MESMAP – 12

The 12th International Mediterranean Symposium on Medicinal and Aromatic Plants

MESMAP – 12

PROCEEDINGS BOOK*

ABSTRACTS & FULL PAPERS

***The symposium had a special session as "Thai Medicinal and Aromatic Plants 2026" -THAI-MAP 2026-**

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Dear Colleagues,

MESMAP Symposium series started on April 17–20, 2013 in Gazimagosa (Famagusta), Turkish Republic of Northern Cyprus (TRNC) and up to now 11 successful meetings were organized in Türkiye, TRNC and Italy. In these meetings worldwide known scientists and researchers found in the international organizing and scientific committees and distinguished research results have been presented as keynote, invited, plenary, oral and poster presentations. All the series presentations and full papers were published in the PROCEEDINGS BOOK with ISBN.

Almost all the MESMAP series collaborated with worldwide reputed scientific journals indexed by SCOPUS and THOMSON REUTERS, and many full papers were published in these journals. MESMAP-2 were indexed by Web of Science Conference Proceedings Citation Index SCIENCE (CPCI-S) / Scopus Index.

Former series of the symposium was MESMAP-11 and it was hosted by MACFRUT-2025 & Spices and Herbs Global Expo during May 6-8, 2025 in Rimini, Italy. Congress Venue was the Rimini - Expo Centre Meeting Halls. Topic of the symposium was "The Science behind the Healthy Flavor and Taste". The MESMAP-11 Symposium marked the first instance of the event being organized within a major EXPO, bringing together farmers, companies, and various stakeholders under a unified platform. The impact of this collaborative setting proved to be highly effective, with participants expressing great experience as a memorable event by the Adriatic seaside. The Organizing Committee extends its sincere appreciation to all participants of the MESMAP-11 Symposium for their valuable contributions and active engagement. We hope that the event provided an enriching experience and lasting memories.

The 12th International Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP-12) was jointly organized under the coordination of Kilis 7 Aralik University (Türkiye) and hosted by the Mediterranean Society (AMAPMED) and School of Allied Health Sciences of the Walailak University (Thailand) during January 21-24, 2026 in (Chon Buri) Bangkok, Thailand. The symposium had a special session as "Thai Medicinal and Aromatic Plants 2026" -THAI-MAP 2026. Symposium Topic was ***"The Spirit behind the Herbal Medicine & Beauty"***. The symposium was organized in the *"Land of Smiles, Happy land, Thailand"* and all the participants turned back their homes with a great happiness.

We are delighted to announce that the 13th edition of the MESMAP series is scheduled to take place in …….. in October 2027. It would be our honor to welcome you once again to the MESMAP-13 Symposium.

Sincerely,

Symposium Chair
Prof. Dr. Nazım ŞEKEROĞLU
President of AMAPMED, General Coordinator of GOFMAP
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- AMAPSEEC – Association for Medicinal and Aromatic Plants of Southeast European Countries
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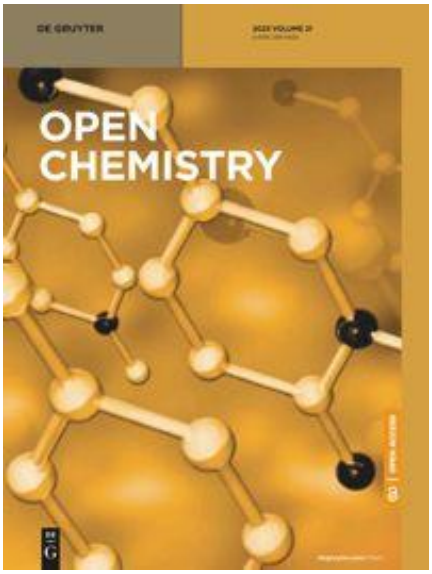

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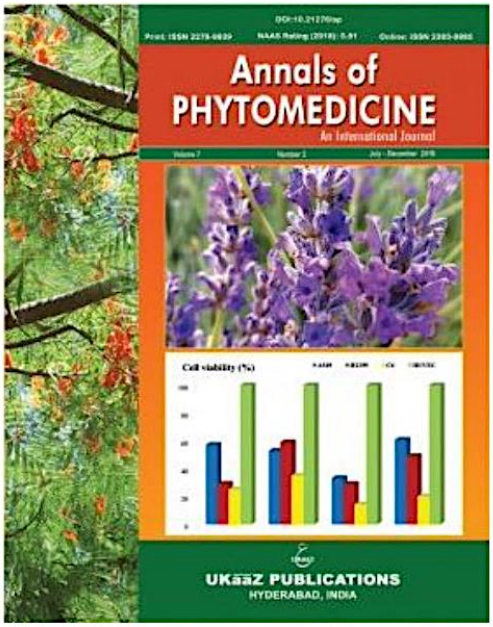

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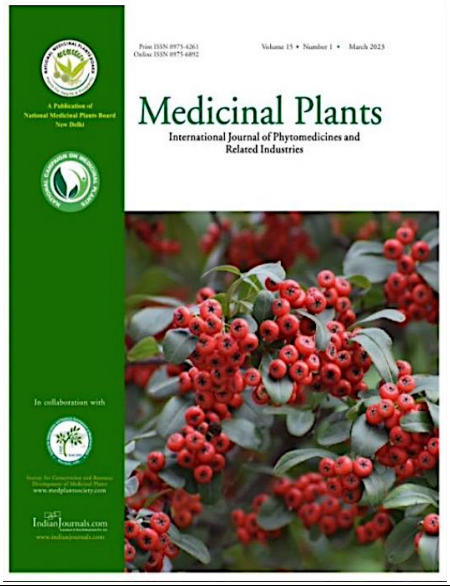

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Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)

