, which is traditionally obtained by tapping the trunk and, after further processing, yields the so-called larch turpentine. In addition, an essential oil is obtained by steam distillation of needles and twigs, with yields of approximately 0.2%. The essential oil is produced only in small quantities and is distinct from the volatile fraction of the oleoresin, which is commercially available as larch turpentine oil [2].

For this study, eight industrial batches of *L. decidua* essential oil produced in Tyrol were analysed, covering a period from 2020 to 2024. The essential oil composition was determined by GC-MS and dual-channel GC-FID. Additionally, the enantiomeric distribution of selected monoterpene hydrocarbons was determined by enantioselective GC on a chiral cyclodextrin-based stationary phase.

In total, 58 compounds were identified, accounting for over 96% of the essential oil composition. The main constituents were α -pinene (45.3 – 52.1%), β -pinene (8.0 – 10.0%), δ -3-carene (8.4 – 10.4%), limonene (3.5 – 5.1%) and β -phellandrene (3.2 – 4.9%).

The enantiomeric distribution of the main chiral constituents showed a predominance of the dextrorotatory enantiomer for α -pinene, whereas the opposite enantiomer was dominant for β -pinene and β -phellandrene. For limonene, a relatively high variability in enantiomeric distribution was observed, with no consistent predominance of either enantiomer.

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PP1-38. Development of a Citrus-Based Perfume Formulation and Evaluation of Its Anti-Breast Cancer Activity

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Keywords: Anticancer, *Citrus spp.*, GC-MS, Perfume

Conventional synthetic perfumes may contain chemical ingredients associated with adverse health effects, including potential carcinogenicity and endocrine disruption (Alblooshi, 2025). In contrast, natural perfume formulations prepared from essential oils are considered safer alternatives due to their plant-derived bioactive constituents and lower synthetic chemical content. The present study aimed to characterize the chemical profile of a citrus-based perfume formulation using Gas Chromatography–Mass Spectrometry (GC–MS) analysis and to investigate its potential anticancer activity against MDA-MB-231 breast cancer cells. The perfume formulation was prepared using commercially available essential oils with dominant citrus aromatic characteristics. Chemical characterization of the formulation was performed by GC–MS analysis to identify major volatile secondary metabolites. The cytotoxic activity of the perfume formulation against MDA-MB-231 breast cancer cells was evaluated using the MTT cell viability assay. Cells were treated with different concentrations of the perfume formulation (100, 10, 1, and 0.1 µg/mL) for 24 h, and cell viability was determined spectrophotometrically according to mitochondrial metabolic activity. GC–MS analysis identified 31 different constituents in the perfume formulation. The major compounds were 1,2-propanediol (18.9%), linalool (14.4%), and linalyl acetate (12.0%). The perfume formulation exhibited concentration-dependent cytotoxic activity, as cell viability decreased from 96.8% ± 1.89 at 0.1 µg/mL to 19.5% ± 2.23 at 100 µg/mL. Cell viability values at 1 and 10 µg/mL were determined as 40.6% ± 6.43 and 28.0% ± 2.39, respectively. The observed cytotoxic activity against MDA-MB-231 breast cancer cells suggests that citrus-based perfume formulations enriched with bioactive essential oil constituents may possess additional functional value beyond their aromatic properties. This activity may be associated with the presence of major compounds such as linalool and linalyl acetate, which have previously been reported to exhibit antioxidant and anti-inflammatory effects in various cancer models (Wu et al., 2025). Therefore, the demonstrated anticancer potential may increase both the pharmaceutical and commercial significance of such natural perfume formulations.

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PP1-39. The Invisible Becomes Visible: The Art & Science of Essential Oils

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Keywords: essential oils, scientific illustration, data visualization, aromatherapy, phytochemistry

Essential oils are complex mixtures of volatile compounds that are primarily perceived through olfaction and have been widely utilized across medicine, pharmacology, and aromatherapy for centuries. Despite their long-standing significance, knowledge in this field is often conveyed through fragmented scientific formats, including chromatographic analyses, textual descriptions, and isolated phytochemical or botanical studies, which may hinder a holistic understanding of the interconnections between historical development, plant sources, and chemical composition [2,3]. This study proposes an integrative scientific illustration approach aimed at synthesizing essential oil knowledge into a unified visual framework. A single comprehensive scientific drawing was developed to simultaneously represent five interrelated domains: (i) the historical evolution of essential oils, (ii) the development of aromatherapy practices, (iii) key botanical species, (iv) major geographical production regions, and (v) fundamental molecular structures. The resulting illustration is structured as a visual knowledge map, where temporal progression, botanical diversity, and chemical information are organized within a coherent compositional system. Historical milestones, from early distillation practices to modern analytical techniques, are visually integrated alongside influential figures in aromatherapy. Concurrently, essential oil-producing species such as *Rosa damascena*, *Lavandula angustifolia*, *Citrus sinensis*, and *Iris germanica* are contextualized according to their geographical origins and representative chemical constituents. The methodological framework combines scientific rigor with principles of graphic design, including visual hierarchy, spatial organization, and abstraction. Complex datasets—such as molecular structures and plant morphology—are translated into accessible visual elements while preserving scientific accuracy. This approach responds to the growing need for innovative methodologies in the visualization and communication of natural product research [1]. By integrating multiple thematic layers into a single visual system, this study enhances cognitive accessibility and supports interdisciplinary understanding. The proposed illustration serves as an effective scientific communication tool, demonstrating how complex, multi-dimensional essential oil data can be synthesized within a constrained visual format.

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PP1-40. A Cross-Domain Capability Architecture for Trustworthy AI for Chemical Risk Assessment

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Keywords: High-risk AI systems, Chemical risk assessment, Data provenance, Agriculture, ECETOC, EFSA, PARC / READYAI, Readiness, Standardisation, Metadata, FAIR

In view of the forthcoming adoption of AI systems* used for chemical risk assessment in the European Union, several actors have independently examined the opportunities and barriers to building trust in the use of AI for regulatory purpose. In a comparative analysis of AI-enabled risk-assessment approaches across agriculture, non-institutional chemical risk assessment (ECETOC), institutional risk assessment (EFSA), and the PARC consortium (READYAI), **data provenance** emerged as a non-negotiable requirement for trustworthy AI.

Each actor contributes a distinct capability: agriculture demonstrates mature data-standardisation practices, ECETOC identifies structural gaps and proposes methodological enablers, EFSA operationalises governance and lifecycle oversight, and READYAI defines explicit readiness criteria. Together, these contributions reveal a **cross-domain AI capability architecture** relevant for regulatory decision-making.

Within this **cross-domain AI capability architecture**, **data provenance** is articulated explicitly by READYAI, where it is defined as a core readiness pillar and scoring system requiring traceable data lineage, documented transformations, and transparent uncertainty reporting. The other actors address data provenance implicitly through domain-specific mechanisms: agriculture through mature, standardised data-generation, harmonised field-trial formats, residue-analysis templates, controlled vocabularies, rich metadata capture, and multi-year, multi-location external validation frameworks; ECETOC - through FAIR-aligned ontologies, reproducibility and transparency-oriented documentation templates, uncertainty estimates and applicability-domain checks; EFSA through governance-driven requirements for AI-ready data, traceability, documentation, and lifecycle oversight.

A unified conceptual model of this cross-domain capability architecture is proposed to demonstrate how these four actors collectively provide the structural foundations required to build trust in AI for chemical risk assessment.

*classified as ‘high-risk’ according to the EU AI Act

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PP1-41. The Aromatic Fusion of Essential Oils and Chewing Gum

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Keywords: Oral candidiasis, *Mentha piperita* L, *Warburgia salutaris* (Bertol.f.) Chiov., biofilm, MIC, chewing gum

Oral candidiasis (oral thrush) is a prevalent fungal infection caused by *Candida albicans* [1]. Although miconazole is commonly used and generally effective, it may induce systemic side effects or promote resistance. The essential oil of *Mentha piperita* L., commonly known as peppermint oil, is rich in menthol and methone, the active compounds that disrupt the fungal membrane, leading to cell death [2]. The “pepper bark tree” or *Warburgia salutaris* (Bertol.f.) Chiov., is traditionally used as a medicinal plant, and the leaves' essential oils are rich in myrcene, which has antifungal activity [3]. The aim of this study was to formulate and manufacture an oil-based, film-coated chewing gum containing essential oils from *Warburgia salutaris* and *Mentha piperita* as an alternative, locally targeted antifungal therapy. *Warburgia salutaris* oil was extracted via hydrodistillation, while peppermint oil was commercially sourced; both oils were evaluated for activity against *C. albicans*. Minimum Inhibitory Concentration (MIC) and biofilm inhibition assays were performed to assess antimicrobial efficacy. The combination of peppermint and *Warburgia* oil demonstrated the strongest antimicrobial activity in both assays. Screening of nine pilot batches with varying excipient concentrations and oil ratios yielded a chewing gum with significant *in vitro* antifungal activity, supporting its potential as a localised oral dosage form.

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PP1-42. The essential oil composition and antimicrobial activity of *Hypericum perforatum* L. from Ağrı, eastern part of Türkiye

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Keywords: *Hypericum perforatum*, essential oil, antimicrobial activity

Among the members of Hypericaceae family, *Hypericum perforatum* (St. John's wort) is undoubtedly the most widely recognized species due to its long-standing traditional usage, particularly for antidepressant and wound healing purposes [1]. Today, *H. perforatum* is considered one of the most commercially important herbal medicines worldwide and is available in various pharmaceutical and herbal formulations, including capsules, ointments, and oral drops. In the present study, the essential oil composition of aerial parts from *H. perforatum* were assessed by GC-FID, GC/MS. Along with the essential oil different solvent extracts (*n*-hexane, chloroform, methanol, water) were prepared and their antimicrobial activities were studied against 6 pathogenic bacteria and 7 yeasts using broth dilution method.

Based on the results, the major components of the oil were determined as β -caryophyllene (13.1%), caryophyllene oxide (7.2%), tridecanol (5.8%), hexadecanoic acid (5.1%), spathulenol (5.0%), undecanol (4.1%), phytol (4.0%) β -Selinene (3.7%), and dodecanol (3.6%). As to the antimicrobial activity results, the MIC values ranged from 31 μ g/mL to 2000 μ g/mL for all tested microorganisms. The findings of our investigation offer important comparative insights into the essential oil composition and antimicrobial properties of *H. perforatum* collected from Eastern part of Türkiye in comparison with samples previously reported from different geographical regions.

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PP1-43. *Helichrysum italicum* essential oil as a Natural Skin Penetration Enhancer: Chemical Insights and Anti-Ageing Potential

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Keywords: *Helichrysum italicum*, skin permeation, elastase, enantiomeric composition, skin ageing

Helichrysum italicum (Roth) G.Don (Asteraceae family) is an aromatic plant typical of the Mediterranean region, widely distributed along the Adriatic coasts, where it grows in dry, sandy environments. Its essential oil (EO) is present in the aerial parts of the plant and has been traditionally used in folk medicine. The species is known for its anti-inflammatory and antimicrobial properties [1,2].

In this study, the essential oil of *H. italicum* from Italy (HEO) was first chemically characterized by both conventional and enantioselective GC–MS analyses and then preliminary evaluated for its potential as a skin permeation enhancer, as well as for its inhibitory activity against tyrosinase and elastase.

The main compounds were α -pinene (28.7%) and neryl acetate (27.1%) together with minor components, such as limonene (4.5%) and italicene (2.6%). Chiral compounds were present mainly in a high enantiomeric excess (i.e. (*R*)- α -pinene 96.5% and (*R*)-limonene 96.7%).

In vitro skin permeation studies of an oil-in-water (o/w) emulsion containing bioactive compounds for skin ageing with and without HEO combination, were performed using static Franz diffusion cells and porcine skin. Preliminary results showed that HEO acts as an effective permeation enhancer for bioactive compounds.

In parallel, the evaluation of enzymatic inhibitory activity through spectrophotometric assays indicated that *H. italicum* EO is inactive against tyrosinase and exhibits weak activity against elastase. Interestingly, the combination of HEO and skin ageing compound led to an increase in elastase inhibitory activity.

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PP1-44. Chemical Profiling and Antibacterial Evaluation of *Mentha × piperita* L. Aetheroleum vs Aromatic Water

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Keywords: *Mentha x piperita* L., hydrosol, gastrointestinal pathogens, antibacterial activity.

Essential oils are well known for their antimicrobial, antioxidant, anti-inflammatory, and antispasmodic effects among others[1,2], however, their potential for gastrointestinal irritation, hepatotoxicity, neurotoxicity, allergic reactions, and drug interactions limit safe oral use. Hydrosols or aromatic waters, obtained during essential oil distillation or by preparation, respectively, contain water-soluble volatile compounds, which are reported suitable for diluted oral use under controlled, microbiologically tested conditions [3,4].

This present study evaluated the chemical composition of *Mentha x piperita* L. temporal changes in hydrosol volatiles, and their antibacterial activity against common gastrointestinal pathogens. Essential oil and aromatic water volatiles were analysed by GC-FID/GC-MS and HS-SPME-GC/MS [5]. Antibacterial activity against pathogenic reference strains of *Escherichia coli* NRRL B-3008, *Bacillus cereus* NRRL B-3711, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 13311 and *Listeria monocytogenes* ATCC 19111 was determined by microdilution methods[6].

Menthol (34.6%), menthone (25.0%), 1,8-cineole (5.6%), and menthyl acetate (5.4%) were verified as the major components of the commercial *M. x piperita* oil. Whereas the aromatic water which was prepared was analysed for 1, 24, and 48 h, resulting in menthol (17.4, 9.8, 13.4%), menthone (30, 27.8, 27.5%), and menthyl acetate (11.5, 9.3, 10.9%) as main components, followed by menthofuran (7.9, 14.3, 11.1%), limonene (6.0, 8.8, 6.6%), and isomenthone (6.1, 5.4, 5.9%), respectively. The essential oil showed moderate activity against *E. coli*, *S. aureus*, and *S. typhimurium* (MIC 1.25 mg/mL) and weaker activity against *B. cereus* and *L. monocytogenes* (MIC 2.5–5.0 mg/mL), compared with ciprofloxacin (MIC 0.01–0.03 mg/mL), the aromatic water antibacterial effects will be presented in detail.

Overall, this study showed moderate to weak antibacterial activity against selected gastrointestinal pathogens. Compositional differences of the essential oil and hydrosol, particularly the time-dependent variation of the menthofuran content, indicate that the biological activity and safety profile of the hydrosol must be evaluated in detail.

Acknowledgements: This study was financially supported by Anadolu University Scientific Research Projects Coordination Unit under grant number OGT-2026-3419.

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PP1-45. Chemical composition and antimicrobial activities of *Ziziphora clinopodioides* Lam. essential oil from Ağrı, Eastern part of Türkiye

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Keywords: *Ziziphora clinopodioides*, essential oil, antimicrobial activity

Ziziphora species belonging to the family Lamiaceae have been traditionally used for their medicinal properties. *Z. clinopodioides* is one of the important edible members of the genus for its use in the treatment of several diseases [1]. Its aerial parts have been commonly utilized for their stomachic, carminative, and antimicrobial effects in Anatolian folk medicine. Due to its strong aromatic character and distinctive flavor, *Z. clinopodioides* is traditionally used by local communities as a culinary herb and spice to enhance the taste and aroma of foods, similarly to mint, and it is sometimes used as an alternative to mint or thyme in traditional dishes [2]. In the current study, the essential oil composition and antimicrobial activity of *Z. clinopodioides* collected from Eastern Anatolia were investigated and evaluated for the first time. The essential oil composition of the aerial parts of plant was analyzed by GC-FID, GC/MS. The antimicrobial activities of the essential oil and several extracts, including *n*-hexane, chloroform, methanol extracts and infusion, were examined by determining their minimum inhibitory concentration (MIC) values. According to the results, the major components of the oil were identified as thymol (56.4%), γ -terpinene (11.5%), *p*-cymene (9.9%), carvacrol (3.7%) and carvacrol methyl ether (3.6%). Regarding antimicrobial activity results, “strong to moderate” inhibitory effects against 6 bacteria and 7 yeasts were observed between the concentrations of 125 to 2000 μ g/mL and 31 to 2000 μ g/mL, respectively. The present study provides valuable comparative data regarding the essential oil composition and antimicrobial activity of *Z. clinopodioides* growing in Eastern Anatolia in comparison with samples reported from different geographical regions. Our future studies will particularly focus on the pharmacological potential of both the extracts and the essential oil, as well as the phytochemical characterization of the active extracts.

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PP1-46. Chemical Profiling and Biological Activity of *Achillea biebersteinii* Afan. Essential Oil and Hydrosol from Türkiye

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Keywords: *Achillea biebersteinii*, Asteraceae, GC-MS analysis, HS-SPME, Camphor, 1,8-Cineole.

Achillea biebersteinii Afan. (Asteraceae) is an ethnobotanically significant perennial herb in traditional Turkish medicine, widely distributed in the Eastern Anatolia region.

In this study, aerial parts were collected during the flowering stage from natural populations in Ağrı, Türkiye. The taxonomic identification was carried out by examining the morphological characteristics of the specimens in accordance with the "Flora of Turkey and the East Aegean Islands"[1]. The aerial parts of the collected plants were carefully air-dried in a shaded environment to preserve the volatile secondary metabolites. The essential oil and hydrosol were obtained by hydrodistillation using a Clevenger apparatus for 3 hours. The chemical profile of the essential oil was analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). The volatile components of the *A. biebersteinii* hydrosol were investigated using the headspace-solid-phase microextraction (HS-SPME) method. Camphor (34.6% and 32.4%) and 1,8-cineole (28.8% and 34.9%) were identified as the major components of the essential oil and hydrosol of *A. biebersteinii*, respectively. The potential antibacterial activity of essential oil was tested by the microdilution method against *Staphylococcus aureus* ATCC 6538, *Escherichia coli* NRRL B-3008, *Bacillus cereus* NRRL B-3711, and *Bacillus subtilis* NRRL-4378. The minimum inhibitory concentration (MIC) values of the samples were in the range of 0.78-12.5 mg/mL, compared with standard antibacterial agents. Lethal and acute toxicities of essential oil were determined by *in vivo* *Caenorhabditis elegans* test. Lethal concentrations for 50% of the nematodes (LC₅₀) and viability % for essential oil and major components were determined. These results indicate that the chemical profile of the species is influenced by the high-altitude environmental conditions of the Ağrı region. This study contributes to the phytochemical characterization of the genus *Achillea* and provides a scientific basis for the biological evaluation of its volatile metabolites.

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PP1-47. Comparative Evaluation of Chemical Composition and Bioactivities of *Helichrysum arenarium* subsp. *erzincanicum*: Influence of Extraction Techniques with Insight into Volatile Profiles

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Keywords: *Helichrysum arenarium* subsp. *erzincanicum*, HS-SPME–GC–MS, enzyme inhibition, antioxidant activity.

Diabetes mellitus and Alzheimer's disease are major chronic disorders associated with metabolic dysfunction and neurodegeneration. *Helichrysum arenarium* subsp. *erzincanicum* is a rare endemic subspecies native to Türkiye with limited phytochemical investigation [1-3]. This study aimed to comparatively evaluate the chemical composition and biological activities of extracts obtained using different extraction techniques. Extracts were prepared using multiple approaches, including polar, non-polar, and supercritical methods. Fatty acid compositions were determined by GC and GC–MS following FAME derivatization, while volatile compounds were analyzed by HS-SPME/GC–MS. Biological activities were assessed using antioxidant (DPPH[•], ABTS⁺) and enzyme inhibition assays (acetylcholinesterase, butyrylcholinesterase, α -amylase and α -glucosidase). The results revealed that both chemical composition and biological activities varied depending on the extraction technique. Major fatty acids included palmitic (29.9%), myristic (9.0%), stearic (8.1%), and linoleic acids (7.9%). Volatile profiles also differed among extracts, with supercritical samples showing higher diversity and enriched levels of compounds such as 2-methyl propanoic acid and 4,4-dimethyl but-2-enolide, while the essential oil was characterized by β -caryophyllene (9.9%) and T-cadinol (6.8%). In the ABTS assay, the methanol extract exhibited the highest antioxidant activity (79.97% inhibition at 60 μ g/mL), whereas no significant activity was observed in the DPPH assay. The strongest α -glucosidase inhibition was detected in the supercritical ethanol extract (90.83%), followed by the hexane extract (88.03%), both exceeding the standard acarbose (77.08%).

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PP1-48. Chemical Profiling and *In-vitro* Bioactivities of *Origanum bilgeri* P.H. Davis

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Keywords: *Origanum bilgeri*, carvacrol, antimicrobial activity, synergistic effect

Origanum bilgeri P.H. Davis is an endemic aromatic species of the genus *Origanum* (Lamiaceae), distributed in the Taurus Mountains of southern Türkiye. This study investigated the chemical composition and *in vitro* biological activities of its essential oil and solvent extracts. The essential oil, obtained by hydrodistillation and analyzed by GC–MS and GC-FID, was dominated by carvacrol (75.50%), followed by *p*-cymene (8.93%) and borneol (6.03%). LC-MS/MS analysis revealed phenolic constituents including rosmarinic acid, salvianolic acids, vicenin-2, and apigenin derivatives [1]. Antioxidant activity assessed by the DPPH[•] assay showed polarity-dependent activity. Antimicrobial evaluation against five bacterial and five yeast strains demonstrated strong antibacterial activity against *Salmonella typhimurium* (MIC=4.9 µg/mL) and *Staphylococcus epidermidis* (MIC=19.5 µg/mL), and antifungal activity against *Candida krusei* (MIC = 78 µg/mL). Synergistic interactions with conventional antimicrobial agents were also evaluated [2]. Cytotoxicity (MTT assay, NIH/3T3 cells) yielded IC₅₀ values of 291.44 ± 4.76 µg/mL and 102.57 ± 1.47 µg/mL for the extract and essential oil, respectively [3]. These findings suggest *O. bilgeri* as a promising natural source of bioactive compounds for pharmaceutical and agricultural applications.

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PP1-49. *Pinus mugo* Turra and *Pinus sylvestris* L. essential oils: two interesting sources of specialized metabolites inhibitors of tirosinase and elastase

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Essential oils (EOs) from conifer species are rich in bioactive terpenes and represent promising natural sources of cosmetic ingredients [1,2]. In this work, the chemical composition and enzyme inhibitory activity of *Pinus mugo* Turra and *Pinus sylvestris* L. essential oils were investigated, with particular attention to elastase and tyrosinase inhibition.

Nine batches of each EO were analyzed by GC-FID and GC-MS, using both conventional and enantioselective stationary phases. Both oils were mainly characterized by monoterpenes, with α -pinene, β -pinene, Δ -3-carene and limonene as the predominant constituents. Although qualitatively similar, the two EOs showed significant quantitative differences, especially in β -myrcene, β -phellandrene and α -pinene contents. The enantiomeric composition revealed that the two species showed an excess of the (1S)-(-)-enantiomer for both α -pinene and β -pinene, although with some interspecific differences. In contrast, limonene exhibited the opposite trend, with an excess of the (S)-(-)-enantiomer in *P. mugo* and the (R)-(+)-enantiomer in *P. sylvestris*. The biological activities were evaluated using spectrophotometric enzymatic assays. Both EOs exhibited remarkable elastase inhibitory activity at 166.7 μ g/mL (concentration in cuvette). *P. sylvestris* showed an average inhibition of 66.0%, while *P. mugo* reached 77.0%, comparable or superior to ursolic acid used as positive control. Conversely, only *P. mugo* EO demonstrated an interesting tyrosinase inhibitory activity, with an average inhibition of 35.9%, whereas *P. sylvestris* was inactive.

Preliminary bioguided fractionation experiments suggested that monoterpene hydrocarbons may play a key role in the observed activities. In particular, the presence of β -myrcene, previously identified as a tyrosinase inhibitor, could contribute to the activity of *P. mugo* EO. These results highlight *P. mugo* EO as a promising natural source of bioactive compounds with potential applications in cosmetic formulations targeting skin aging and hyperpigmentation processes.

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PP1-50. Composition and antimicrobial activity of essential oils from natural populations of *Lychnophora pinaster* Mart. (Asteraceae)

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Keywords: arnica; GC×GC; antimicrobial activity; *Lychnophora pinaster*

Lychnophora pinaster Mart., popularly known as arnica-mineira, is a medicinal plant endemic to the Brazilian state of Minas Gerais. The species has recognized therapeutic and aromatic potential, popularly used as an anti-inflammatory. Therefore, this study aimed to investigate the chemical composition and antimicrobial activity of leaf essential oils obtained from three natural populations of *L. pinaster* occurring in different geographic regions. The leaf essential oils from populations (P1, P2 and P3) were extracted by hydrodistillation (3h) and analyzed by GC×GC-MS/FID and multivariate statistical analysis. Antibacterial activity of the essential oils was investigated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/ MFC) against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The essential oils showed high abundance of oxygenated sesquiterpenes. The main constituents were 14-hydroxy- α -humulene for P1 (15.34%) and P2 (16.93%), and, for P3, the 4-hydroxy-9-epi- β -caryophyllene (21.13%) and 4-oxo-15-nor-eudesman-11-ene (18.50%). Multivariate analysis (PCA and HCA) separated the populations into two distinct chemical phenotypes: group 1 (P1 and P2) and group 2 (P3). The essential oils showed antibacterial activity exclusively against *E. coli*, with MIC values ranging from 0.25 to 0.50 mg/mL and MBC values from 0.50 to 1.00 mg/mL. The essential oil from population P1 showed the highest efficacy, with an MIC of 0.25 mg/mL and an MBC of 0.50 mg/mL. The results demonstrate that the antimicrobial potential of *L. pinaster* essential oils is directly influenced by chemical variability among natural populations.

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PP1-51. Comparative Essential Oil Chemotypes and Biological Potential of Five Cultivated *Ocimum basilicum* L. Cultivars

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Keywords: *Ocimum basilicum*, linalool, methyl chavicol, eugenol, geranial, chemotype.

Ocimum basilicum L. (basil) is among the most economically important aromatic plants worldwide and is widely cultivated for its culinary, medicinal, and industrial applications [1, 2]. The present study aimed to comparatively evaluate the essential oil composition and selected biological activities of five cultivated basil cultivars (cv. Dino, cv. Large Sweet, cv. Compact, cv. Moonlight, and cv. Midnight) grown under greenhouse conditions. Essential oils obtained from flowering aerial parts by hydrodistillation were analyzed using GC-FID and GC-MS. Between 95 and 99% of the total oil composition was identified in all cultivars. GC-MS analysis revealed distinct chemotypic differences among cultivars. Linalool was the predominant constituent in cv. Midnight (34.1%), cv. Moonlight (22.4%), cv. Large Sweet (12.2%), and cv. Compact (11.8%), whereas cv. Dino was characterized by a methyl chavicol/geranial chemotype containing methyl chavicol (32.0%), geranial (25.0%), and neral (16.4%) as major components. Other noteworthy constituents included methyl eugenol (up to 13.9%), eugenol (up to 12.6%), germacrene D (up to 7.8%), β -elemene (up to 7.3%), and τ -cadinol (up to 15.4%). The biological relevance of these chemotypic variations was further investigated through antioxidant, antimicrobial, and enzyme inhibitory assays. Several extracts exhibited remarkable α -glucosidase inhibitory activity, with the methanolic root extract of cv. Midnight showing 97.65% inhibition under the tested conditions. Strong ABTS radical scavenging activity was observed in methanolic root extracts of cv. Large Sweet (96.21%) and cv. Moonlight (95.51%). Furthermore, selective antifungal activity was detected against *Candida* species, with the methanolic flowering aerial part extract of cv. Moonlight exhibiting the strongest effect against *Candida parapsilosis* (MIC 156.25 μ g/mL).

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PP1-52. High-throughput SFC separation of target furocoumarins for the quality control of *Citrus* essential oils

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Keywords: *Citrus* essential oils, Furocoumarins, Green analytical chemistry, Bio-based solvents for chromatography, Supercritical fluid chromatography

Citrus essential oils serve as fundamental ingredients in the global production of fragrances and topical cosmetic products; however, their widespread use necessitates rigorous safety standards due to the presence of phototoxic compounds in the non-volatile fraction, namely furocoumarins. To address the quality control required by the cosmetic and fragrance industries, this study focused on a screening method based on supercritical fluid chromatography.

By leveraging recycled carbon dioxide as the primary mobile phase, the study exploits low fluid viscosity to achieve rapid, eco-friendly separations of target analytes in cold-pressed bergamot, bitter orange, lemon, and grapefruit essential oils. A systematic evaluation of stationary phases and co-solvents was conducted to optimize retention and separation against key operational parameters, including flow rate, temperature, pressure, and modifier concentration.

The optimized method was characterized by an analysis time of 3.5 min per sample and reduced solvent consumption to 840 μ L per run (bio-based methanol). Furthermore, hazardous solvents and additives were successfully replaced by a mobile phase containing 6% methanol with 2.5% water, isocratically.

Processing 17 samples per hour with a significantly reduced environmental footprint, this high-efficiency workflow provides a high-throughput and more sustainable alternative to conventional liquid chromatography for routine industrial quality control of *Citrus* essential oils.

Acknowledgements: The authors gratefully acknowledge the support from Merck Life Science (Merck KGaA, Darmstadt, Germany) and Shimadzu Europa (Duisburg, Germany).

PP1-53. RSM and ANN based optimization of microwave assisted hydrodistillation of *Cinnamomum zeylanicum* bark: A circular economy approach to essential oil extraction and residual water valorization

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Keywords: *Cinnamomum zeylanicum* essential oil, microwave assisted hydrodistillation, response surface methodology, artificial neural network

Cinnamomum zeylanicum Blume, bark essential oil (CZBEO) is highly valued for its aromatic and bioactive properties in food and pharmaceutical industry. This study optimized microwave-assisted hydrodistillation (MAHD) using response surface methodology (RSM) and artificial neural network (ANN) to enhance CZBEO yield. The optimized conditions were 800 W microwave power, 1:1 solvent-to-plant ratio and 85 min extraction time. The best ANN model obtained via hyperparameter optimization was a 3:2:2:1 multilayer perceptron (R^2 :0.993), outperforming RSM (R^2 :0.987) prediction accuracy. Kinetic modelling confirmed that the Weibull model accurately describes the CZBEO extraction under optimized condition, indicating a biphasic mechanism. MAHD yielded 2% (v/w) with reduced time and energy relative to hydrodistillation (HD) (1.8% v/w). HD and MAHD methods exhibited comparable physical properties, while GC-MS and FTIR analysis confirmed similar compositional profile. HPLC analysis revealed higher bioactive constituents in MAHD residual water. MTT assay revealed non-cytotoxic effect of MAHD residual water on 3T3-L1 cells and displayed enhanced antiadipogenic and antibacterial activity compared to HD residual water. SEM demonstrated extensive glandular disruption under MAHD. AGREEprep and GAPI underscored the greenness and sustainability of MAHD, supporting higher yield of CZBEO. Overall, MAHD can be considered as a rapid, eco-friendly and sustainable alternative technique for extraction of CZBEO.

POSTERS

SESSION 2 : Tuesday 8th Sept.



PP2-1. Decoding *Citrus* Essential Oils: Chemical Signatures and Anti-Aging Efficacy

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Keywords: *Citrus*, limonen, GC–MS, enzyme inhibition, limonene, green chemistry

Türkiye hosts a rich diversity of *Citrus* taxa characterized by high-value phytochemical constituents that remain largely underutilized [1]. This study aimed to evaluate the chemical composition and anti-aging potential of essential oils and extracts obtained from locally sourced *Citrus* species, focusing on the valorization of fruit peel by-products (flavedo/albedo) within a green chemistry and circular economy framework [2]. The study material comprised 13 *Citrus* taxa collected from Türkiye (*Citrus limon* (L.) Burm. f. cv. Kütdiken, *C. limon* (L.) Burm. f. cv. Lamas, *C. limon* (L.) Osbeck (Grande), *C. paradisi* Macfadyen cv. Star Ruby, *C. paradisi* Macfadyen (Red), *C. maxima* (Burm.) Merr., *C. sinensis* (L.) Osbeck cv. Washington Navel, *C. sinensis* (L.) Osbeck cv. Mystrimson, *C. fortunella*, *C. reticulata* Blanco cv. Murcott, *C. floridana*, *C. bergamia*, and *C. aurantium*). To assess extraction impacts on chemical profiles, cold pressing, hydrodistillation, maceration, and supercritical CO₂ extraction were applied and analyzed via GC–MS/GC–FID. Anti-aging potential was evaluated spectrophotometrically through tyrosinase, elastase, collagenase, and hyaluronidase inhibition assays [3, 4].