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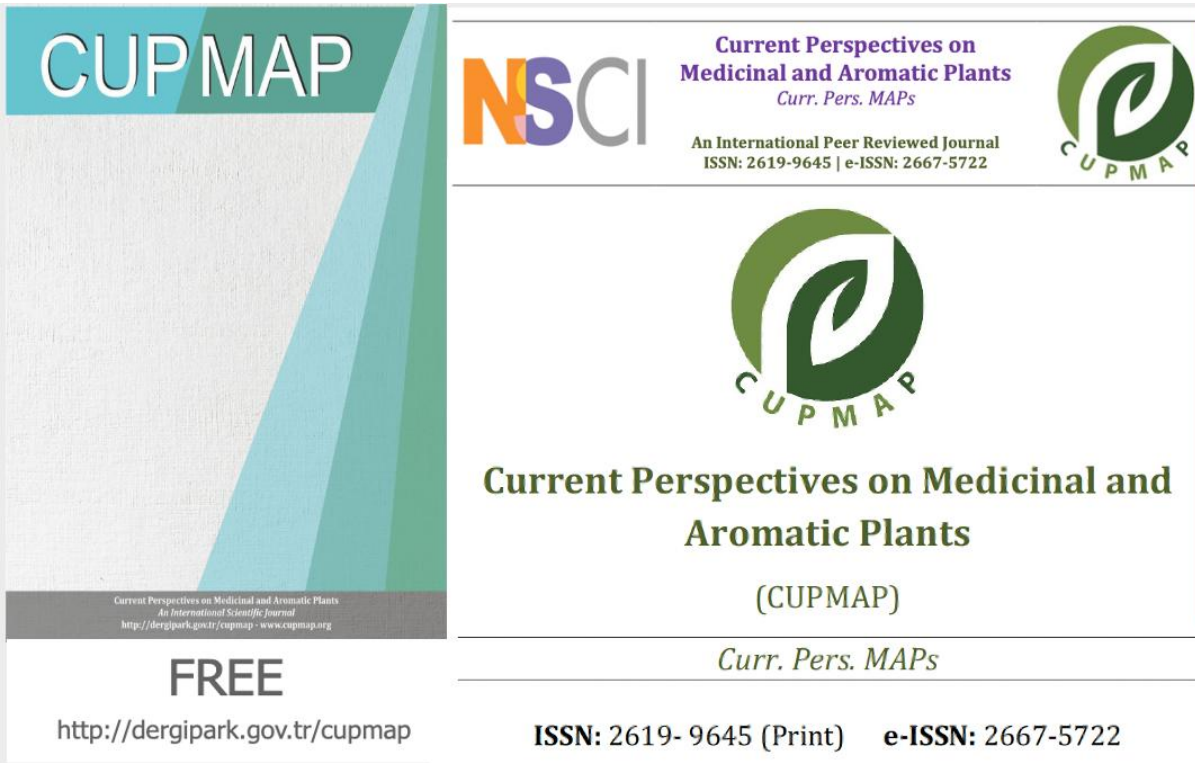

CUPMAP is an open access, peer-reviewed and refereed international journal published by MESMAP scientific group. The main objective of the CUPMAP is to provide an intellectual outlook on the scientific researches on Medicinal and Aromatic Plants. CUPMAP have distinguished goals to promote interdisciplinary scientific studies in which results could easily be used in industrial production on MAPs. This international scientific journal publishes research papers related to Medicinal and Aromatic Plants in the fields of science and technology such as Biology, Molecular Biology and Genetics, Chemistry, Agriculture, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition and Food Science, Pharmaceutical Sciences, and so on. CUPMAP publishes original research papers, applied studies, and review articles in MAPs science and technology. Special Issues devoted to important topics in the MAPs science and technology could also be published. CUPMAP Journal publishes Biannually (on June and December) in both print and on-line versions. The publication language of the journal is English. Journal of CUPMAP welcomes article submissions and does not charge any article submission or processing charges. CUPMAP is inviting papers for Volume 9 Issue 1, which is scheduled to be published on June, 2026. **Last date of submission: May 29, 2026.**

MESMAP-12 Sempozyumunda **toplam 131 bildiri** sunulmuş olup, bunların 99 tanesi sözlü, 32 tanesi ise poster sunum şeklindedir. Sunulan **sözlü bildirilerin %53,54'lük kısmı yabancı katılımcılar** tarafından sunulmuştur. Sempozyuma yaklaşık **27 farklı ülkeden** bilim insanı katılım sağlamıştır. **Sempozyuma katılım sağlayan ülkeler şunlardır:** Thailand, Poland, Romania, Morocco, China, South Africa, Pakistan, Portugal, Italy, Uman, Kuwait, Ireland, Uzbekistan, India, Brazil, Singapore, Croatia, USA, Peru, Lithuania, Slovakia, Ukraine, Nigeria, Iran, Turkish Republic of Northern Cyprus, Bulgaria, and Türkiye.

Sempozyuma katılım sağlayan katılımcılar ve sunum başlıkları 'MESMAP-12 Abstracts & Proceedings Book' kitabının içindekiler kısmında ayrıntılı olarak sunulmuştur. MESMAP-12 Sempozyumu, aşağıda yer alan YÖK Akademik Teşvik ve Yükselme kriterlerini sağlamaktadır.

İlgili YÖK akademik teşvik yönetmeliği; 17/1/2020 tarihli ve 31011 sayılı Resmî Gazete'de yayımlanan 16/1/2020 tarihli ve 2043 sayılı Cumhurbaşkanı Kararı uyarınca:

(9) (Değişik: RG-17/1/2020-31011-CK-2043/3 md.) Tebliğlerin sunulduğu yurt içinde veya yurt dışındaki etkinliğin uluslararası olarak nitelendirilebilmesi için Türkiye dışında en az beş farklı ülkeden sözlü tebliğ sunan konuşmacının katılım sağlaması ve tebliğlerin yarıdan fazlasının Türkiye dışından katılımcılar tarafından sunulması esastır. Ayrıca etkinliğin uluslararası niteliği haiz olup olmadığı hususunda, ödemeye esas teşkil etmek üzere üniversite yönetim kurulu kararının olması gerekir. Tebliğlerin değerlendirilmesinde tebliğin ilgili etkinlikte sunulmuş ve bunun belgelendirilmiş olması (etkinlik programı ve etkinliğe tebliğde ismi yer alan en az bir araştırmacının katılım sağladığını gösterir belge) esastır. Ayrıca değerlendirme için tebliğin elektronik veya basılı olarak etkinlik tebliğ kitapçığında yer alması ve yayımlanmış tam metninin sunulması gerekir.

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HONORARY SPEAKER

CARVACROL AND ITS BIOLOGICAL ACTIVITIES: AN UPDATED REVIEW

Kemal Husnu Can Baser[1], Ismail Celil Haskologlu[2] and Emine Erdag[3]

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Carvacrol, a monoterpenic phenol found abundantly in essential oils of *Origanum*, *Thymus*, *Satureja*, *Thymbra* and *Lippia* genera, is recognized for its extensive range of biological and pharmacological activities. This bioactive compound, primarily responsible for the health-promoting properties of oregano essential oil, exhibits diverse functionalities including antimicrobial, antitumor, antimutagenic, analgesic, anti-inflammatory, antioxidant, and neuroprotective effects. Its therapeutic applications extend to managing gastrointestinal ailments, reducing oxidative stress, and serving as an insecticidal agent. Furthermore, carvacrol has demonstrated potential as a feed additive and in honeybee breeding. Advances in encapsulation and nanotechnology have enhanced its stability and bioavailability, broadening its utility across food, pharmaceutical, and agricultural industries. This presentation reviews the evidence for carvacrol's biological activities and explores its possible *in vivo* mechanisms of action, emphasizing its promise as a natural therapeutic agent.

Key Words: Oregano, monoterpenic phenol, biological activity, carvacrol

KEYNOTE SPEAKER

HEALING THROUGH BEAUTY: AFRICAN MEDICINAL FLORA IN WELLNESS AND SKINCARE

Alvaro Viljoen

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KEYNOTE SPEAKER

NANO-PHYTOCOSMECEUTICALS: A SYNERGISTIC APPROACH TO ADVANCED SKIN SOLUTIONS

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Nanocosmetics is a relatively new and transformative field that integrates nanotechnology to enhance the performance of beauty and personal care products. This approach frequently employs nanocarriers to improve the delivery and efficacy of active ingredients. By encapsulating and transporting substances to targeted areas of the skin, nanocarriers maximize therapeutic impact while minimizing potential side effects. Nanocosmetics also simplify skincare regimens by combining multiple benefits—such as hydration, anti-aging, and sun protection—into single products. Beyond cosmetics, nanoforms hold significant promise for medical device formulations as well.

Our research is dedicated to developing innovative, plant-based cosmeceuticals at a laboratory scale using nanotechnology. We employ rigorous screening methods, including in vitro enzyme inhibition assays, in silico molecular docking, toxicity assessments, and cell-based studies, to identify effective plant extracts and natural compounds. For instance, we have successfully incorporated *Cotinus coggygria* extracts into niosome, nanofiber, and nanoemulgel formulations. In one application, we developed polycaprolactone (PCL) electrospun nanofibers loaded with *C. coggygria* extract and hyaluronic acid (HA) for wound healing. We have also created a patent-pending anti-acne formulation that blends plant extracts specifically designed to target Propionibacterium acnes. In another study, we investigated the 5α-reductase inhibitory activity of several *Lavandula* species, with plans to formulate them for use against alopecia.

This talk will highlight our most recent results in developing novel phytocosmeceutical formulations, achieved through our innovative interdisciplinary approach.

Key Words: Natural products, cosmetics, phytocosmetics, nanotechnology, nanocosmetics

KEYNOTE SPEAKER

ETHNOPHARMACOLOGY AND TRADITIONAL INDIAN HEALING PRACTICES: A RASAYANA LEARNING LEGACY FOR SUSTAINABLE SOCIOECONOMIC DEVELOPMENT

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Phytomedicinal products have been recognized around the world for their potential functions in management of a variety of health problems. As interdisciplinary scientific exploration, ethnopharmacology has multidimensional potentials towards development of phytomedicinal products derived from traditionally employed biologically active agents and evidence-based medicines. This includes field observations, descriptions of the utilization and bioactivities of folk remedies, botanical identification of the plant material as well as scientific exploration including phytochemical and pharmacological research. Rasayana treatment is a specialized therapeutic approach in Ayurveda, the traditional system of medicine from India. In Ayurveda, Rasayana drugs are substances used to promote longevity, enhance well-being, and improve overall health, including disease prevention. Traditional healing practices constitute an important component of global healthcare systems, rooted in indigenous knowledge, cultural traditions, and long-standing community experience. These practices offer significant potential for advancing sustainable socioeconomic development by promoting accessible and affordable healthcare, particularly in resource-limited settings. The utilization and conservation of medicinal plants support biodiversity preservation while creating livelihood opportunities through cultivation, processing, and value-added enterprises. Moreover, the increasing global demand for natural and traditional health products presents opportunities for local entrepreneurship, rural development, and inclusive economic growth. When supported by scientific validation, appropriate regulatory frameworks, and ethical commercialization, traditional healing practices can contribute to improved public health outcomes, cultural preservation, environmental sustainability, and resilient local economies. Integrating traditional knowledge with modern research and development thus represents a viable pathway toward holistic and sustainable socioeconomic development. My talk include, ethnopharmacological aspects of herbal medicine, Rasayana and potentials of Indian medicinal plants and a case study with reference to the traditional medicines towards sustainable Socioeconomic Development.

Key Words: Ethnopharmacology, Traditional Indian Healing Practices, Rasayana Learning

KEYNOTE SPEAKER

ERGOTHIONEINE: A DIET-DERIVED NUTRIENT FOR HEALTHY LONGEVITY

Barry Halliwell

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INVITED SPEAKER

FROM MEDICINAL PLANT TO MOLECULAR TARGET: *PLECTRANTHUS* ABIETANES INHIBITING P-GLYCOPROTEIN

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Cancer remains a major global health challenge, with multidrug resistance (MDR) — often driven by P-glycoprotein (P-gp) overexpression — limiting the effectiveness of chemotherapy. Africa, home to five major medicinal biodiversity hotspots, offers untapped potential for drug discovery. Among its rich flora, the genus *Plectranthus* (Lamiaceae) stands out for its bioactive abietane diterpenes. The compound 7α-acetoxy-6β-hydroxyroyleanone (Roy), isolated from *Plectranthus grandidentatus* Gürke, has shown cytotoxicity against various cancer cell lines. To enhance its activity, Roy ester derivatives were synthesized and evaluated for P-gp inhibition. A ligand-based pharmacophore model and structure–activity relationship (SAR) analysis using molecular interaction field (MIF) descriptors helped identify key features for P-gp modulation. Additionally, 6,7-dehydroroyleanone (DeRoy), isolated from *P. madagascariensis* (Pers.) Benth, was explored for its modifiable hydroxyl group.