GC–MS revealed limonene as the predominant compound (53.7–94.1%). Extraction methods significantly impacted chemical stability; for example, thermal degradation during hydrodistillation reduced bergamot linalyl acetate from 16.6% (cold pressing) to 7.2%, and converted it to secondary alcohols like linalool in red grapefruit. Biological assays showed solvent type and plant part heavily influenced enzyme inhibition. Methanolic extracts exhibited superior elastase inhibition (Kütdiken pulp: 92.6%, pomelo peel: 91.1%, Grande peel: 87.8%) exceeding the standard (62.1%). Collagenase inhibition in Lamas aqueous (34.5%) and Murcott methanolic (32.1%) pulp extracts surpassed EGCG (31.7%). While hyaluronidase inhibition remained below EGCG (90.7%), Lamas methanolic pulp (56.6%) and Mystrimson cold-pressed samples (51.9%) showed notable activity.

Conclusively, extraction techniques directly influence the chemical integrity and biological activity of *Citrus* fractions. These findings highlight *Citrus*-based compounds as sustainable cosmeceutical candidates, supporting the valorization of local agricultural by-products.

Acknowledgements: This study was supported by the Atatürk University Scientific Research Projects (BAP) Coordination Unit under project number BAP TYL-2026-16706.

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PP2-2. Synergistic Antibacterial Activity of Linalool and Kynurenic Acid Against Major Otitis Media Pathogens

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Keywords: *Castanea sativa*, *Lavandula angustifolia*, otitis media, linalool, kynurenic acid

The widespread and inappropriate use of antibiotics has contributed to the emergence of resistant bacterial strains, posing a major challenge in clinical practice. Otitis media is among the most common childhood infections and a leading indication for antibiotic therapy, with frequent pathogens including *Haemophilus influenzae*, *H. parainfluenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*. Natural-origin products, such as certain honeys and essential oils, exhibit significant antibacterial activity through multiple mechanisms of action, which may reduce the risk of antibiotic resistance development. Therefore, their combination may represent a promising adjunctive approach alongside conventional antibiotic treatment.

Lavender essential oil, rich in linalool, and chestnut honey, containing kynurenic acid, are both valued for their antibacterial, anti-inflammatory, and antioxidant properties. This essential oil-honey combination, as well as combining their major components can be expected to exert synergistic antibacterial effects. Potential synergistic interactions were evaluated using checkerboard assays to assess the therapeutic relevance of this natural compound combination for potential topical applications in otitis media management.

Synergistic interactions between linalool and kynurenic acid were observed against *H. influenzae* (FICI = 0.25), *M. catarrhalis* (FICI = 0.37), and *S. pneumoniae* (FICI = 0.25). In contrast, additive effects were detected against *H. parainfluenzae* and *P. aeruginosa* (FICI = 0.75).

These findings suggest that the combined application of the two compounds may enhance antibacterial efficacy against clinically relevant otitis media pathogens.

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PP2-3. Anti-biofilm Activity of Selected Essential Oils Against *Streptococcus mutans*

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Keywords: *Streptococcus mutans*, *Origanum vulgare*, *Nepeta pannonica*, *Achillea millefolium*

The control of microbial biofilms remains a major challenge in both oral health and dermatological conditions, as biofilm-associated bacteria exhibit increased resistance to conventional antimicrobial agents. Natural volatile compounds, including essential oils and monoterpenes, have attracted considerable interest due to their ability to disrupt microbial adhesion, membrane integrity, and quorum sensing mechanisms.

The present study aimed to investigate the anti-biofilm activity of selected essential oils and borneol against *Streptococcus mutans*, a key biofilm-forming microorganism. Biofilm formation was evaluated using a crystal violet colorimetric assay in 24-well plates after 24 h incubation under anaerobic conditions. The strain *S. mutans* UA159 was cultured in Todd Hewitt broth supplemented with 1% sucrose. Essential oils and borneol were tested at concentrations ranging from 2 to 10 mg/mL, and optical density was measured at 595 nm. Statistical analysis was performed using one-way ANOVA ($p < 0.05$).

All tested samples exhibited significant inhibitory effects on biofilm formation. *Origanum vulgare* essential oil showed the highest activity, reducing biofilm biomass by up to 98% at concentrations of 4–10 mg/mL. High inhibition levels were also observed for *Nepeta pannonica* (up to 97%), indicating strong anti-biofilm potential of monoterpenoid-rich compositions.

Other essential oils, including *Hyssopus ambiguus*, *Achillea millefolium*, *Juniperus sabina*, demonstrated concentration-dependent effects, suggesting a dose-related interaction with bacterial adhesion and extracellular matrix formation. In contrast, *Melissa officinalis* showed weaker activity at lower concentrations. The solvent dimethyl sulfoxide exhibited moderate inhibition but remained significantly less effective than the tested natural compounds.

The results indicate that essential oils rich in phenolic and terpenoid constituents, as well as borneol, can effectively suppress *S. mutans* biofilm formation, likely through disruption of cell membrane integrity and inhibition of biofilm maturation processes. These findings highlight their potential application in the development of innovative antimicrobial and cosmeceutical formulations targeting biofilm-related disorders.

This work was supported by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP23488250).

PP2-4. Skin-targeted biological profile of *Pluchea grevei* essential oil: antimicrobial, antioxidant and toxicity evaluation

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Keywords: *Pluchea grevei*, antimicrobial activity, skin microbiota, cytotoxicity, antioxidant capacity

The *Pluchea* genus (Asteraceae) includes a group of aromatic species traditionally applied in ethnomedicine, particularly in the management of inflammatory and skin-associated conditions. Despite its recognized therapeutic relevance, detailed and consistent data on individual species remain limited. In particular, *Pluchea grevei*, a Madagascar-endemic plant and a source of commercially available essential oil, has not yet been comprehensively characterized in terms of its chemical profile and biological activity.

The present study aimed to investigate the properties of *P. grevei* essential oil through combined chemical and biological evaluation. Its composition was examined using chromatographic techniques (GC-MS), while functional properties were assessed by determining antimicrobial activity against selected skin-associated microorganisms, antioxidant capacity, cytotoxic effects on human keratinocytes, and *in vivo* toxicity.

The antimicrobial activity was assessed using a broth microdilution method, revealing selective effects with MIC values ranging from 100 to 3200 µg/mL, including the highest activity against *Acinetobacter baumannii* (MIC 100 µg/mL), and intermediate activity against other microbial strains. Antioxidant assays showed relatively low radical scavenging capacity (DPPH IC₅₀ = 31.78 ± 0.18 mg/mL) and modest reducing power (FRAP = 1881.55 ± 49.37 µmol TE/g), while both cytotoxicity testing on HaCaT cells and *in vivo* toxicity assessment using the brine shrimp lethality assay (BSLA) indicated moderate toxicity, with IC₅₀ = 124.74 µg/mL and a 24h LC₅₀ value of 325.9 mg/L (95% CI: 282.4–401.3 mg/L), respectively.

Commercial *P. grevei* EO exhibits selective antibacterial activity, particularly against *A. baumannii*, as well as moderate cytotoxicity toward keratinocytes and low antioxidant capacity. These findings suggest a targeted but limited biological potential, warranting further chemical characterization and comprehensive safety evaluation.

PP2-5. Natural fragrance materials as modulators of cell viability and gingipain activity: cytotoxic and enzymatic insights

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Keywords: human keratinocytes, cytotoxicity, *Porphyromonas gingivalis*, gingipains, enzymatic kinetics

Natural fragrance materials, including essential oils (EOs), are complex mixtures of compounds with diverse structures that exhibit a wide range of biological activities. Their effects cover many biological levels, from molecular targets and enzymes to mammalian cells. This study aimed to evaluate the cytotoxic effects of selected EOs on human keratinocytes and to assess their inhibitory activity against gingipains - cysteine proteases that are key virulence factors of *Porphyromonas gingivalis*.

Inhibition of cell viability was investigated using the HaCaT human keratinocyte cell line over a concentration range of 5-160 µg/mL. Cellular metabolic activity was measured using the MTS assay according to the manufacturer's protocol (Promega), and cell viability was expressed relative to untreated controls. A concentration-dependent decrease in cell viability was observed. The strongest effects were recorded for New Caledonia sandalwood (*Santalum austrocaledonicum*) and patchouli (*Pogostemon cablin*) EOs, reducing cell viability to 7.86% and 7.95% at 40 µg/mL, respectively. In contrast, minimal or no effects were observed at 5 µg/mL for ylang-ylang I (*Cananga odorata*) EO (100% viability) and blue cypress (*Callitris intratropica*) EO (91.66% viability).

In parallel, enzymatic assays are being conducted to evaluate the inhibitory potential of fragrance materials against gingipain RgpB (0.145 µg/mL). High-throughput screening of over 800 samples was performed in 96-well plates (4 µg/mL per well) using the fluorogenic substrate Cbz-F-R-AMC (20 µM). Preliminary results indicate strong inhibitory activity for vetiver (*Chrysopogon zizanioides*) EO (98.74%), artichoke (*Cynara cardunculus*) absolute (97.11%), and nettle (*Urtica urens*) absolute (97.75%). Further work will include GC-MS analysis to identify active constituents, followed by activity-guided evaluation of individual compounds.

These findings demonstrate the multidimensional biological activity of natural fragrance materials and highlight their potential relevance in biomedical and toxicological research, supporting further studies on their mechanisms of action and safety profiles.

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PP2-6. *Veronica dillenii* Crantz – antioxidant activity of volatile compounds

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Keywords: *Veronica dillenii* Crantz, caryophyllene oxide, caryophyllene, γ -eudesmol, hexahydrofarnesyl acetone, ORAC, DPPH

Veronica dillenii Crantz (Dillenius' speedwell) (Plantaginaceae) is a rare annual herbaceous plant found in Croatia only in the Sands Botanical Reserve (Đurđevački pijesci). It is strictly protected and listed in the Red Book of Croatia as a rare and endangered species [1]. Therefore, we requested and received permission from the Nature Protection Agency of the Republic of Croatia to research this species. The free volatile compounds in the composition obtained by microwave extraction were investigated, and extract consist of a lipophilic (essential oil) and an aqueous phase (hydrosol). The composition of the isolates was determined by gas chromatography-mass spectrometry. The lipophilic fraction is dominated by hexahydrofarnesyl acetone (8.86%), hexadecanoic acid (5.32%) and phytol (9.56%), while the hydrosol is dominated by caryophyllene oxide (24.32%), *E*-caryophyllene (20.05%) and γ -eudesmol (12.46%). These sesquiterpene compounds were also identified in the oil fraction of *V. dillenii*, but at a much lower percentage than in the hydrosol fraction. The composition of volatile compounds in other studied species of the genus *Veronica* is also dominated by these compounds [2]. The antioxidant activity of essential oil and hydrosol was investigated using two methods, ORAC (oxygen radical absorbance capacity) and DPPH (2,2-diphenyl-1-picrylhydrazyl). The obtained ORAC result for *Veronica dillenii* EO (80.65 μ mol/g of EO) show moderate activity when compared to other *Veronica* species for the same extracts (essential oils and hydrosols) [3].

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PP2-7. Antimicrobial activity and mutagenic effect of natural raw materials derived from *Calendula officinalis* L.

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Keywords: *Calendula officinalis* L., Ames MPF™ 98/100, *Salmonella typhimurium*, *Staphylococcus epidermidis*, *Staphylococcus aureus*.

Calendula officinalis L. is an annual plant from the Asteraceae family, valued for ornamental and medicinal use. Its yellow–orange flowers contain bioactive compounds (flavonoids, carotenoids, triterpenoid saponins) with anti-inflammatory, antibacterial, and wound-healing properties.

In our laboratory, studies were conducted to evaluate the antimicrobial activity of the absolute, concrete, wax, essential oil, and extract obtained from *Calendula officinalis* L (A. Fakhry & CO). In this study, minimum inhibitory concentration (MIC) values were determined against two bacterial species: *Staphylococcus epidermidis* ATCC 12228 and *Staphylococcus aureus* ATCC 6538. The assays were performed in 96-well plates using a series of twofold dilutions and the alamarBlue® reagent. The Ames MPF™ 98/100 mutagenicity test was carried out on two strains of *Salmonella typhimurium*, TA98 and TA100, both in the presence and absence of the S9 liver fraction. The experiments were conducted in accordance with the Ames MPF 98–100 Instruction for Use, version 7.0.

The results showed low MIC values against *S. aureus* and higher values against *S. epidermidis* (for concrete: 800 µg/mL vs. 3200 µg/mL). None of the tested materials exhibited mutagenic activity at any concentration, either in the presence or absence of the S9 fraction.

Based on the conducted studies, it can be concluded that compounds obtained from *Calendula officinalis* L. demonstrate differential antimicrobial activity, being more effective against *S. aureus* than *S. epidermidis*, as confirmed by the lower MIC values for the former microorganism. Furthermore, the absence of mutagenic activity of the tested materials, both in the presence and absence of the metabolic S9 fraction, indicates their potential genotoxic safety and supports their further application in various types of formulations.

Acknowledgements:

This work was supported by the project “Valorization of raw materials, including waste, from *Calendula officinalis* for applications in modern skin and hair cosmetics” (INNOGLOBO Programme, INNOGLOBO/4/76/CALENDERM/2025) financed by the National Centre for Research and Development – Poland.

PP2-8. Chemical Characterization of Essential Oils in Clinical Studies and Case Reports: Associations Between Compositional Characterization, Pharmacological Effects, and Adverse Events

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Keywords: *Citrus bergamia*, *Lavandula angustifolia*, *Mentha × piperita*, clinical studies, adverse effects, safety.

Clinical studies evaluating the pharmacological effects of essential oils frequently report beneficial effects; however, inclusion of analytical characterization of the oils used is often lacking or incomplete. This review systematically evaluated clinical studies involving bergamot (*Citrus × bergamia*), lavender (*Lavandula angustifolia*), and peppermint (*Mentha × piperita*) essential oils, with emphasis on compositional reporting, route of exposure, pharmacological effects, and adverse events. Most studies investigated inhalation exposure, followed by topical and oral use. A subset of studies included gas chromatography-based compositional characterization, commonly reporting linalool and linalyl acetate in lavender oils, limonene and linalyl acetate in bergamot oils, and menthol and menthone in peppermint oils.

Composition-characterized oils generally demonstrated chemically plausible profiles consistent with authentic botanical sources and tended to be associated with more consistent beneficial outcomes and fewer reported adverse effects than studies lacking compositional information. Lavender oils characterized by relatively high linalyl acetate and balanced linalool content were most consistently associated with sleep-promoting and calming effects, while a subset of studies reporting less consistent oil compositions appeared to be associated with a greater frequency of mild adverse effects. Similarly, bergamot oils rich in limonene, linalyl acetate, and linalool were associated with alleviated mild anxiety or stress, and better mood and sleep quality. Peppermint oils demonstrated benefits primarily related to gastrointestinal health. Reported adverse events were uncommon overall and were more frequently described in studies lacking detailed compositional characterization. In bergamot oil case reports, topical application followed by ultraviolet radiation exposure produced classical phototoxic reactions consistent with furocoumarin-mediated toxicity. Studies using bergapten- or 5-methoxypsoralen-reduced bergamot oils appeared to demonstrate improved safety profiles, particularly with respect to phototoxicity risk.

Collectively, these findings underscore the critical role of analytical characterization and standardization in ensuring reproducibility, facilitating interpretation of clinical outcomes, and strengthening safety and efficacy evaluation in essential oil research.

PP2-9. Biological activity of parsley essential oils against tomato pathogens

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Keywords: *Petroselinum crispum*, *Orthotospovirus tomatomaculae*, *Botrytis cinerea*, biological effects

Parsley (*Petroselinum crispum*; Apiaceae) represents a complex phytochemical resource beyond its common culinary use. Its fruits are the primary source of the volatile oil known as 'seed oil' that is widely used as a flavouring agent in the food industry and differs markedly from the leaf-derived "herb oil" because of its distinct biochemical composition [1].