A library of Roy and DeRoy derivatives was screened for cytotoxicity and P-gp inhibition in MDR cancer cell lines. Several compounds significantly increased intracellular doxorubicin (DOXO) accumulation, with the 12-*p*-chlorobenzoyl Roy derivative showing the strongest P-gp inhibition and selective cytotoxicity, particularly in lung cancer models. To address solubility and bioavailability limitations, Roy and a phenylpropanoate derivative were incorporated into gold nanoparticle (AuNP)-based nanosystems. These Roy-functionalized AuNPs were characterized and tested in vitro on breast cancer cell lines (MDA-MB-231, 4T1, MCF7), showing enhanced cytotoxicity and selectivity compared to free Roy. This work highlights the potential of *Plectranthus*-derived abietanes as promising leads for overcoming MDR in cancer therapy, combining phytochemistry, computational modeling, semisynthetic derivatization, and nanotechnology for improved efficacy and delivery.

Key Words: Cancer, molecular targeting, P-glycoprotein, Plectranthus-derived abietanes

INVITED SPEAKER

BIOTECHNOLOGICAL BIOFORTIFICATION OF KALE: NOBLE METAL NANOPARTICLES ENHANCE THE PRODUCTION OF HEALTH-PROMOTING METABOLITES

Łukaszyk Aleksandra[1], Kwiecień Inga[1], Kanik Anita[1], Blicharska Eliza[2], Tatarczak-Michalewska Małgorzata[2], Kozak Joanna[3], Tkaczyk Sandra[3], Jędraszek Krzysztof[3], Czarnek Katarzyna[3], Maerek Dariusz[4], Kusznierewicz Barbara[5], Szopa Agnieszka[1]

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Kale (*Brassica oleracea* var. *acephala*) is a nutritionally valuable plant, rich in glucosinolates, phenolic acids, and flavonoids, which contribute to its antioxidant, anti-inflammatory, and anticancer properties. The aim of this study was to develop an *in vitro* culture system of kale shoots and evaluate the effects of noble metal nanoparticles (NPs) (gold-AuNPs, platinum-PtNPs, silver-AgNPs, and copper-CuNPs) on biomass growth, metal bioaccumulation, and the biosynthesis of secondary metabolites. Kale shoots were cultured in liquid Murashige and Skoog medium supplemented with AuNPs, PtNPs, AgNPs, and CuNPs at concentrations of 5, 10, and 15 ppm. Biomass growth was assessed via growth index (Gi), elemental accumulation via ICP-MS, and phytochemical profiling via UHPLC-Orbitrap and HPLC-DAD. Antioxidant activity was evaluated using FRAP, DPPH, CUPRAC, and ABTS assays. Cytotoxicity was tested on human skin fibroblasts (CCD-1090Sk).

The highest biomass increase was observed with 15 ppm AuNPs Gi = 81.61), compared to the control (Gi = 72.22). PtNPs showed the highest bioaccumulation (396.45 ppm Pt; BCF = 26.43). Nine glucosinolates were identified, with neoglucobrassicin reaching 2.15 µmol/g DW (10 ppm PtNPs). Among phenolic acids, rosmarinic acid peaked at 58.49 mg/100 g DW (15 ppm PtNPs), while caffeic acid reached 26.04 mg/100 g DW (10 ppm PtNPs). Kaempferol was the dominant flavonoid, with a maximum of 24.80 mg/100 g DW (10 ppm AgNPs). Antioxidant activity was highest for PtNPs in DPPH (3.16 mg TE/g DW) and ABTS (3.68 mg TE/g DW) assays. Cytotoxicity assays confirmed the safety of nanoparticle-enriched extracts at tested concentrations. To conclude, noble metal nanoparticles act as effective elicitors in *in vitro* cultures of kale, enhancing biomass, bioaccumulation, and the production of health-promoting compounds. This strategy supports green biofortification and the development of functional foods with improved antioxidant potential.

Key Words: *Brassica oleracea* var. *acephala*, noble metal nanoparticles, *in vitro* culture, glucosinolates, antioxidant activity, biofortification

Acknowledgements: This research was funded by the National Science Centre (NCN), Poland: OPUS project no. 2023/49/B/NZ7/02428.

INVITED SPEAKER

CURCUMIN DERIVATIVE CL3 FOR TARGETING TAU OLIGOMER TOXICITY IN NEURODEGENERATIVE DISEASES

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The misfolding and aggregation of tau plays a central role in the pathogenesis of tauopathies, including Alzheimer's disease (AD). Studies have identified that soluble tau oligomers (TauO) accumulate early in disease progression (i.e., prior to the formation of neurofibrillary tangles (NFTs) and onset of AD symptoms), act like a seed for misfolding and spread of the tau pathology. Therefore, approaches that inhibit TauO formation can act as effective therapeutics for AD treatment. Curcumin, a polyphenol, plays an important role in the treatment of various diseases and studies have shown its therapeutic potential against AD. However, its low solubility in water and low bioavailability in brain per os, have restricted its therapeutic application. To overcome these challenges, we synthesized a library of novel curcumin derivatives and evaluated their effect on the aggregation behavior of TauO.

Our findings revealed that these novel curcumin derivatives interact with TauO, modulate their conformation to form larger, less toxic species. We further extended these investigations to brain-derived TauO (BDTO), isolated from the brain tissue of AD, dementia with Lewy bodies (DLB) and progressive supranuclear palsy (PSP), for diagnostic and therapeutic applications. The biochemical and biophysical characterization BDTO in the presence of the novel curcumin derivatives showed that one derivative CL3 effectively reduced BDTO toxicity as demonstrated by reduced hydrophobicity and increase in size of aggregates formed. The uptake of BDTO by the cells, seeding potency in primary cortical neurons and FRET-biosensor cells was also reduced in the presence of CL3. The results support the therapeutic potential of CL3 and lay the groundwork for our ongoing studies *in vivo* studies on animal models of tauopathies.