The aim of this study was to evaluate the potential antiviral and antifungal activity of essential oils extracted from the leaves and fruits of a cultivated local ecotype of parsley from the province of Trento, Italy [2] against pathogens affecting tomato, a widely cultivated crop highly susceptible to biotic stressors.

Essential oil extract from parsley fruits was dominated by phenylpropanes such as apiole and myristicin, whereas the leaf essential oil consisted predominantly of 1,3,8-*p*-mentathriene. *In vivo* assays on tomato plants demonstrated that the fruit essential oil, applied at 100 ppm (w/w), exhibited significant antiviral and antifungal activity without inducing phytotoxic effects. In contrast, the leaf essential oil did not show any relevant biological activity.

In conclusion, parsley seed oil represents a promising source of bioactive compounds with potential applications in sustainable and green agriculture.

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PP2-10. Synergistic Effects of Essential Oils and Ampicillin Against *Streptococcus pyogenes*

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Keywords: thyme, tea tree, checkerboard titration, *Streptococcus pyogenes*, synergy

The rapid emergence of antibiotic resistance has become a major global healthcare concern, significantly threatening the effectiveness of current antimicrobial treatments [1]. Essential oils (EOs) have attracted significant scientific interest because of their established antimicrobial activity and their potential application as complementary agents in antimicrobial therapies [2]. Among these, thyme (*Thymus vulgaris* L.) and tea tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel) EO have demonstrated significant antibacterial activity against a wide range of pathogens.

The present study aimed to investigate the synergistic antibacterial effects of these EOs, both in combination with each other and in combination with ampicillin, against *Streptococcus pyogenes*. Minimum inhibitory concentrations (MICs) of the individual agents were determined using the broth microdilution method. Subsequently, combination MIC values were established using the checkerboard assay, and the fractional inhibitory concentration index (FICI) was calculated to evaluate the nature of the interactions between the tested compounds.

The MIC values demonstrated that tea tree EO exhibited greater antibacterial activity against *Streptococcus pyogenes* (MIC=0.078 mg/mL) than thyme EO (MIC=0.156 mg/mL). The combinational effects revealed synergistic interactions in two cases. The combination of the two EOs demonstrated a marked synergistic effect (FICI=0.25), while the combination of tea tree EO and ampicillin also showed synergistic activity (FICI=0.31). In contrast, the thyme EO-ampicillin combination exhibited an additive effect (FICI=0.62). Future studies will focus on the nanotechnological formulation of the synergistic combinations to improve their therapeutic applicability, including their potential use as wound dressings for chronic wound infections.

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PP2-11. Essential oil of the native Brazilian species *Croton compressus*: chemical profile and antibacterial activity

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Keywords: *Croton compressus*; Euphorbiaceae; Brazilian biodiversity

Native Brazilian species are important sources of bioactive natural products. Among them, the genus *Croton* stands out due to its wide distribution in tropical environments and remarkable chemical diversity [1]. *Croton compressus* Lam. (family Euphorbiaceae), native to Brazil, still has no reports describing the chemical composition of its essential oil. Therefore, this study investigated the chemical profile and antibacterial potential of the essential oil distilled from *C. compressus* leaves collected in 2025 at Tijuca National Park, Rio de Janeiro (SisGen authorization: A3BA2C3). The essential oil was obtained from fresh leaves by hydrodistillation using a Clevenger-type apparatus and was analyzed by GC-MS and GC-FID. The components were identified by comparison of their linear retention indices (LRI) and mass spectra with those from spectral libraries and literature data, and the relative composition was determined by peak-area normalization from FID data [2]. Antibacterial activity was evaluated by both microdilution (1000 - 7.81 µg/mL) against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213), following CLSI guidelines [3]. Oil yield was 0.12% w/w. Bicyclogermacrene (8.6%), (*Z*)- α -trans-bergamotol (6.9%), germacrene D (5.9%), γ -terpinene (5.5%), and limonene (4.3%) were identified as the major constituents. For the antibacterial assay, *S. aureus* showed susceptibility to the oil at concentrations below 1000 µg/mL, whereas no inhibitory effect was observed against *E. coli*. This study reports, for the first time, the chemical composition and antibacterial activity of the essential oil from *C. compressus*.

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PP2-12. Insecticidal and phytotoxic effects of the terpenic fraction of *Piper aduncum* essential oil

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Keywords: Dillapiole, toxicity, terpenoids, phenylpropanoid, Noctuidae, *Piper aduncum* L.

The fall armyworm [*Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae)] is an endemic pest in the Americas that attacks 186 hosts, such as maize and beans. Resistance to synthetic insecticides drives the use of essential oils, although their phytotoxicity remains a challenge. *Piper aduncum* essential oil (Oepa), rich in dillapiole and synergistic terpenoids, is a promising alternative. To adjust the dillapiole content, fractional distillation was applied [1], generating three distinct fractions. This study aimed to determine the lethal doses of the Oepa terpenic fraction against the pest and evaluate its phytotoxicity in common beans. The essential oil was obtained from *P. aduncum* plants via steam distillation. Fractional distillation of the oil generated three fractions characterized by dillapiole content: terpenic (26%), intermediate (59.1%), and propanoic (85.3%). Toxicological assays to determine the LD₅₀ and LD₈₀ of the terpenic fraction followed established methodologies [1]. For phytotoxicity, BRS FC310 common beans grown in vermiculite were used. At the V4 phenological stage, plants were sprayed until runoff with the emulsified terpenic fraction (Tween 20) at six concentrations (0.5 to 3.0% v.v⁻¹), plus a control. The experimental design was completely randomized (CRD) with four replications. Phytotoxicity was evaluated daily for 8 days using a zero-to-three scale [2], and the Area Under the Disease Progress Curve (AUDPC) was calculated [3]. Data were subjected to ANOVA, with means compared by Tukey's test ($p < 0.05$) and regression analysis. Lethal doses of the Oepa terpenic fraction for *S. frugiperda* were LD₅₀ = 1.83% (1.70%-2.02%) e LD₈₀ = 2.54% (2.25%-3.11%), indicating high toxicity and a rapid mortality increase with small dose variations. Regarding phytotoxicity (AUDPC), concentrations of 0.5% and 1.0% showed the lowest damage (0.25 and 1.42), with no statistical difference. From 1.5% onwards, damage was significantly higher, peaking at 3.0% (15.55). Linear regression confirmed a dose-dependent effect ($R^2 = 0.88$; $p < 0.0001$), with the equation $AUDPC = -3.13 + 6.66x$, indicating that each 1% dose increment increased severity by 6.66 units. Phytotoxicity can be explained by the cytotoxic action of monoterpenes (74% of the fraction), which reduce mitochondria and Golgi complexes, impairing respiration, photosynthesis, and cell membrane integrity, resulting in tissue injuries [4]. It is concluded that the *P. aduncum* terpenic fraction is effective in controlling *S. frugiperda* in common beans, with recommended doses up to 1.0% v/v to avoid significant phytotoxic effects.

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PP2-13. Terpenoid composition and antioxidant activity of Lithuanian *Tanacetum vulgare* (Tansy) essential oils

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Keywords: *Tanacetum vulgare*, 1,8-cineole, borneol, antioxidant activity, square wave voltammograms, medicinal plants.

Tanacetum vulgare L. (Asteraceae) is an aromatic medicinal plant recognized for its diverse essential oil composition and broad spectrum of biological activities. The present study aimed to investigate the chemical composition and antioxidant properties of *T. vulgare* essential oils (EOs) obtained from herbs collected in Lithuania. “Tansy” leaf and inflorescence EOs were isolated by hydrodistillation and analyzed using gas chromatography–mass spectrometry (GC–MS). Antioxidant activity was evaluated using in vitro assays, including DPPH• and ABTS•+ radical scavenging, and less used electrochemical methods [1].

GC–MS analysis demonstrated qualitative and quantitative differences between leaf and inflorescence essential oils. The major constituents identified in leaf essential oil were 1,8-cineole (28.65 %), borneol (18.53 %), and caryophyllene oxide (4.44 %). Meanwhile, the inflorescence essential oil was dominated by 1,8-cineole (19.58 %), borneol (10.86 %), β -thujone (8.91 %), *E*-dihydrocarvone (8.45 %), and camphor (8.26 %). Monoterpenes and oxygenated monoterpenes predominated in both essential oils. The antioxidant assays (Figure 1) revealed that the investigated essential oils possessed promising radical scavenging activity, which may be associated with the synergistic effects of their major terpenoid constituents.

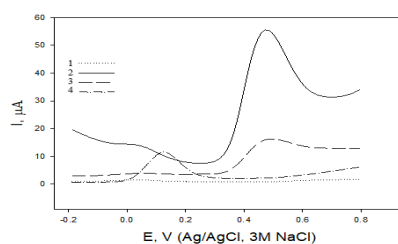


Figure 1. Square wave voltammograms of carbon paste electrode: 1 - phosphate buffer at pH 7.3; 2 - inflorescence EO modified electrode; 3 - leaf EO modified electrode; 4 - quercetin.

The obtained results contribute to the phytochemical characterization of Lithuanian *T. vulgare* chemotypes and support the potential application of its essential oils as natural antioxidant agents for pharmaceutical, cosmetic, and food industry applications.

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PP2-14. Model optimization and pilot study assessing the effects of essential oils on burn wound healing

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Keywords: *Lavandula angustifolia* Mill., burn wound

Burn injuries are caused by extreme heat, electricity, chemicals, friction, or radiation, with children under five years of age being the most frequently affected population. Burns constitute characteristic damage to the skin and are classified as superficial (first-degree), partial-thickness; superficial and deep partial-thickness (second-degree); and full-thickness (third-degree) injuries. Certain essential oils may exert beneficial effects on burn wound healing due to their antibacterial, anti-inflammatory, and tissue-regenerative properties.

The aim of our present study was to optimize and extend a second-degree burn model [1] by incorporating Laser Speckle Contrast Analysis (LASCA) and to conduct a preliminary test using lavender essential oil.

Prior to the in vivo experiments, chemical characterization of the essential oil was performed using GC–MS and GC–FID. Burn wounds were induced under anesthesia using a soldering device applied to the center of the shaved nape area. Contact burn wounds were subsequently treated with vehicle alone (negative control), a zinc hyaluronate-containing gel (positive control), or *Lavandula angustifolia* Mill. essential oil formulated in the vehicle. Cutaneous blood flow in the superficial burn area was measured using a laser speckle imaging device at time points optimized during the pilot study. Conventional and thermal images, as well as skin tissue samples, were collected to evaluate visible and histological parameters.

Perfusion in the affected area increased over the course of the experiment; treatment with the positive control attenuated the rate of this increase, while lavender essential oil treatment resulted in a higher initial perfusion followed by a slow decrease.

Although further evaluation of imaging and histological data, together with an increased sample size, is required to confirm the effects of lavender essential oil, the preliminary results suggest that the optimized model is suitable for assessing the potential effects of essential oils in the treatment of burn wounds.

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PP2-15. Terpene-based cocrystals for sustainable control of honeybee pathogens

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Keywords: cocrystals, terpenes, sustained delivery, vapor-phase activity, honeybee pathogens, green pesticides.

Terpenes, the main bioactive components of essential oils, are well known for their antimicrobial activity and are promising green substitutes of synthetic pesticides in apiculture [1,2]. However, their high volatility, low stability and limited bioavailability have greatly limited their practical application in realistic conditions [3]. In this context, crystal engineering provides a useful approach for tuning the physicochemical properties of terpenes by the formation of cocrystals, thereby attaining enhanced stability and prolonged release [3]. In this work, selected essential oils were screened against honeybee pathogens, with thyme, oregano and mint identified as the most active. Their main components, thymol, carvacrol and menthol, were further evaluated to assess their individual effects and potential interactions and used for the design of terpene-based cocrystals with selected sustainable coformers. The antimicrobial performance of the resulting systems was tested through vapor-phase exposure assays, which constitute a realistic model for applications in confined environments such as beehives. Biological assays revealed that selected cocrystals displayed notable antimicrobial activity, while differences in efficacy across systems underscored the potential of crystal engineering to finely tune terpene release, offering a strategy for sustained and controlled antimicrobial action. Results indicate that terpene-based cocrystals represent promising candidates for the development of sustained-release antimicrobial systems, offering a novel, environmentally compatible strategy for the prevention of hive infections.

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PP2-16. Long-Term EO–Antibiotic Interactions Shape Cefotaxime Resistance and EO Sensitivity

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Keywords: *Lavandula angustifolia*, linalol, *Origanum vulgare*, carvacrol, *Escherichia coli*, cefotaxime, development of antimicrobial resistance.

The use of essential oils (EOs) in aromatherapy to manage microbial and neuropsychological conditions is rapidly increasing. This trend is driven by the search for novel antimicrobial compounds capable of counteracting the rise of antimicrobial resistance, identifying alternatives with fewer side effects. *Origanum vulgare* (OV) and *Lavandula angustifolia* (LA) are among the most widely used EOs, yet the consequences of their prolonged oral exposure, alone or combined with antibiotics, on microbial resistance remain poorly understood. This study aimed to assess whether extended exposure of *Escherichia coli* ATCC 25922 to sub-MIC concentrations of cefotaxime (CE), OV, LA, and their major constituents (carvacrol, CA; linalool, LI), individually or in combination (CE+OV, CE+LA, CE+CA, CE+LI), could induce resistance to antibiotics or EOs. This objective aligns with the need to better define both the beneficial and adverse biological effects of EOs when used alone or in combination with conventional antimicrobials. EO composition was assessed by GC-MS, and MIC values were determined according to EUCAST guidelines. The strain was exposed for 80 days, with susceptibility testing performed at T0, T10, T30, T50, and T80 through antibiograms (19 antibiotics), aromatograms (15 EMA/ISO-listed EOs) standardized according to EUCAST guidelines, and dedicated assays for CE, OV, LA, CA, and LI. CE-resistant mutants were quantified at T0, T50 and T80 on agar containing CE at 2MIC, 8MIC, 16MIC and 32MIC. By T80, the strain became resistant to CE, with a time-dependent increase in CE-resistant CFUs. CE exposure also induced resistance to ampicillin as early as T10, an effect not suppressed by LA or LI, whereas OV and CA completely prevented its emergence. At T80, cultures exposed only to CE displayed markedly reduced susceptibility to penicillins and cephalosporins, an effect absent in cultures treated with CE+OV or CE+CA and only partially mitigated by CE+LA or CE+LI. Sensitivity to EOs and their constituents remained stable over time, except for a selective reduction in susceptibility to *Coriandrum sativum* EO (leaves) and *Mentha x piperita* in the CE+LI and CE-LA groups from T50. Overall, these findings demonstrate that combining EOs with CE does not induce antibiotic resistance, and that OV and CA effectively prevent CE-driven resistance development. LA and LI provide only partial protection. Conversely, the CE+LI and CE+LA interactions highlight a reduced susceptibility to alcohol-based EOs. Further studies will be necessary to investigate this phenomenon in greater depth.

PP2-17. Alginate-based edible coatings enriched with essential oils for enhancing postharvest quality and bioactive stability of cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*)

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Keywords: Edible coatings; Essential oils; Postharvest quality.

This study evaluated the effectiveness of sodium alginate-based edible coatings enriched with essential oils and their main bioactive compounds in preserving the postharvest quality of cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*). Fruits were treated with alginate coatings containing lemongrass (*Cymbopogon citratus*) essential oil, citral, bay laurel (*Laurus nobilis*) essential oil, and 1,8-cineole, and stored at 7 °C for up to 21 days followed by 3 days of shelf-life simulation at room temperature. Physicochemical parameters, including color, firmness, total soluble solids, and weight loss, were monitored alongside bioactive compounds such as lycopene, β -carotene, and total phenolics, as well as microbial quality. Results showed that alginate coatings, particularly those combined with citral, lemongrass, and bay laurel essential oils, effectively maintained fruit firmness and reduced weight loss, while delaying ripening-related color changes. The coatings had limited influence on total soluble solids and did not significantly alter lycopene and β -carotene contents, although temporal variations were observed during storage. Total phenolic content increased over time in all treatments, suggesting enhanced antioxidant potential during storage. Overall, alginate-based coatings enriched with essential oils demonstrated potential as sustainable and natural postharvest technologies to maintain quality and extend the shelf life of cherry tomatoes without compromising their nutritional value.

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PP2-18. *Artemisia absinthium* L.– cytotoxic activity of volatiles

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Keywords: *Artemisia*, epoxy-ocimene, cytotoxic activity

Free volatile compounds isolated from *Artemisia absinthium* L. (wormwood) (Asteraceae) are used in folk medicine [1]. Volatile compounds in wormwood obtained by microwave extraction were investigated, and the extract consists of essential oil and hydrosol. The composition of the isolates was determined by gas chromatography-mass spectrometry. Oxygenated monoterpenes are the dominant class in the essential oil of *Artemisia absinthium* L. from different geographical areas of Europe with variations in the percentage [1]. In our investigation, oxygenated monoterpenes dominate in both phases accounting for 70.63% of the total identified compounds in the essential oil and 62.51% in the hydrosol. The compound trans + cis epoxy-ocimene constitutes almost 50% of the free volatile compounds in both fractions. Cytotoxic activity was tested on human colon cancer (HCT116) and cervical cancer (HeLa) cell lines. The essential oil showed considerably higher cytotoxic activity than the hydrosol in both cell lines. The IC₅₀ values for the essential oil were 169.77 µg/mL for HCT116 and 431.94 µg/mL for HeLa cells, while the hydrosol exhibited significantly lower activity, with IC₅₀ values of 1.24 mg/mL and 2.25 mg/mL, respectively. The stronger activity of the essential oil is likely due to the combined effects of its major compounds, suggesting possible synergistic interactions among the volatile components [2].

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PP2-19. Characterization of *Rosa damascena* Oil and Distillation Products: Phytochemical Profile, Bioactivity, and Anti-Aging Formulations

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Keywords: Anti-aging, Antimicrobial, Formulation, Genotoxicity, *R. × damascena*, Skin

Rosa damascena, from which rose essential oil is obtained and which holds an important place in industry, is one of Türkiye's most important combined products. [1] Rose oil obtained from *R. × damascena*, together with its distillation by-products—rose water, rose yeast, and rose distillation wastewater—were evaluated as herbal raw materials. Both the original products and the formulations prepared from them at different concentrations were subjected to comprehensive content analysis and biological activity assessments, including antioxidant, anti-aging (tyrosinase, collagenase, elastase, and hyaluronidase inhibition), antimicrobial (microdilution), genotoxicity studies to ensure safety for potential dermal use. Volatile compounds were analyzed by GC–MS, secondary metabolites by LC–MS/MS, and elemental composition by ICP–MS. Major constituents of essential oil were identified as citronellol (16.6%), geraniol (15.6%), γ -muuroles, nerol (11.5%). Phenylethyl alcohol was detected at 33.8% in rose yeast and 44.6% in rose water. Rose distillation wastewater was sparticularly rich in secondary metabolites, notably quinic acid, gallic acid, quercetin, and exhibited the highest total phenolic (119.5 μg GAE/mg) and tannin contents (264.8 μg TAE/mg). Strong antioxidant activity was observed with IC₅₀ values of 87 $\mu\text{g}/\text{mL}$ for DPPH[•] and 4.67 $\mu\text{g}/\text{mL}$ for ABTS^{•+}. A remarkable collagenase inhibition rate of 79.51% indicated significant anti-aging potential. Formulation-3 and Formulation-4 showed the highest hyaluronidase inhibition (62.30% and 60.44%, respectively).

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PP2-20. Protective effect of sweet orange essential oil and (+)-limonene in an in vitro co-culture neurodegeneration model

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Keywords: *Citrus sinensis* (L.) Osbeck, (+)-limonene, neurodegeneration, inflammation, oxidative stress, SH-SY5Y-microglia co-culture

The prevalence of neurodegenerative diseases, including Parkinson's disease, is continuously increasing worldwide, creating a growing demand for novel and natural complementary therapies. Recent studies have increasingly focused on the potential role of essential oils (EOs) in neurodegenerative disorders [1]. Inflammation and oxidative stress are key mechanisms contributing to disease development and progression [2]. The present study investigates the effects of sweet orange [*Citrus sinensis* (L.) Osbeck] EO and its main component, (+)-limonene, on oxidative stress and inflammation in a 6-hydroxydopamine (6-OHDA)-induced in vitro neurodegeneration model.

Commercially obtained (Herby's Ltd., Hungary) sweet orange EO was chemically analyzed by GC-MS. Experiments were performed on a co-culture of all-*trans* retinoic acid-differentiated SH-SY5Y cells and BV-2 microglia, with rasagiline used as a positive control. Cell viability, ROS production, antioxidant capacity, iron content, ATP levels, and inflammatory markers were assessed.

At 500x dilution, neither the EO nor (+)-limonene affected cell viability. Both treatments significantly reduced ROS production, while (+)-limonene increased antioxidant capacity and decreased iron accumulation. The EO and (+)-limonene restored intracellular ATP levels and reduced the fractalkine, TNF- α , and IL-6, with stronger effects observed for (+)-limonene. These findings suggest that sweet orange EO and (+)-limonene are promising neuroprotective agents for complementary therapy of Parkinson's disease.

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PP2-21. Antimicrobial activity of essential oil from *Cryptomeria japonica* foliage

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Keywords: *Cryptomeria japonica* (Thunb. ex L.f.) D. Don, antimicrobial, disk diffusion, circular bioeconomy

Antimicrobial resistance (AMR), accelerated by the COVID-19 pandemic, is a growing global concern as bacteria increasingly resist existing antibiotics, causing treatment failures and higher mortality. This underscores the need for new antimicrobial agents, with natural products like essential oils (EOs) remaining a valuable source of antibacterial compounds.

This study investigated the antimicrobial activity of EOs hydrodistilled from Azorean *Cryptomeria japonica* foliage. Growth inhibition zone (GIZ) was determined via disc diffusion according to the Kirby–Bauer method against several microorganisms.

The EOs exhibited activity primarily against Gram-positive bacteria (GIZ: 9.0 – 11.0 mm), with effectiveness decreasing in the following order: *Staphylococcus aureus* > *Bacillus licheniformis* > *Bacillus subtilis* > *Enterococcus faecium*, as well as the Gram-negative *Vibrio cholerae*. Additionally, the EOs demonstrated activity against *Candida albicans* and *Candida tropicalis* (GIZ: 8.5 – 13.5 mm).

These findings highlight the potential of these natural products as sources of antimicrobial compounds. However, further studies are necessary to support their potential applications.

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PP2-22. Anti-inflammatory activity of essential oil from Azorean *Cryptomeria japonica* foliage harvested in two different seasons

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Keywords: *Cryptomeria japonica* (Thunb. ex L.f.) D. Don, anti-inflammatory, seasonal variation, sustainable circular bioeconomy

The Azorean wood industry sectors produce significant amounts of *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Cupressaceae) biomass residues, particularly foliage (Az–CJF), which are often misused sources of natural products, such as essential oils (EOs), with varied profiles and potentialities that can be affected via distinct environmental factors.

This study evaluated the influence of seasonal variation on the anti-inflammatory efficacy of Az–CJF EOs, obtained from hydrodistillation of autumn (Aut–EO) and spring (Spr–EO) Az–CJF samples [1]. A colorimetric assay for cyclooxygenases (COX) inhibition was used for measurement, expressed through IC₅₀ values, of the mentioned bioactivity.

Results indicate that Spr–EO demonstrated greater inhibitory activity against both COX isoforms (IC₅₀ COX-1: 284 µg/mL; IC₅₀ COX-2: 750 µg/mL) than Aut–EO (IC₅₀ COX-1: 560 µg/mL; IC₅₀ COX-2: 1074 µg/mL), with both EOs exhibiting stronger inhibition of COX-1. Quantitative differences in the EOs' chemical composition [1] may account for the observed differences in their anti-inflammatory activities.

This study demonstrates that the anti-inflammatory efficacy of Az–CJF EO is influenced by seasonal variation, highlighting also its potential for future use in therapeutic practices.

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PP2-23. Essential Oil–Based Technologies for Sprout Control in Stored Potato

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Keywords: *Solanum tuberosum*, volatiles, sprout suppression

Potato is one of the most important food crops globally and a cornerstone of the agricultural economy in the United States, contributing approximately \$100.9 billion annually (National Potato Council [NPC], 2023). However, postharvest sprouting during storage remains a major challenge, leading to quality deterioration, economic losses, and reduced marketability (Gumbo et al., 2021). Currently, sprout suppression in stored potato tubers relies primarily on synthetic plant growth regulators such as chlorpropham (CIPC) (Paul et al., 2016). While effective, the use of CIPC is increasingly constrained by regulatory changes and market demands. Anticipated reductions in maximum residue limits (MRLs) by regulatory agencies, along with bans in key international markets including the European Union, United Kingdom, and Japan, are limiting its use and affecting global trade. In addition, concerns regarding environmental persistence and potential human exposure are driving the need for safer and more sustainable alternatives. In response, our research focuses on the discovery and development of essential oil–based technologies for sprout control in stored potato. We are investigating bioactive compounds derived from natural sources and developing innovative formulation and delivery systems to enhance their efficacy, stability, and duration of action under storage conditions. This work is actively driven by graduate research, including the design and execution of storage experiments, evaluation of treatment efficacy, and generation of data supporting technology development. This presentation will highlight recent advances from our program, including results from controlled storage studies, and will outline future research directions aimed at developing effective, scalable, and environmentally responsible alternatives to conventional sprout suppressants.

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PP2-24. Comparative Antitumor Activity of the Liquid and Vapor Phases of Juniper Essential Oil

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Keywords: *Juniperus communis* L., H292 cells; MRC-5 cells; Cell viability, Selectivity index; Scratch assay.

Essential oils (EOs) possess a wide range of pharmacological properties and are composed of volatile compounds that, upon inhalation, can be directly delivered to respiratory tract tissues. This characteristic highlights the relevance of investigating the biological effects of their vapor phase in comparison with the liquid phase for potential therapeutic applications via the inhalation route. The present study aimed to evaluate the antitumor potential of the essential oil of *Juniperus communis* L. (Juniper) in its liquid phase (LP) and vapor phase (VP). Juniper OE chemical analysis by GC/MS confirmed α -pinene (69.94%) as the major compound. Human lung carcinoma cells (NCI-H292) and human lung fibroblasts (MRC-5, non-tumor control) were used as experimental models to assess antitumor activity. Cells were exposed for 24 h to EO treatments in the LP (5–2000 $\mu\text{g/mL}$) or VP (25–16,000 $\mu\text{g/mL}$). To VP treatments, cells were seeded in the eight central wells of 24-well culture plates at a concentration of 1×10^5 cells/well, after complete cell adhesion, were exposed for 24 h to the vapor generated from liquid EO solutions placed in the sixteen surrounding cell-free wells as described to Seal et al. [1]. Cell viability was evaluated using the sulforhodamine B (SRB) assay, from which IC50 values and selectivity indices (SI) were determined. In addition, a scratch wound-healing assay was performed to evaluate cell migration and proliferation. Both EO phases reduced tumor cell viability in a dose-dependent manner. In LP treatment, the IC50 value obtained for the H292 cell line were $633.79 \pm 97.06 \mu\text{g/mL}$. The concentrations of EO required to generate sufficient vapor to reduce cell viability by 50% (vapor-phase IC50) were $1843 \pm 600.5 \mu\text{g/mL}$. LP exhibited SI values of 5.32, whereas VP showed SI value of 7.01. Furthermore, the scratch assay demonstrated reductions in cell migration/proliferation of 66.0% and 35.7% for LP and VP, respectively. These findings suggest that both the liquid and vapor phases of Juniper EO exhibit promising antitumor activity, with VP demonstrating greater selectivity toward tumor cells.

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PP2-25. Phytochemical Profiling and Antimicrobial Potential of *Tamarix parviflora* (Tamaricaceae) Influence of Floral Color and Morphological Parts on Essential Oil Composition

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Keywords: *Tamarix parviflora*, antimicrobial activity, antifungal activity

In this study, the phytochemical profile and biological activities of *Tamarix parviflora* were evaluated, considering its traditional use in diuretic and gastrointestinal disorders [1–2]. Essential oils (EOs) and solvent extracts from the aerial parts and flowers were analyzed by GC-MS/MS. The results showed clear differences depending on flower color and plant part. In pink flowers, the main compounds were farnesyl acetone (23.4%), hexahydrofarnesyl acetone (20.0%), and heptacosane (10.3%), while aerial parts contained farnesyl acetone (10.1%), nonanal (8.7%), and hexahydrofarnesyl acetone (7.4%), along with 1-octadecanol (6.5%) and linalool (4.7%). In white flowers, hexahydrofarnesyl acetone (19.1%) and hexadecanoic acid (14.4%) were dominant, whereas aerial parts showed heptacosane (8.4%), hexadecanoic acid (6.4%), and benzaldehyde (5.0%). Biological activity results indicated that EOs exhibited moderate antibacterial effects with MIC values of 625–1250 µg/mL, but weak antifungal activity against *Candida albicans* (MIC >2500 µg/mL). In contrast, fractionated extracts showed improved activity, with antibacterial MIC values of 312.5–1250 µg/mL and strong antifungal activity against *C. albicans* (MIC: 9.76–39.02 µg/mL). These results suggest that fractionation enhances the concentration of active antimicrobial compounds.

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PP2-26. A synergistic essential oil blend alleviates inflammatory response via NF- κ B/MAPK/Nrf2 signalling pathways in murine macrophages.

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Keywords: Macrophages, *Melaleuca alternifolia*, *Eucalyptus globulus*, reactive oxygen species, cytokines, GC-MS

Inflammation is a highly complex, multifactorially regulated phenomenon in the body that responds to cellular dysfunction caused by noxious bacterial or viral pathogenesis. However, the molecular underpinnings of inflammation remain largely unclear due to a lack of detailed preliminary evaluations of anti-inflammatory agents that could meet the diagnostic standards. The therapeutic application of essential oils (EOs) and their synergistic anti-inflammatory potential remains largely underexplored. In the present study, we evaluated the anti-inflammatory efficacy of ten commercially used essential oils and their binary combinations in LPS-stimulated murine macrophages (RAW 264.7 cells). Among all EO combinations assessed in varying ratios, the combination of *Eucalyptus globulus* and *Melaleuca alternifolia* EOs in equal proportion demonstrated the most potent synergistic activity (CI). This optimized blended essential oil, i.e., BL-10, was non-cytotoxic and maintained cell viability across a broad concentration range. BL-10 significantly reduced reactive oxygen species generation, pro-inflammatory cytokines secretion, and COX-2 and iNOS gene expressions. JC-1 staining confirmed mitochondrial membrane potential depolarisation, while intracellular antioxidant enzymes were markedly elevated. Confocal assays revealed reduced nuclear translocation of NF- κ B-p65 and Nrf2 proteins in BL-10-treated cells. Furthermore, BL-10 attenuated inflammation by interfering in the TLR4/MyD88/NF- κ B/MAPK signalling pathways and additionally upregulated the Nrf2/HO-1 antioxidant axis. GC-MS analysis of BL-10 identified major bioactive constituents including 1,8-cineole (33.60%), terpinen-4-ol (24.69%), and γ -terpinene (11.57%). These results show that by targeting several intracellular signalling pathways, the synergistic EO blend has strong anti-inflammatory effects. Future in vivo research and the therapeutic development of EO-based formulations for the treatment of inflammatory disorders have a promising basis attributable to this initial work.