Key Words: Curcumin, neurodegeneration, CL3, TauO, toxicity

INVITED SPEAKER

ZEBRAFISH SCREEN FOR CLINICALLY RELEVANT NEUROACTIVE EFFECTS OF TRADITIONAL CHINESE MEDICINE HERBS

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Traditional Chinese Medicine (TCM) continues to play an important role in modern medicine. TCM herbs are commonly used to treat various brain disorders, including anxiety and depression. Complementing rodent studies, the zebrafish (*Danio rerio*) has emerged as a promising new model organism for modeling brain disorders and neuroactive drug discovery. Here, we discuss adult zebrafish-based screens to test potential neurotropic effects of extracts of selected neuroactive TCM herbs, including ginseng, saffron, St. John's wort, lavender, passiflora and albizia. All TCM extracts tested evoked overt sedative-like effects in zebrafish assays, consistent with their neuroactive profiles known clinically. We observe high sensitivity of zebrafish to neuroactive effect of TCM herbs, suggesting these fish as a valid, promising, feasible and clinically relevant *in vivo* screening platform for investigating neurotropic activity of TCM herbs.

Key Words: Traditional Chinese Medicine (TCM), zebrafish (*Danio rerio*), neurotropic activity

INVITED SPEAKER

CHAMOMILE LARGE-SCALE PRODUCTION IN UKRAINE

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Chamomile, рамашка лікарська, (*Matricaria recutita* L.), is one of the most popular medicinal plants in Ukraine. Already in the Kievan Rus (9th-11th centuries), chamomile flowers were used in the folk and domestic medicine. A tea (infusion), decoction, or tincture has long been used for treating colic, convulsions, croup, diarrhea, fever, indigestion, insomnia, infantile convulsions, toothache, bleeding, swollen gums, a folk cancer remedy, and many other uses. Gradually, between the years 1995-1999 the chamomile variety Perlyna Lisostepu (Сорт Перлина Лісостепу) was bred at the Research Station of Medicinal Plants of the Ukrainian Academy of Agrarian Sciences in Berezotocha, Lubny District, Poltava Region. The variety is characterized by its low percentage of /-/-α-Bisabolol (7.0 ±1.20 %; 0.18 ±0.05 mg. g^{-1}) and Chamazulene (0.9 ±0.10 %; 0.02 ±0.01 mg. g^{-1}) with total essential oil content in dry chamomile inflorescences about (0.25 ± 0.05 %; 2.5 ± 0.5 mg. g^{-1}). Yield of fresh plant raw materials is declared from 0.76 to 2.1 t. ha^{-1}.

After the improvement of this variety, chamomile flower drug production has been directed at special, large-scale cultivation. Chamomile plants are picked only in the stage of developed anthodia, using various types very simple harvesters or cutting machines. Sorting the chamomile biomass is not performed by sorting machines. Drying is provided mostly on imperfect on hot-air driers. The dry chamomile drug is delivered directly to processing enterprises. Unfortunately, the remaining plant material and the waste are not used to produce essential oil and extracts. Chamomile flowers, Chamomillae flos, are widely used in the preparation of herbal teas. The commercially available Ukrainian teas in the retail network, which are of low medicinal quality for the consumer.

Key Words: Cultivation, flower heads, chamomile product, long tradition

INVITED SPEAKER

HIMALAYAN MEDICINAL PLANTS OF HIGH THERAPEUTIC VALUE: A REVIEW OF PHARMACOLOGICAL PROPERTIES, TRADE OPPORTUNITIES AND CONSERVATION STRATEGIES

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The Himalayan region, stretching across eight countries and covering 18% of the Indian subcontinent, is home to approximately 1,748 medicinal plant species. These plants are crucial to the lives of tribal communities, providing essential products for food and medicine. However, over-harvesting and climate change have pushed many of these species to the brink of extinction. This review highlights the traditional, therapeutic, and pharmacological values of most important medicinal plants of high altitudes, *Picrorhiza kurroa, Aconitum ferox, A. heterophyllum, Saussurea costus, Nardostachys jatamansi, Swertia chiryata, Podophyllum hexandrum, Arnebia euchroma, Inula racemosa* and *Dactylorhiza hatagirea* from the Indian Himalayas that are being endangered species.

To protect these medicinal plants, conservation efforts are necessary, including establishing protected areas, sustainable harvesting, cultivation practices and market regulation. The conservation of these medicinal plants is crucial for maintaining traditional health systems and supporting the global pharmaceutical industry. Additionally, these plants play a vital role in maintaining ecological balance and supporting local communities. Therefore, it is essential to take urgent action to protect these plant species and ensure their sustainable use for future generations.

Key Words: Herbal plants, therapeutic values, pharmacological values, high altitudes, conservation, market potentials

INVITED SPEAKER

UNLOCKING THE POTENTIAL OF MEDICINAL PLANTS FROM UZBEKISTAN: PHYTOCHEMICAL INSIGHTS AND BIOACTIVITY ASSESSMENT

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Uzbekistan possesses a remarkably rich and unique flora, comprising 4,344 native plant species, about 10% of which are endemic. Nearly 500 wild plant species are currently cultivated across the Republic for medicinal, nutritional, and industrial uses. This rich plant diversity offers vast opportunities for systematic research aimed at discovering and characterizing valuable plant secondary metabolites. The largely unexplored chemical diversity of Uzbekistan's flora positions these species as promising resources for developing functional foods, nutraceuticals, and pharmaceutical agents. Using analytical platforms including NMR spectroscopy, UHPLC-MS, and GC-MS has enabled detailed screening of complex natural mixtures such as crude extracts, resins, and essential oils, facilitating the identification of structurally diverse compounds.

In this presentation, we have highlighted our recent phytochemical investigations of selected species from the Caryophyllaceae, Lamiaceae, and Apiaceae families. Using advanced analytical methodologies, we have revealed distinct and often markedly different metabolite profiles among species belonging to the same genus. The identified metabolites have shown relevance to several biological activities, including antifungal, antioxidant, enzyme-inhibitory, and cytotoxic effects. Our findings have confirmed that these analytical approaches are highly effective for characterizing plant chemical diversity and can be broadly applied to support natural product discovery, chemotaxonomic studies, and the development of health-promoting plant-derived products.

Key Words: Medicinal plants, phytochemical, GC-MS, UHPLC-MS, essential oils, biological activity

Acknowledgements: Financial support was provided by the Academy of Sciences of Uzbekistan.