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PP2-27. Lipid peroxidation inhibitory potential of *Nepeta nuda* L. essential oils and it's most abundant volatiles

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Keywords: *Nepeta nuda*, 1,8-cineol, lipid peroxidation, TBA-MDA assay

Oxidative stress and resulting lipid peroxidation (LP) are related to a detrimental process that can cause significant cellular damage and has been implicated in the pathogenesis of various diseases, including rheumatoid arthritis, atherosclerosis, ischemia, carcinogenesis, and aging. As a result, the role of antioxidants remains a to be of interest, and the capacity of various natural compounds and synthetic supplements to neutralize radicals has been extensively evaluated. *Nepeta* species are rich in compounds with extensive biological activities, some of which have a sedative effect on humans, and are widely used in medicine, the food industry, and perfumery. In the present work, we have examined *in vitro* antioxidant activity of essential oil obtained by the hydrodistillation of above-ground parts of *Nepeta nuda* ssp. *nuda* gathered from four distinct locations in SE Serbia, along with its main components, several of which have been previously recognized for their anti-inflammatory effects [1-3], on the LP process. To measure LP, a thiobarbituric acid-malondialdehyde (TBA-MDA) assay was used. The percentage of LP inhibition in the presence of tested compounds was evaluated spectrophotometrically, by measuring the absorbance of the TBA-MDA conjugate at 530 nm, according to the slightly modified procedure described by Lazarević et al. [4] and Brizzolari et al. [5]. The same experiments were done using Trolox, caffeic acid, vitamin C, and rutin as standards. All tested samples showed a LP reduction, although the effect was less significant compared to the standard antioxidants.

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PP2-28. Compositional analysis of *Nepeta nuda* L. volatiles from SE Serbia

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Keywords: *Nepeta nuda* volatiles, chemotypes, 1,8-cineol, germacrene D

Nepeta nuda L. (syn. *N. pannonica* L.) is a perennial forest-steppe plant widespread across Europe and Asia, with four registered *N. nuda* subspecies. Two out of four *N. nuda* subspecies: ssp. *nuda* and ssp. *albiflora* have a wide growth area across Europe and Asia, with the Balkans being their region of overlap. Research shows that essential oils of *N. nuda* have great bioactive potential, which is mainly associated with the presence of nepetalactones and oxygenated monoterpenes [1].

The chemical composition of the essential oils of a *Nepeta* taxon can be subject to variation due to environmental and geographical factors. According to the referenced literature, the hierarchical clustering is currently suggesting four *N. nuda* essential oil chemotypes: 1) the nepetalactone chemotype group, 2) 1,8-cineole group, 3) mixed chemotype group (high content of nepetalactone+1,8-cineole+germacrene D), and 4) nonspecific chemotypes group [2]. Understanding chemotypes is crucial because the dominant chemical composition drastically alters the therapeutic, aromatic, or toxic properties of a substance, which ultimately determines its practical application.

The essential oils of seven geographically distinct SE Serbian specimens of *N. nuda* were prepared by hydro-distillation, and analysed by GC and GC/MS. One hundred sixty-seven compounds were identified, accounting for 94.6–99.0% of the oils. Analyzed samples contained varying amounts of 1,8-cineole and/or germacrene-D as dominant components; since producing two different chemical profiles of volatiles, SE Serbia *N. nuda* samples belong to two genetically or ecologically distinct groups.

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PP2-29. Antibacterial Activity of Flower-Derived Essential Oils Against Acne-Associated Bacteria

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Keywords: Acne vulgaris, Essential oils, Antibacterial activity

Acne vulgaris is a multifactorial inflammatory skin disorder in which bacterial colonization and biofilm formation contribute to disease progression and therapeutic resistance [1,2,3]. Due to the increasing prevalence of antimicrobial resistance and the adverse effects associated with long-term antibiotic therapy, there is growing interest in natural antimicrobial agents, including essential oils (EOs). However, many highly active EOs possess strong odors or may cause skin irritation, limiting their cosmetic applicability.

In the present study, we investigated the antibacterial activity of selected flower-derived essential oils against acne-associated bacterial strains. Broadleaved lavender (*Lavandula latifolia* L.) and ylang-ylang (*Cananga odorata* Lam.) essential oils were tested against *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, and *Cutibacterium acnes* using broth microdilution assays for minimum inhibitory concentration (MIC) determination.

Both essential oils exhibited antibacterial activity against the tested strains, although susceptibility varied among microorganisms. *P. aeruginosa* showed the lowest sensitivity, while *S. epidermidis* and *C. acnes* were more susceptible to the investigated oils. Wild lavender EO demonstrated MIC values of 2.5 mg/mL against *P. aeruginosa*, 1.25 mg/mL against MRSA and *C. acnes*, and 0.625 mg/mL against *S. epidermidis*. Ylang-ylang EO showed particularly promising activity against *C. acnes* with an MIC value of 0.3125 mg/mL, while MIC values of 1.25 mg/mL were observed against *P. aeruginosa* and MRSA, and 0.625 mg/mL against *S. epidermidis*.

Our findings suggest that flower-derived essential oils, especially ylang-ylang EO, may represent promising candidates for the development of complementary cosmetic formulations targeting acne-associated bacteria. Future studies will focus on antibiofilm investigations and mixed-species biofilm models relevant to acne vulgaris.

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PP2-30. The antibiotic adjuvant potential of various essential oil components against respiratory pathogens

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Keywords: *Streptococcus pneumoniae*, *S. pyogenes*, *Haemophilus influenzae*, *H. parainfluenzae*, *Moraxella catarrhalis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, thymol, eugenol, trans-cinnamaldehyde, terpinen-4-ol

Respiratory tract infections are commonly treated with antibiotics; however, increasing antimicrobial resistance and biofilm formation substantially reduce therapeutic efficacy. The ability of the bacteria causing these infections to form biofilms makes it difficult to treat them effectively.

The selected EO components (thymol, eugenol, trans-cinnamaldehyde and terpinen-4-ol) possess well-documented antibacterial and antibiofilm activities and may enhance the efficacy of conventional antibiotics.

In our study, we investigated the effect of these EO components against biofilm-forming bacteria commonly found in respiratory infections: *Streptococcus pneumoniae*, *S. pyogenes*, *Haemophilus influenzae*, *H. parainfluenzae*, *Moraxella catarrhalis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*.

Methods: The minimum inhibitory concentration (MIC) was determined using the microdilution method. To investigate synergistic interactions with clinically relevant antibiotics, we employed the checkerboard method and fractional inhibitory concentration index (FICI) analysis. Finally, the effect of EO components, as well as EO components combined with antibiotics, on early-stage biofilms (6 h) was determined using crystal violet staining.

Results: Gram-positive strains showed greater susceptibility to the tested components. Synergistic interactions (FICI $\leq$ 0.5) were most frequently observed between β -lactam antibiotics and phenolic compounds. We observed strong synergism between vancomycin and eugenol against MRSA, as well as between amoxicillin/clavulanic acid and cinnamaldehyde against *M. catarrhalis*. Synergistic combinations significantly enhanced the inhibition of early biofilm formation compared with antibiotic monotherapy ($p < 0.05$).

These findings highlight the promising antibiotic adjuvant potential of EO components and support their possible application in the management of biofilm-associated respiratory infections.

PP2-31. Bioactivity of *Origanum vulgare* essential oils against phytopathogenic *Xanthomonas campestris*

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Keywords: black rot, crucifers, antimicrobial, oregano, sustainable control.

Xanthomonas campestris is a Gram-negative phytopathogenic bacterium responsible for black rot in economically important cruciferous crops, including cabbage, broccoli, and cauliflower. Its virulence is primarily associated with the production of extracellular polysaccharides (xanthan gum), hydrolytic enzymes, and effector proteins that promote host colonization and suppress plant immune responses. Current chemical control strategies rely on copper-based treatments; however, their efficacy is limited and prolonged use can contribute to the development of resistant strains. Essential oils (EOs) have emerged as promising eco-friendly alternatives, representing natural and biodegradable sources of antimicrobials. Their multi-target modes of action reduce the likelihood of developing resistance. In this study, EOs extracted from *Origanum vulgare* were evaluated against a virulent *X. campestris* isolate (CPBF 278) to determine its microbiocidal potential. The EOs were obtained by hydrodistillation for 3 h and chemically characterized using GC-FID and GC-MS. Antimicrobial activity was assessed using the Alamar Blue assay. The EOs analyzed showed chemical variability that directly influenced antimicrobial activity against *X. campestris* at a concentration of 1 µL/mL. The most inhibitory (94–99%) were rich in carvacrol (40%), thymol (21–43%) and *p*-cymene (13–24%), while the least active (56%) was composed of linalool (80%). The results suggest *O. vulgare* EOs rich in chemically related phenol-like monoterpenes, particularly carvacrol and thymol along with their precursor *p*-cymene, proved effective against *X. campestris*, highlighting the importance of selecting chemical structural features for improving EO microbiocidal efficacy.

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PP2-32. Antibacterial and efflux pump modulating effects of Scots pine (*Pinus sylvestris* L.) essential oil against resistant pathogens

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Keywords: *Pinus sylvestris* L., nosocomial infections, antibacterial activity, efflux pump modulation

The increasing prevalence of resistant Gram-negative bacteria poses a major global health challenge, highlighting the urgent need for novel antimicrobial strategies [1]. Plant-derived essential oils have gained attention due to their complex composition and multitarget mechanisms of action, including efflux pump inhibition. Scots pine (*Pinus sylvestris* L.) essential oil (SPEO) has been reported to exhibit antibacterial activity against various bacterial species [2]. This study investigated the antibacterial and efflux pump inhibitory effects of SPEO against *Escherichia coli* and *Klebsiella pneumoniae* strains. The chemical composition of SPEO was characterized using gas chromatography-mass spectrometry (GC-MS) [3]. Antibacterial activity was determined using the broth microdilution method [3], and efflux pump inhibition was evaluated using the ethidium bromide accumulation assay [4]. GC-MS analysis identified α -pinene (38.66%) as the predominant component of SPEO. The essential oil exhibited moderate antibacterial activity with MIC values of 2.5 mg/mL (*E. coli*) and 5.0 mg/mL (*K. pneumoniae*). Antibiotic control was included (meropenem MIC for both strains: 0.007 μ g/mL). Efflux pump inhibition was observed with RFI values of 0.6 (*E. coli*) and 1.6 (*K. pneumoniae*). The positive control was carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) with RFI values of 0.911 (*E. coli*) and 1.040 (*K. pneumoniae*). A higher RFI indicates stronger efflux pump inhibition. These *in vitro* findings demonstrate that SPEO possesses antimicrobial properties against resistant strains and may interfere with bacterial efflux pump mechanisms. After further *in vivo* examinations, SPEO may be used against nosocomial pathogens in clinical practice as a surface disinfectant.

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PP2-33. Chemical Profiling and Biological Activity of *Sanguisorba officinalis* L. Volatile Extracts

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Keywords: *Sanguisorba officinalis* L., volatiles, GC-MS, antioxidant activity, antidiabetic activity.

Sanguisorba officinalis L. (Rosaceae) is a perennial medicinal plant widely distributed across Europe and Asia, commonly found in grasslands and moist habitats. It has long been used in traditional medicine for its hemostatic, wound-healing, and anti-inflammatory properties [1]. The therapeutic potential of this species is largely attributed to its rich phytochemical composition. Previous studies have shown that *S. officinalis* contains significant amounts of tannins, flavonoids, triterpenoids, and phenolic acids, which contribute to a wide range of biological activities, including antioxidant and enzyme inhibitory effects [2]. These characteristics highlight its potential as a valuable source of bioactive compounds for applications in phytotherapy and functional products.

In the present study, the chemical composition and biological activity of the essential oil and hydrolate of *S. officinalis* from Bosnia and Herzegovina were investigated. The essential oil and hydrolate were extracted from plant material by hydrodistillation. Solid-phase microextraction (SPME) was used for the isolation of volatiles from the hydrolate. Volatile compounds were analyzed by gas chromatography–mass spectrometry (GC–MS). The dominant constituents identified in both samples were salicylaldehyde (27.2% and 15.8%), lavandulol (23.3% and 25.5%), methyl salicylate (6.4% and 11.7%), and linalool (6.2% and 12.3%) in the essential oil and hydrolate, respectively. Biological activity was evaluated using FRAP and DPPH assays, as well as through inhibition of α -amylase and α -glucosidase enzymes. The results indicate good antidiabetic activity and comparatively lower antioxidant activity of the tested essential oil and hydrolate samples in relation to the reference compounds.

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PP2-34. Anti-inflammatory effects of *Gaultheria fragrantissima* L. essential oil, *Nigella sativa* L. oil, and their combination in an *in vivo* psoriasis model

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Keywords: *Nigella sativa* L., *Gaultheria fragrantissima* L., *in vivo*, psoriasis

Psoriasis is a chronic, immune-mediated inflammatory skin disease characterized by erythematous, scaly plaques associated with impaired skin barrier function. Although corticosteroids are widely used, their long-term application is limited by adverse effects, prompting the exploration of plant-derived alternatives, including essential oils.

This study aimed to evaluate the anti-inflammatory potential of *Gaultheria fragrantissima* L. essential oil and *Nigella sativa* L. fixed oil, applied individually and in combination, in an imiquimod-induced psoriasis-like mouse model.

Chemical characterization was performed prior to *in vivo* experiments. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that *Gaultheria fragrantissima* L. essential oil was predominantly composed of methyl salicylate (96.35%), whereas gas chromatography with flame ionization detection (GC-FID) showed a thymoquinone content of 209 ± 18 µg/mL in *Nigella sativa* L. oil.

Psoriasis-like dermatitis was induced by the topical application of imiquimod. Clobetasol propionate ointment and paraffin oil were used as the positive and negative controls, respectively. Skin thickness, cutaneous blood flow, transepidermal water loss (TEWL), and histological analysis were evaluated.

Imiquimod treatment significantly increased skin thickness, perfusion, and TEWL. Treatment with *Gaultheria* essential oil moderately reduced skin thickness. Combined treatment did not significantly affect skin thickness or TEWL; however, a measurable reduction in cutaneous blood flow was observed.

These findings suggest that the investigated oils exert partial anti-inflammatory effects, with the most pronounced impact on microcirculation. The high methyl salicylate content of *Gaultheria* oil and the presence of thymoquinone in *Nigella sativa* L. may have contributed to the observed effects. Further studies are required to elucidate their mechanisms of action and therapeutic relevance.

PP2-35. Chemical composition and antimicrobial activity of essential oils from aromatic plants: support for a state phytotherapeutic program in São Paulo, Brazil

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Keywords: *Mentha* spp.; *Cymbopogon citratus*; *Pogostemon cablin*; *Rosmarinus officinalis*; public policy.

The World Health Organization (WHO) supports the integration of traditional, complementary, and integrative medicine into public healthcare systems. In Brazil, aromatherapy was officially incorporated into the Unified Health System (SUS) in 2018, reinforcing the need for scientific validation of plant-derived raw materials intended for therapeutic use. In this context, and as part of efforts to establish a State Program for Medicinal, Aromatic, and Herbal Plants (PMAF) in the State of São Paulo, this study investigated the chemical composition and antimicrobial activity of essential oils produced and/or commercialized in the region. Essential oils from *Mentha* spp., *Cymbopogon citratus*, *Pogostemon cablin*, and *Rosmarinus officinalis* were analyzed. Chemical characterization was performed by gas chromatography coupled with mass spectrometry and flame ionization detection (GC–MS and GC–FID). Antibacterial and antifungal activities were evaluated through minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) assays against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans*. Distinct chemical profiles were observed among the oils. *Mentha* spp. samples were dominated by geraniol (>75%), *C. citratus* samples showed high citral content (neral + geranial ≈85%), *P. cablin* oils contained predominantly patchoulol (≈36%), and *R. officinalis* oils presented 1,8-cineole and camphor as major constituents. *Mentha* spp. oils exhibited the broadest and strongest antibacterial activity (MIC 0.25–1.0 mg mL⁻¹), with predominantly bacteriostatic effects. *C. citratus* showed moderate activity, whereas *P. cablin* displayed selective inhibition against *E. coli* and *B. subtilis*. These findings provide scientific support for establishing quality standards and prioritizing species and chemotypes within the PMAF.

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PP2-36. Component Composition and Antimicrobial Activity of Essential Oils of *Ferula varia* and *Ferula songarica*

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Keywords: *Ferula varia*, *Ferula songarica*, pinene, guaiol, bornyl acetate

Plants of the genus *Ferula* are used in traditional medicine for the treatment of various diseases due to their antioxidant, antidepressant, antibacterial, antifungal, antileishmanial, and anti-inflammatory properties. In addition, these plant species are used for dizziness, asthma, bronchitis, and gastrointestinal discomfort. All pharmacological effects of these plants are associated with the presence of various phenolic compounds, including flavonoids, sesquiterpenes, coumarins, and polysulfides. However, the chemical composition and pharmacological activity of plants of this genus remain insufficiently studied. A total of 51 species of the genus *Ferula* occur in Kazakhstan, including 15 endemic species, while 15 species are listed in the Red Book of Kazakhstan flora.