INVITED SPEAKER

METAGENOMIC INSIGHTS INTO SECONDARY METABOLITE BIOSYNTHESIS FOR DRUG DISCOVERY

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The investigation of natural products derived from microorganisms has long been a cornerstone of pharmaceutical and biotechnological advancement. Traditionally, the discovery of microbial natural products has depended largely on the laboratory cultivation of microorganisms. However, this conventional culture-based method has accessed only a limited portion of microbial diversity estimated at roughly 1% in soil ecosystems and 0.1% in aquatic environments- leaving the vast majority of microorganisms and their immense chemical and biological potential largely inaccessible. Microorganisms that remain uncultivated, particularly those residing in extreme or insufficiently characterized environments, are thought to encode previously unknown biosynthetic pathways that give rise to structurally diverse and biologically potent secondary metabolites. Nonetheless, the inability to culture most of these microorganisms has posed a major barrier to natural product discovery. To address this challenge, a range of innovative culture-independent strategies have been developed. In the late twentieth century, metagenomics emerged as a transformative approach, enabling the direct retrieval and analysis of DNA from environmental samples without the need for cultivation.

In recent years, metagenomic approaches have enabled the identification of previously uncharacterized biosynthetic gene clusters, thereby facilitating the discovery of novel biocatalysts, antibiotics, and other bioactive metabolites. This approach is further strengthened by single-cell genomics, which provides detailed insights into the metabolic potential of individual microbial cells. In addition, advances in synthetic biology have enabled the reconstruction and heterologous expression of complex biosynthetic pathways in suitable host organisms. The convergence of these technologies with high-throughput screening methods and sophisticated analytical platforms has substantially accelerated the discovery of novel natural products with significant therapeutic potential. In this presentation, state-of-the-art approaches for accessing uncultivated microorganisms from diverse environmental niches are presented, and recent advances in natural product discovery are reviewed. In addition, the vast and largely unexplored potential of these microbial resources is underscored. Harnessing the hidden chemical diversity encoded by uncultured microorganisms is proposed as a powerful avenue for addressing critical challenges in medicine and biotechnology, thereby facilitating the development of next-generation pharmaceuticals.

Key Words: Natural products, uncultured microorganisms, metagenomics, novel therapeutics

INVITED SPEAKER

PHYTOCONSTITUENT CONTRIBUTED SYNERGY IN DUAL DRUG LOADED HOLLOW GOLD NANOPARTICLES IN EFFECTIVE MANAGEMENT OF GLIOBLASTOMA

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Glioblastoma multiforme (GBM) remains one of the most aggressive and treatment-resistant brain tumors, with current therapies hampered by the blood-brain barrier (BBB), poor tumor specificity, and drug resistance mechanisms such as MGMT overexpression. Our lab is involved in exploring the potential of glutathione-functionalized hollow gold nanoparticles (GSH-HAuNPs) as a targeted nanocarrier system for co-delivery of temozolomide (TEM) with some phytoconstituents like resveratrol (RES), quercetin and thymoquinone aiming to enhance cytotoxicity, overcome resistance, and enable photothermal therapy (PTT) via near-infrared (NIR) excitation. HAuNPs were synthesized via a sacrificial galvanic replacement method and optimized for size, zeta potential, and stability. These were PEGylated and surface-functionalized with glutathione for selective uptake via xCT receptors, overexpressed in GBM cells. TEM and RES were co-loaded, exploiting RES's p53 pathway activation to enhance TEM-induced apoptosis. The nanocarriers were characterized for drug loading, release kinetics, and photothermal response. *In vitro* cytotoxicity, apoptosis, cell cycle arrest, and uptake studies were conducted on GBM cell lines (C6, U87, LN229). *In vivo* efficacy was assessed in a Wistar rat ectopic GBM model. GSH-HAuNPs demonstrated high drug encapsulation efficiency, controlled release under near-infrared (NIR) irradiation, and significant tumor cell uptake. Combination therapy showed synergistic cytotoxicity and induced marked cell cycle arrest and apoptosis. *In vivo* studies confirmed tumor volume reduction, improved survival, and minimal systemic toxicity. The multifunctional GSH-HAuNP platform offers a promising strategy for overcoming chemoresistance and delivering synergistic therapy in GBM. This nanotheranostic system has potential for future clinical translation in glioblastoma treatment.

Key Words: Glioblastoma, gold nanoparticles, photothermal therapy, temozolomide, resveratrol, drug resistance, targeted delivery

INVITED SPEAKER

ORAL PHYTOCOSMECEUTICALS IN TRADITIONAL PERSIAN MEDICINE

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The use of cosmeceuticals has a rich and longstanding tradition in Iran, particularly among royal families. To the extent that tourists who traveled to Iran have mentioned this issue in their travelogues. One of the most used medicinal plants for this purpose has been henna, with the scientific name of *Lawsonia inermis*, which has been used for hair dying and nail polishing. Moreover, beyond its value as a beautifying agent, it has some health benefits, such as acting as a hair tonic and helping prevent hair loss, as well as helping prevent fungal skin infections. Remarkably, oral pharmaceutical products have also been used alongside topical agents for beauty and skincare. For example, Rhazes, in his book Alhavi, recommended triphala, an oral paste made from 3 medicinal plants, including *Terminalia chebula*, *Phylanthus emblica*, and *Terminalia bellerica,* for preventing hair whitening. The use of oral phytocosmeceuticals in Traditional Persian Medicine (TPM) stems from its holistic philosophy, which emphasizes the interconnectedness of internal organs, particularly the gastrointestinal tract and liver, with skin and hair health. According to TPM, cleansing the body with oral medications prior to applying topical treatments is essential for preventing the worsening of dermatological conditions. Oral phytopharmaceuticals exert their beneficial effects through various pharmacological mechanisms, including antioxidant activity, enhanced cutaneous microcirculation, prebiotic effects, immunomodulation, reduction of proinflammatory cytokines, and protection against UVB radiation. Several clinical trials have validated the efficacy of phytopharmaceuticals used in TPM; for example, *Phyllanthus emblica* fruit in female androgenic alopecia, *Ziziphus jujuba* fruit in brightening of facial skin, triphala in sculp seborrhea, and *Melissa officinalis* leaves in psoriasis. Thus, integrating oral phytopharmaceuticals with conventional treatments may offer a more comprehensive approach to promoting skin health and managing dermatological conditions effectively.