In the present study, the chemical composition and antimicrobial activity of the essential oils obtained from *Ferula varia* Schrenk ex Fisch and *Ferula songarica* Pallas ex Sprengel growing in Kazakhstan were investigated. Essential oils were isolated by hydrodistillation using a Clevenger apparatus. The component composition was determined by GC–MS analysis. Antimicrobial activity was evaluated using the disc diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*.

The essential oil of *Ferula varia* was characterized by a predominance of sesquiterpenoids, with guaiol (29.61%), bornyl acetate (14.60%), and nerolidol (11.13%) identified as the major constituents. The oil exhibited moderate antimicrobial activity against Gram-positive bacteria and *Escherichia coli*, as well as weak activity against *Pseudomonas aeruginosa* and *Candida albicans*.

The essential oil of *Ferula songarica* demonstrated a complex multicomponent composition containing up to 113 constituents, and its biological activity depended on the type of plant material used. β -Pinene (up to 34%) was identified as the major component. Samples obtained from underground organs exhibited moderate antibacterial activity, whereas some samples showed pronounced activity against Gram-positive bacteria and *Candida albicans*. The root essential oil was characterized by high antifungal activity.

The obtained results demonstrate that essential oils of *Ferula varia* and *Ferula songarica* are promising natural sources of biologically active compounds with significant antimicrobial and antifungal potential, which may be considered for further development of pharmaceutical, cosmetic, and antimicrobial formulations based on plant-derived raw materials.

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PP2-37. Phytochemical Characterization and Essential Oil Profiling of *Alkanna tinctoria* and *Alkanna orientalis* with Enzyme Inhibitory and Cytotoxic Activities

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Keywords: *Alkanna tinctoria*, *A. orientalis*, enzyme inhibition, cytotoxicity, diabetes

Diabetes mellitus, Alzheimer's disease and cancer are major chronic disorders linked to aging, oxidative stress and metabolic dysfunction [1]. In this study, the biological activities and essential oil compositions of methanolic extracts, their solvent fractions, lipid extracts (butter and olive oil) and the essential oils obtained from the aerial parts of *Alkanna tinctoria* and *A. orientalis* were comprehensively evaluated for their therapeutic potential against diabetes, Alzheimer's disease and cancer. Essential oils obtained by hydrodistillation and analyzed by GC-MS/MS revealed distinct chemotypes: *A. tinctoria* aerial parts were rich in oxygenated sesquiterpenes and diterpenes (spathulenol, *p*-cymene, manool), whereas *A. orientalis* aerial parts were dominated by long-chain alcohols and hydrocarbons (1-octadecanol, 1-hexadecanol). Biological assays demonstrated strong α -glucosidase inhibition by *A. orientalis* root methanol extract (90.09%), exceeding acarbose, while moderate α -amylase inhibition was observed, particularly in lipid extracts. Anticholinesterase activity was moderate for AChE but pronounced for BChE. Non-polar fractions showed the highest anti-aging enzyme inhibition, with notable tyrosinase, hyaluronidase, collagenase and elastase inhibitory effects. Cytotoxicity assays revealed selective activity depending on plant part and cell line; *A. tinctoria* flower extract showed strong activity against SH-SY5Y cells, while root extracts were more toxic to L929 fibroblasts.

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PP2-38. Cytotoxicity assessment of *Calendula officinalis* essential oil and related lipophilic natural complex substances

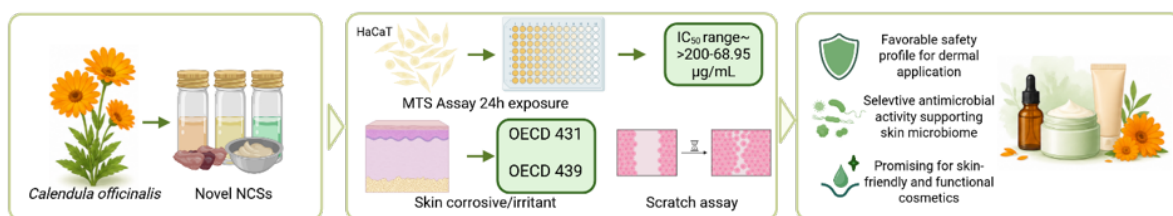
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Keywords: *Calendula officinalis*, cytotoxicity, HaCaT, skin safety

Calendula officinalis L. is a source of diverse lipophilic natural complex substances (NCSs), including essential oil (EO) and other industrially relevant aromatic plant products. Despite their growing commercial interest, information regarding their skin safety remains limited. Therefore, five proprietary lipophilic NCSs (EO, absolute (A), concrete (C), wax (W), and extract (E)) derived from *C. officinalis* and provided by A. Fakhry & Co. were evaluated for their cytotoxicity.



Scheme 1 – From plant to product: safety assessment of lipophilic NCSs from *Calendula officinalis* for cosmetic use

Cytotoxicity was estimated for human keratinocytes HaCaT cells via the MTS test after 24 h of treatment. It should be noted that the investigated materials had different cytotoxic activity: the EO and A possessed rather pronounced toxicity with IC₅₀ values within the range of 68.95±0.35 and 78.70±0.86 µg/mL, whereas E, W and C were significantly less toxic (respectively, IC₅₀ = 128.30±1.11; 187.97±0.35 and >200 µg/mL).

Preliminary classification of their skin irritant potential according to OECD TG 439 indicates that at least one of the tested materials (W) can be considered non-irritant, while evaluation of the remaining materials is ongoing. Additional studies, including skin corrosion testing (OECD TG 431) and scratch assay, are planned to further assess their corrosion potential, regenerative capacity and compatibility with cosmetic ingredients.

Together, these findings indicate that selected lipophilic NCSs derived from *C. officinalis* possess a favorable safety profile and may represent promising ingredients for cosmetic applications. The results support the valorization of EO-related and other lipophilic natural products from aromatic plants, contributing to their further development and safe use.

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PP2-39. Anti-inflammatory activity of essential oils and hydrosol from Okinawan *Alpinia zerumbet*

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Keywords: *Alpinia zerumbet*, anti-inflammatory, Okinawa

The genus *Alpinia* is widely utilized in traditional medicine, food, and cosmetics across tropical regions. In Okinawa, Japan, two types of *Alpinia zerumbet* occur naturally: *A. zerumbet* (AZ) and *A. zerumbet* var. *excelsa* (AZe) [1]. These two types differ in aroma, essential oil (EO) composition, and floral morphology. However, comparative studies on EOs and hydrosols (HS) from different types of *Alpinia zerumbet* remain limited. Therefore, this study aimed to compare the chemical compositions of EOs and HS obtained from Okinawan *A. zerumbet* and to evaluate their anti-inflammatory activities using murine macrophage cells.

EOs and HS were obtained from leaves by steam distillation for 2 h. EO compositions were analyzed by GC-MS and expressed as relative peak area percentages. Major volatile constituents in AZeHS were quantified by GC-MS using calibration curves of terpinen-4-ol and 1,8-cineole. AZEO was mainly composed of *p*-cymene (19.3%), 1,8-cineole (15.5%), α -pinene (10.5%), and limonene (10.1%), whereas AZeEO was characterized by terpinen-4-ol (21.7%), 1,8-cineole (18.4%), γ -terpinene (13.2%), and sabinene (11.6%). AZeHS contained terpinen-4-ol (397.4 ± 15.3 μ g/mL) and 1,8-cineole (109.5 ± 2.8 μ g/mL) as major components.

LPS-stimulated RAW264 murine macrophage cells were treated with the samples to evaluate anti-inflammatory activity. Both AZEO and AZeEO significantly inhibited nitric oxide (NO), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α) production. AZEO exhibited stronger inhibition of NO production (approximately 40% at 200 μ g/mL) compared with AZeEO (approximately 20% at 400 μ g/mL). AZeHS at 20% in the culture medium decreased NO production by approximately 10%. Authentic compounds, including methyl cinnamate, terpinen-4-ol, and sabinene, showed varying degrees of inhibitory effects on inflammatory mediator production.

These results demonstrate that EOs and HS from Okinawan *A. zerumbet* possess anti-inflammatory potential, with differences in activity likely attributable to variations in volatile composition. Further studies using GC-FID and Western blot analysis are ongoing to clarify the quantitative composition of EOs, investigate the contribution of individual constituents, and elucidate the underlying molecular mechanisms, particularly NF- κ B and MAPK signaling pathways.

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PP2-40. Chemical Profile and Biological Activities of *Salvia officinalis* L. Essential Oils from Herzegovina

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Keywords: sage, *Salvia officinalis* L., α -thujone, α -amylase, α -glucosidase, cholinesterase inhibition.

Sage (*Salvia officinalis* L.) is a prominent medicinal and aromatic plant widely utilized in the pharmaceutical and cosmetic industries. While its pharmacological and biological properties are well-documented, this study focuses on the specific chemical profile of *S. officinalis* essential oils indigenous to the Herzegovina region [1]. The aim of this research was to characterize the chemical composition of these oils and evaluate their antioxidant, antidiabetic, and neuroprotective effects. Essential oils obtained by steam distillation from dry plant material, alongside a commercially available *S. officinalis* oil, were investigated. The chemical constituents were determined by GC-MS/FID analysis. Antioxidant activity was evaluated using DPPH and CUPRAC assays. Furthermore, *in vitro* tests were employed to evaluate the inhibition of α -amylase, α -glucosidase, and cholinesterases (AChE and BChE).

A variation in the relative proportions of major components was observed between the distilled and commercial essential oils: α -thujone (30.84% and 23.13%), camphor (15.90% and 16.70%), β -thujone (12.76% and 2.29%), and 1,8-cineole (12.51% and 13.63%). The antioxidant capacity of the essential oils was poor ($IC_{50} > 4.00$ mg/mL). The sage essential oils exhibited moderate inhibitory effects against α -amylase and α -glucosidase ($IC_{50} < 1.00$ mg/mL). Both samples showed slight inhibitory activity ($IC_{50} \leq 1.50$ mg/mL) on acetylcholinesterase (AChE), with the commercial sample demonstrating higher efficacy than the laboratory distillate. Inhibition of butyrylcholinesterase (BChE) was significantly weaker in both cases.

In conclusion, medicinal sage from the Herzegovina region exhibits a specific chemical profile characterized as an α -thujone / β -thujone / camphor / 1,8-cineole chemotype. This chemotype showed mild to moderate biological activity.

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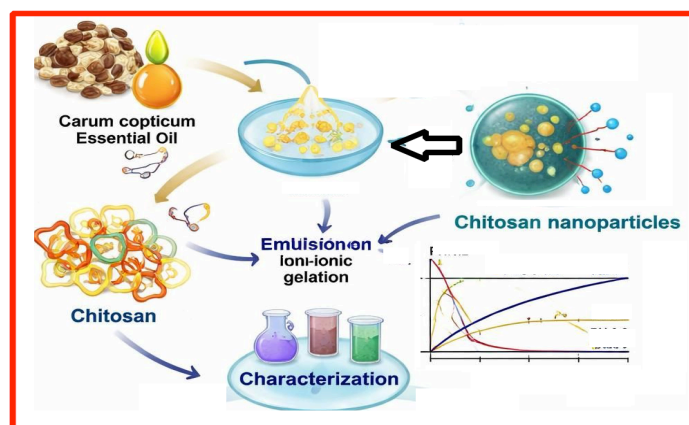
PP2-41. Revolutionizing *Carum copticum* oil: Chitosan nanoparticle encapsulation and its biological impact

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Keywords: *Carum copticum* oil, chitosan nanoparticles, antibacterial properties, antioxidant properties.

The unique functional properties of essential oils have attracted substantial interest across sectors, yet their use is often limited by environmental sensitivity and poor solubility. This study examines the encapsulation of *Carum copticum* essential oil in chitosan nanoparticles via an emulsion-ionic gelation method, using sodium tripolyphosphate and sodium hexametaphosphate as crosslinking agents. Nanoparticle characterization by FTIR, UV-vis spectroscopy, DSC, SEM, and DLS shows that encapsulation efficiency (EE) and loading capacity (LC) increase with higher essential oil content. The nanoparticles display spherical morphology, with sizes of 30 to 80 nm and a uniform size distribution. Release studies show an initial burst, followed by sustained release of the essential oil across a range of pH levels. Antioxidant and antibacterial activities of the encapsulated *Carum copticum* oil were assessed using the DPPH assay and disk diffusion method, respectively. Findings indicate that encapsulation in chitosan nanoparticles not only preserves essential oil quality but also enhances its functional applications [1-4].



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PP2-42. Antifungal and anti-inflammatory activities of an essential oil blend against *Malassezia furfur*: A synergistic approach

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Keywords: *Cymbopogon martinii*, *Mentha × piperita*, *Varronia curassavica*, seborrheic dermatitis.

Seborrheic dermatitis is a chronic inflammatory condition primarily triggered by *Malassezia* yeast species, affecting body regions with high sebaceous gland density. Current treatments rely on synthetic antifungals, which often face issues with recurrence and resistance, highlighting the need for new bioactive compounds derived from aromatic plants. This study aimed to evaluate the activity of essential oils (EOs) against *Malassezia furfur* in both planktonic and biofilm forms [1]. Additionally, the anti-inflammatory potential (TNF- α ; IL-8), mutagenicity (Ames test), and cytotoxicity toward human keratinocytes (HaCaT) and bone marrow-derived macrophages (BMDMs) were assessed. Out of 40 EOs individually screened, a specific blend of essential oils (BEO) was developed using *Cymbopogon martini* (Roxb.) Will. Watson (MIC = 0.5 mg/mL) and *Mentha × piperita* L. (MIC = 1.0 mg/mL), which demonstrated a synergistic effect determined by the checkerboard assay. The inclusion of *Varronia curassavica* Jacq. oil maintained the fungicidal activity. The BEO significantly inhibited biofilm formation ($\cong 80\%$) and partially eradicated established biofilms ($\cong 30\%$), outperforming fluconazole. No mutagenic potential was observed, and the blend did not compromise the viability of HaCaT or BMDM cells. While individual EOs reduced TNF- α levels (except for *V. curassavica*), this anti-inflammatory property was preserved in the final blend. The BEO did not affect IL-8 levels. These findings suggest that the combination of *C. martini*, *M. × piperita*, and *V. curassavica* is a promising alternative for treating seborrheic dermatitis.

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PP2-43. Essential Oil Blends as Natural Post-Harvest Preservatives Against *Aspergillus flavus* in Brazil Nuts

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Keywords: *Cinnamomum cassia*; *Foeniculum vulgare*; aflatoxin B1; post-harvest preservation.

Brazil nuts (*Bertholletia excelsa* H.B.K.) are an important non-timber forest product from the Amazon region and are highly susceptible to contamination by aflatoxigenic fungi during post-harvest handling and storage. *Aspergillus flavus* is one of the major producers of aflatoxin B1 (AFB1), representing a significant food safety concern. Natural preservatives based on essential oils (EOs) may represent an alternative strategy for controlling fungal contamination. In this study, the antifungal activity of different EOs and their blends against *A. flavus* isolated from Brazil nuts was evaluated using a broth microdilution assay [1]. Commercial essential oils from *Cinnamomum cassia*, *Foeniculum vulgare*, *Eugenia caryophyllata*, *Thymus vulgaris*, *Citrus aurantium*, *Cymbopogon citratus*, and *Mentha piperita* were tested to determine minimum inhibitory concentrations (MICs). Among the oils, *C. cassia* exhibited the highest antifungal activity, with MIC values below 200 µg/mL. Blends containing *C. cassia* showed similar MIC values, indicating the major contribution of this oil to fungal inhibition. Based on antifungal activity, cost, and feasibility for industrial application, a 1:1 blend of *C. cassia* and *F. vulgare* was selected for *in vivo* application assays on the nuts, which were immersed in aqueous solutions containing the blend at concentrations of 5×, 50×, 150×, and 200× MIC, inoculated with *A. flavus*, and incubated at 25 °C for 19 days. Qualitative analysis demonstrated concentration-dependent inhibition of fungal growth, with complete inhibition observed at 200× MIC (4 g/L). Quantification of AFB1 by HPLC with fluorescence detection showed reduced toxin levels at higher blend concentrations, reaching values comparable to the natural initial contamination of the nuts. These results demonstrate the potential of EO blends as natural post-harvest preservatives for improving the safety of Brazil nuts and highlight the importance of concentration optimization for the control of aflatoxigenic fungi in food systems.

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PP2-44. Antibacterial activity of a blend of essential oils against three bacterial species associated with chronicity in acne vulgaris

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Keywords: Essential oils, antibacterial activity, acne vulgaris.

Acne vulgaris is one of the most common skin diseases and has a multifactorial origin. It is characterized by obstruction and inflammation of the pilosebaceous units due to follicular hyperkeratosis, excessive sebum production triggered by hormonal changes, and bacterial activity. Treatment includes retinoids, antimicrobials, hormonal therapies, and alternative approaches; however, in chronic cases, treatment is often complicated by bacterial resistance and the production of virulence factors. The present study aimed to evaluate *in vitro* the antibacterial activity of essential oils (EOs) against *Propionibacterium acnes*, *Staphylococcus aureus*, and *S. epidermidis* in both planktonic and biofilm forms. Among 21 plant species evaluated, a blend of bioactive essential oils (BEOs) was selected, including *Cymbopogon martini* (Roxb.) Will. Watson, *Eugenia uniflora* L., and *Varronia curassavica* Jacq. [1]. In addition, the anti-inflammatory potential (TNF- α modulation) and the effects of the EOs on the viability of human keratinocytes (HaCaT), murine fibroblasts (3T3-L1), and bone marrow-derived macrophages (BMDMs) were assessed. Of the oils evaluated, nine showed activity against all three bacterial species (MIC = 0.125–2.0 mg/mL). In biofilm inhibition assays, the BEOs showed inhibition rates of $\leq 50\%$ for *S. epidermidis*, $\leq 30\%$ for *S. aureus*, and $\leq 14\%$ for *P. acnes*. In biofilm eradication assays, the rates reached up to $\leq 58\%$ for *S. epidermidis*, $\leq 54\%$ for *S. aureus*, and $\leq 11\%$ for *P. acnes*. These results demonstrated greater efficacy than the antibiotic chloramphenicol against the three target species. The BEOs did not affect the viability of the evaluated cell lines, and TNF- α levels were reduced. These preliminary results support further antibiofilm activity studies and the subsequent development of a natural topical formulation to aid in the treatment of acne vulgaris.

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PP2-45. Modulation of the inflammatory response by *Pectis brevipedunculata* essential oil

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Keywords: *pectis brevipedunculata*; monoterpenes; cell viability; cytokines

Pectis brevipedunculata (Gardner) Sch.Bip (PB) is a plant found in the north and northeast of Brazil, belonging to the *Asteraceae* family and the genus *Pectis* L., popularly known as field rosemary [1]. Although its nematicidal, larvicidal, and antimicrobial properties have been explored in delivery systems, the influence of its essential oil on inflammatory wound modulation remains poorly studied. This study investigates the role of PB essential oil in vitro on wound healing and cytokine response. For this, the plant material was collected in São Luís, Maranhão, Brazil (2°33'20.5" S/44°18'32.7" W). The oil was extracted by hydrodistillation and characterized by gas chromatography coupled with mass spectrometry (GC/MS) using headspace analysis. Cell viability in human fibroblasts (MRC-5) and keratinocytes (HaCaT) was performed by the resazurin assay, and cytokine profiling was performed using Cytometric Bead Array (CBA). A total of 27 compounds were identified, with monoterpene hydrocarbons comprising 83.87% of the composition. The major compounds were α -pinene (56.47%), limonene (19.98%), neral (3.78%) and geranial (3.46%). Both cell lines-maintained viability above 75% at concentrations ranging from 0.00375 μ g/mL to 7 μ g/mL. CBA analysis showed a dose-dependent and selective modulation of cytokine production, with a notable reduction of IL-6 (a key mediator of acute inflammation) at higher concentrations in both MRC-5 and HaCaT cells [2]. In addition, positive correlations between cytokines were observed, suggesting that PB essential oil also remodels cytokine network connectivity.

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PP2-46. Antibacterial evaluation of Amazonian essential oils

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Keywords: *Copaifera langsdorffii*; *Pectis brevipedunculata*; *Protium heptaphyllum*; Disk diffusion.

The increasing bacterial resistance has stimulated the search for plant-derived antimicrobials, including Amazonian aromatic species rich in terpenic compounds affecting microbial growth and membrane integrity [1]. This study evaluated copaiba essential oil obtained from *Copaifera langsdorffii* oleoresin, pectis essential oil from *Pectis brevipedunculata* aerial parts, and essential oil from *Protium heptaphyllum* (breu-branco) resin against *Escherichia coli* (NEWP 0022), *Enterococcus faecalis* (NEWP 0013), and *Pseudomonas aeruginosa* (NEWP 0053). The species were registered in the Brazilian National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under codes A4D47E3 and AAFB38B. The oils were diluted in DMSO to final concentrations of 50, 25, 12.5, 6.25, and 3.125% (v/v), with DMSO as a negative control. Antimicrobial activity was evaluated by the agar disk diffusion method using 0.5 McFarland inocula, following CLSI recommendations for inoculum standardization [2]. Chloramphenicol, amikacin, and meropenem were positive controls. Pectis essential oil showed the best profile, inhibiting *E. coli* and *E. faecalis* with zones of 11.0 ± 1.0 mm at 50%, decreasing to 5.0 ± 1.0 and 3.7 ± 3.2 mm at 3.125%, respectively. The essential oil from breu-branco resin mainly inhibited *E. coli*, with zones from 9.3 ± 0.5 to 5.0 ± 0.5 mm, while *E. faecalis* was inhibited only at 50, 25, and 12.5%. Copaiba essential oil selectively inhibited the Gram-positive strain *E. faecalis* with zones from 9.0 ± 0.5 to 5.0 ± 0.5 mm, without detectable activity against *E. coli*. None inhibited *P. aeruginosa*, and DMSO showed no antimicrobial activity. Overall, Pectis essential oil exhibited the broadest antibacterial spectrum among the evaluated species, showing inhibitory activity against both *E. coli* and *E. faecalis*. This effect may be related to terpenes previously reported for *P. brevipedunculata* in the literature, including α -pinene, limonene, neral, and geranial, which have been associated with membrane disruption and antimicrobial activity [3].

Acknowledgements: FAPESPA (no 106/2024) and CNPq (no 443840/2024-5)

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PP2-47. Insecticidal Potential of Plant Essential Oils for the Sustainable Management of *Spodoptera frugiperda*

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Keywords: *Piper divaricatum*, *Piper rivinoides*, *Spodoptera frugiperda*, Biopesticides

The fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is a highly polyphagous pest whose management relies heavily on synthetic insecticides, increasing concerns about resistance and environmental safety. This study evaluated the larvicidal activity of essential oils (EO) from *Piper rivinoides* (Pr), *Piper divaricatum* (Pd), and *Melaleuca alternifolia* (Ma), as well as their binary combinations, against second-instar larvae of *S. frugiperda*. Larvae were obtained from a laboratory colony maintained on an artificial diet. Essential oils were extracted by hydrodistillation for 4 h and characterized by GC-MS and GC-FID using an HP-5MS column with helium as carrier gas. The oven temperature ranged from 60 °C to 240 °C at a heating rate of 3 °C min⁻¹. The constituents of the EO were identified by comparing the mass spectra and arithmetic index with the literature. Bioassays were performed by manual spraying EO solutions prepared in acetone at a concentration of 2.0%. After treatment, larvae were individually transferred to 24-well ELISA plates containing artificial diet, with one larva per well and 20 larvae per experimental unit. Acetone and chlorfenapyr (Pirate[®] 0.1%) were used as negative and positive controls, respectively. Mortality was recorded daily and evaluated at 24, 48, and 72 h after treatment. Chemical characterization revealed distinct EO profiles. *Piper rivinoides* EO was dominated by arylpropanoids (72.97%), whereas *Pd* EO consisted mainly of sesquiterpenes (33.21%) and arylpropanoids (16.78%). In the larvicidal bioassay, mortality differed among treatments. *Piper divaricatum* and *Ma* EO caused corrected mortality of 88.6% and 87.4%, respectively, with no statistical difference compared with chlorfenapyr (88.6%). The *Ma* + *Pd* combination also resulted in 88.6% mortality, indicating compatibility between these oils. Conversely, *Pr* EO showed the lowest activity (38.6%), whereas the *Ma* + *Pr* mixture caused intermediate mortality (54.9%), significantly lower than that caused by *Ma* alone, suggesting an antagonistic interaction. These results indicate that *Pd* and *Ma* EO, alone or in combination, are promising candidates for further development as botanical insecticides against *S. frugiperda*.

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PP2-48. Genome-Wide Mining of BAHD Genes in Lavender and Functional Validation Related to Linalyl Acetate Biosynthesis

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Keywords: *Lavandula angustifolia*; *Lavandula* × *intermedia*; BAHD; terpenoids; linalyl acetate

Lavandula plants, especially *L. angustifolia*, *L. latifolia* and their natural hybrid *L. × intermedia*, are abundance sources of essential oils (EOs), possesses significant economic and ecological value. Volatile esters especially linalyl acetate are crucial determinants of the distinctive aroma profiles and ecological functions of lavender EOs, and BAHD acyltransferases play pivotal roles in their biosynthesis. This paper introduces the lavender genome, function of LaBAHDs, their transcriptional regulatory networks, and their ecological significance by Professor Lei Shi's team.

1. Presented chromosome-level genomes for *L. angustifolia* ‘Jingxun 2’ and the hybrid lavandin ‘Super’ (with two fully phased subgenomes), unveiled the evolutionary history and the genetic components responsible for the distinct EO composition of *Lavandula* species through genome phasing^[1,2].

2. 166 LaBAHD genes were identified from the lavender genome, five crucial candidate LaBAHD genes closely associated with the biosynthesis and regulation of linalyl acetate were determined. Functional validation via in vitro enzyme assays, stable overexpression in tobacco, and transient expression in *L. latifolia* demonstrated that LaBAHD57 catalyzes the production of linalyl acetate from linalool, also increased glandular trichome density in transgenic tobacco^[2,3].

3. Two transcription factors, LaMADS-box1 and LaMYB3, were identified to directly bind the promoter of *LaBAHD57*, with *LaMADS-box1* expression paralleling the induction pattern of *LaBAHD57* and suggesting that LaMADS-box1 may act as a key positive regulator in volatile ester biosynthesis.

4. Olfactory choice assays and electroantennogram (EAG) recordings using *bombus terrestris* (*European bumblebee*) revealed that linalyl acetate significantly attracts the pollinator, with both behavioral preference and EAG response intensity increasing in a concentration-dependent manner, thus identifying it as the core volatile responsible for pollinator attraction in lavender.

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PP2-49. *Thymus mastichina*: A Promising Source of Anti-Inflammatory and Anti-Senescent Agents for Cardiovascular Health

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Keywords: *Thymus mastichina*, macrophages, cardiomyoblasts, senotherapeutic

Thymus mastichina, an Iberian endemic species traditionally used as an anti-inflammatory and antiseptic remedy, remains poorly characterized regarding its underlying mechanisms of action. This study aimed to validate the anti-inflammatory potential of its essential oil in lipopolysaccharide (LPS)-stimulated macrophages and, for the first time, investigate its senotherapeutic potential in cardiomyocyte-like cells, exploring its relevance to age-related cellular dysfunction and cardiovascular health.

The anti-inflammatory activity was assessed by quantifying nitric oxide production using the Griess assay and evaluating the expression of inflammatory mediators (iNOS, pro-IL-1 β , and COX-2) by western blot. Cellular senescence was induced with low-dose doxorubicin and analyzed through SA- β -galactosidase activity, γ H2AX nuclear accumulation, nuclear morphology, and the expression of p53 and p21 proteins.

T. mastichina essential oil significantly reduced nitric oxide production and suppressed inflammatory mediators expression in a dose-dependent manner. In addition, it attenuated key senescence hallmarks by decreasing SA- β -galactosidase activity and DNA damage signals, improving nuclear morphology, and downregulating the p53/p21 pathway.

Overall, these promising findings support the anti-inflammatory and senotherapeutic potential of *T. mastichina* essential oil and warrant further investigation for cardiovascular health applications.

PP2-50. Anticancer Potential of Hyssop Essential Oil: *In Vitro* Study on LS-174 Cell Lines

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Keywords: *Hyssopus officinalis* L. essential oil, anticancer potential, LS-174

Hyssopus officinalis L., or hyssop, is an aromatic plant in the Lamiaceae family with a sharp, pleasant smell, growing wild in Mediterranean-like areas [1]. Traditionally, it is used in culinary arts for flavoring, food preservation, and easing digestion. Due to antiseptic, antibacterial, and antifungal bioactivity, hyssop essential oil (EO) is utilized in pharmaceutical and cosmetic preparations [2, 3] and in treatment for respiratory infections and their symptoms [4]. The aim of the research was to use hyssop that grows wild in Bosnia and Herzegovina (BiH) to obtain EO, chemically characterize it, and evaluate its cytotoxicity in vitro. EO was extracted from 50 g of dried plant subjected to hydrodistillation in Clavanger for 2 h and phytochemically profiled by GC-MS. In vitro cytotoxic activity of EO against human colorectal adenocarcinoma cell lines (LS-174) was investigated by an MTT cell viability assay and expressed via the IC₅₀ value. Following the assay protocol [5], cancer cells were seeded and cultured in nutrient medium, incubated for 24 h, transferred to microplate wells, and then treated with five concentrations of EO ranging from 0.03125 to 1.000 µL/mL. Nutrient medium with non-treated cells was the control. MTT was added after 72 h of cell exposure to EO and incubated together for another 24 h. The IC₅₀ value was calculated based on absorbance measured at 570 nm. All experiments were performed in triplicates. Among the 98.9% of EO compounds identified, terpenes accounted for 78%. The main compound, eucalyptol, was closely followed by methyl eugenol and limonene. EO exhibited a concentration-dependent, fairly strong anticancer effect against LS-174 cell lines, with IC₅₀ values of 10.02 µL/mL. To the best of our knowledge, this is the first such research of *H. officinalis* EO in BiH, so further experimental studies are required to validate its anticancer mechanisms, optimize lead compounds, and assess the influence of chemotypic variations.

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PP2-51. Major Constituents and Antiproliferative Potential of *Salvia officinalis* L. Essential Oil

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Salvia officinalis L. is one of the most important aromatic genera of the Lamiaceae family, distributed worldwide. Species of the genus *Salvia* are rich in essential oils (EOs) and bioactive compounds, which explains their extensive use in folk and modern medicine [1,2] and cosmetics. This study aimed to evaluate the composition and antiproliferative potential of *S. officinalis* EO. Aerial parts were collected in the post-flowering period in the sub-Mediterranean region of Bosnia and Herzegovina. The EO obtained after 2h of hydrodistillation was analyzed by GC coupled with MS detector for chemical characterization. Samples were injected into the GC system using a split/splitless injector set to 250°C and operated in split mode at a ratio of 20:1. Separation of volatile compounds was achieved on a 30 m DB-FFAP capillary column (0.25 mm i.d., 0.25 µm film thickness). The oven temperature started at 40°C with a 10-minute hold, followed by a gradual increase of 2°C/min up to 250°C, which was maintained for an additional 5 minutes under a 1.0 mL/min flow rate. The EO antiproliferative potential was evaluated by the MTT test. The percentage reduction of the yellow tetrazolium dye was determined, and the IC₅₀ values calculated. GC-MS identified 93 constituents, accounting for 97.53% of the total EO. Main compounds were 2-bornanone (19.15%), thujone (15.57%) and eucalyptol (10.58%). The antiproliferative potential was evaluated on cervical cancer cells (HeLa). Given IC₅₀ value of 0.42 µL/mL of EO suggest strong cytotoxicity against the HeLa. The potential of synergistic effects of all identified components on the overall bioactivity of EO should not be overlooked. To obtain a more comprehensive understanding of *S. officinalis* EO activity, further investigations using theoretical calculations, followed by animal model research, are required, which will be our focus in the future.

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