Keywords: Persian medicine, oral cosmeceuticals, medicinal plant, beauty, skincare

INVITED SPEAKER

NLRP3 INFLAMMASOME AND THE ANTI-INFLAMMATORY ACTION OF ESSENTIAL OILS

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The NLRP3 inflammasome is a crucial multiprotein complex in the innate immune system that responds to infection, cellular stress, and damage by activating the protease caspase-1. Upon assembly, the NLRP3 inflammasome triggers the release of pro-inflammatory cytokines, such as IL-1b and IL-18, inducing apoptosis. NLRP3 overactivation is linked to numerous inflammatory diseases, including arthritis[1]. Certain essential oils, such as the ones obtained from *Mentha arvensis* and garlic, as well as essential oil components such as eucalyptol, and β-caryophyllene, can inhibit the NLRP3 inflammasome[2]. *Capparis cartilaginea* is a species belonging to the *Capparaceae* family, which is known for its various traditional medicinal uses. In Saudi Arabia, the leaves and roots of *C. cartilaginea* are used in Saudi traditional medicine, primarily as an analgesic for conditions such as osteoarthritis and rheumatoid arthritis and gout, often prepared as an infusion (tea). There is limited scientific information directly linking the NLRP3 inflammasome and *Capparis*, although *C. cartilaginea* produces a very distinct essential oil rich in isothiocianates and nitriles[3].

The chemical composition of the essential oil extracted from *C. cartilaginea* was previously analysed using Gas Chromatography-Mass Spectrometry (GC-MS). The anti-inflammatory activity of the oil and its main constituents was evaluated using an *in vitro* model of lipopolysaccharide (LPS)-induced inflammation in murine and human macrophages. Inflammasome NLRP3 activation was also investigated. Previous analysis of the essential oil via GC-MS identified 41 constituents[3]. Biological activity assays revealed that *C. cartilaginea* oil and major compounds (isopropyl isothiocyanate and 2-methylbutylnitrile) exhibited significant anti-inflammatory activity, with notable reductions in MMP-9, inflammatory cytokines (IL-6, TNF-α, IL-1β), and nitric oxide (NO) production by up to 50-60% in LPS-stimulated macrophages. Inhibition of NLRP3 inflammasome activation was also observed.

This study has successfully validated the traditional medicinal use of *C. cartilaginea* essential oil for treating inflammatory conditions. Biological assays confirmed that *C. cartilaginea* oil possesses significant anti-inflammatory properties. These findings not only support traditional uses but also provide a scientific basis for potential therapeutic applications. Further *in vivo* studies and clinical trials are warranted to confirm these effects and explore their clinical relevance.

Key Words: NLRP3 inflammasome, anti-inflammatory, essential oil, *Capparis cartilaginea*

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INVITED SPEAKER

NEUROTROPHIC FACTOR-MIMICKING COMPOUNDS: VASCULAR AND NEUROPROTECTIVE EFFECTS

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INVITED SPEAKER

GREEN EXTRACTION SOLVENTS – STEP FORWARD FOR ACTIVE COSMETICS

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Cosmetic market is recording an everlasting growth, and the segment related to natural cosmetics is not an exception. As a result, the research on medicinal plants with potential use in cosmetics increases almost exponentially. However, a growing concern for the future of our planet has also led to a significant increase in research on the design of green and sustainable methods for the extraction of plant bioactive principles. Classical solvents used for extraction of plant secondary metabolites are often irritant or even toxic for the skin. They should be evaporated using elevated temperature that sometimes leads to the degradation of the extracted metabolites, often at a significant cost of time, energy, budget and the environment. However, numerous liquids of natural origin can not only be used for an efficient extraction of plant bioactive principles, but they can also participate in the overall activity of the extract and consequently the final cosmetic product. This work will focus on the plant extracts prepared using green solvents, including water, its mixture with glycerol or cyclodextrins, as well as natural deep eutectic solvents. Results related to their good extraction potential, their ability to increase the stability of the prepared extracts and their participation in the overall biological activity related to the dermatological and cosmetic potential will be presented. Examples using various medicinal plants, such as *Glycyrrhiza glabra*, *Satureja montana*, *Helichrysum italicum*, and many others will be discussed.

Key Words: Green extraction, solvent extraction, active cosmetics, secondary metabolites

INVITED SPEAKER

MEDICINAL PLANTS AS SOURCES OF BIOACTIVE MOLECULES: PHYTOCHEMICAL CHARACTERIZATION AND PRECLINICAL EVALUATION

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Medicinal plants represent an exceptional reservoir of structurally diverse natural products that continue to drive innovation in pharmaceutical research. Their secondary metabolites, including flavonoids, phenolic acids, alkaloids, lignans, terpenoids, and steroids, display a wide range of chemical architectures, from simple phenolic frameworks to highly complex polycyclic structures that frequently surpass the capabilities of current synthetic chemistry. Owing to this inherent diversity, plant-derived compounds exert selective and potent biological activities mediated through well-defined molecular mechanisms. In contemporary drug discovery, increasing emphasis is placed on elucidating the phytochemical constituents responsible for the biological effects of medicinal plants. This includes comprehensive analytical profiling, mechanistic investigations, and the development of systematic bioactivity-guided workflows. In our recent research, multiple medicinal plant species were examined through an integrated framework combining *in vitro*, *in vivo* and *in silico* studies. These investigations yielded reproducible and pharmacologically meaningful outcomes, demonstrating anti-inflammatory, wound healing, neuroprotective, and cytotoxic effects depending on the plant species evaluated.

Following the identification of active extracts, bioactivity-guided fractionation is employed to isolate the major active principles. The structures of these compounds are elucidated using advanced chromatographic and spectroscopic techniques, including HPLC, LC-MS/MS, and NMR spectroscopy. In parallel, mechanistic studies are conducted to investigate the molecular pathways underlying the observed activities, providing a deeper understanding of their biological relevance. This presentation will summarize these ongoing preclinical efforts, emphasizing the value of combining phytochemical characterization, biological evaluation, and targeted isolation strategies. Collectively, our findings highlight the substantial potential of medicinal plants as sources of new bioactive compounds and underscore their continued relevance in the search for innovative therapeutic agents.

Key Words: Biological activity, natural compounds, phytochemistry

INVITED SPEAKER

TRADITIONAL VAN HERBY CHEESE AND HERBS USED ITS PRODUCTION

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Traditional Van Herby Cheese is a unique fermented dairy product originating from the Van region of Eastern Anatolia, Türkiye, and constitutes an important component of the region's culinary heritage and rural economy. This traditional cheese is predominantly produced from raw sheep's milk and is distinguished by the incorporation of various wild and cultivated herbs during the manufacturing process. The herbs most commonly used include species from the genera *Allium*, *Thymus, Ziziphora*, *Ferula*, and *Anthriscus*, which are added either fresh or dried at specific stagesvof production. These herbs play a crucial role in shaping the characteristic sensory profile of the cheese, particularly its aroma, flavor, and texture, while also contributing to its natural preservation.

In addition to their technological and sensory functions, the herbs used in Van Herby Cheese are known for their bioactive properties, including antimicrobial, antioxidant, and anti-inflammatory effects. These properties may positively influence the microbiological stability, shelf life, and potential functional value of the cheese. The type, quantity, and combination of herbs vary according to local knowledge, seasonal availability, and traditional household practices, resulting in notable variations in chemical composition and sensory characteristics among products. Preserving traditional production techniques and documenting the ethnobotanical knowledge associated with Van Herby Cheese are essential for maintaining this culturally significant product and for supporting sustainable rural development.

Key Words: Ethnobotany, food heritage, functional foods, herby cheese, medicinal and aromatic herbs

ORAL PRESENTATION

Oral Presentations

ORAL PRESENTATION

PHYTOCHEMICAL PROFILING, CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF STOLONS EXTRACT AND FRACTIONS FROM THE MOROCCAN *SEARSIA ALBIDA* (SCHOUSB.) MOFFETT

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Searsia albida (Schousb.) Moffett, an endemic Moroccan species from Essaouira, is known for its phenolic richness and promising pharmacological potential. This study provides the first comprehensive profiling of *S. albida* stolons using UHPLC–MS/MS and evaluates their antioxidant and cytotoxic activities. Methanolic extract, dichloromethane, and ethyl acetate fractions were tested for antioxidant capacity via DPPH, FRAP, ABTS, and TAC assays, and for cytotoxicity on P3 myeloma cells. UHPLC–MS/MS analysis revealed 20 phenolic compounds. Principal Component Analysis (PCA) showed that the ethyl acetate fraction, characterized by a high chlorogenic acid content (45.36%), exhibited the strongest antioxidant and cytotoxic effects. These findings highlight *S. albida* as a promising source of natural bioactive compounds with potential applications in oxidative stress-related disorders and cancer research.

Key Words: *Searsia albida*; UHPLC–MS/MS analysis; Antioxidant activity; Cytotoxic activity

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ORAL PRESENTATION

INNOVATIVE BIOVALORIZATION TECHNIQUES: BIOCATALYTIC TRANSFORMATIONS OF PHENOLIC COMPOUNDS

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The INBIOWAL international research network pioneer's sustainable valorization of agro-industrial by-products through advanced biotechnological methods. A key focus is the biocatalytic transformation of phenolic compounds derived from oil-industry residues, such as oil cakes. Wrocław University of Environmental and Life Sciences in collaboration with Politecnico di Milano investigate biocatalytic processes to convert low-molecular-weight phenolic precursors into high-value bioactive molecules with antioxidant, antimicrobial, and anti-inflammatory properties. Methods currently under investigation include enzymatic esterification/transesterification of phenolic acids/esters (e.g. *p*-coumaric, caffeic, ferulic, sinapic) using lipases as well as whole cell fungal transformations of phenolic acids to produce corresponding aldehydes and vinylphenols with flavor and fragrance properties. The project emphasizes sustainable biocatalysis, enzyme immobilization and optimization, and scalability toward continuous-flow reactors for industrial application. The resulting bioactive phenolic derivatives hold potential for use in food preservation, cosmetics, nutraceuticals, and pharmaceuticals. By integrating green chemistry principles and interdisciplinary collaboration, INBIOWAL aims to demonstrate that biocatalytic valorization of phenolic waste streams can offer a viable, eco-friendly alternative to conventional chemical synthesis, contributing to circular bioeconomy and waste-to-value strategies.

Key Words: Biovalorization, green chemistry, biocatalysis, phenolics

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ORAL PRESENTATION

SALT-INDUCED IMPROVEMENT OF MEDICINAL AND NUTRITIONAL VALUE IN THE HALOPHYTE *LIMBARDA CRITHMOIDES* L.

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With increasing interest in sustainable crop production under saline conditions, halophyte species such as *Limbarda crithmoides* L. (golden samphire) are gaining attention for their dual value as functional foods and sources of bioactive compounds. This study explores the effect of sodium chloride (NaCl) elicitation on the metabolic profile and physiological resilience of *in vitro* shoot cultures of *L. crithmoides*, a medicinal halophyte native to coastal ecosystems. Shoots were exposed to 0, 50, 100, and 200 mM NaCl for four weeks. Growth parameters, pigment content, oxidative stress markers, and primary (soluble sugars, proteins, proline) and secondary metabolites (phenolics, flavonoids, hydroxycinnamic acids, PAL activity, shikimic acid) were analyzed, alongside detailed phenolic profiling by HPLC-DAD. While shoot growth remained unaffected, root development declined under higher salinity. Notably, NaCl treatments led to a dose-dependent increase in chlorophylls and carotenoids, alongside a reduction in hydrogen peroxide levels, suggesting improved oxidative stress management. Elicitation also triggered significant accumulation of proline and phenolic compounds, particularly at 200 mM NaCl. Phenolic acids and flavonoid derivatives with known therapeutic properties, such as gentisic acid, chlorogenic acid, 4-hydroxybenzoic acid, luteolin-7-O-glucoside, and naringenin-7-O-glucoside, were substantially enhanced. These findings highlight the effectiveness of salinity-based elicitation to stimulate the production of high-value compounds in *L. crithmoides*, without compromising shoot biomass. The results support the use of halophyte species in saline agriculture and underline their potential for biotechnological exploitation in nutraceutical and functional food sectors.

Key Words: Golden samphire, Saline cultivation, Stress physiology, Phenolic bioactives, Medicinal halophytes

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ORAL PRESENTATION

FROM TRADITIONAL KNOWLEDGE TO MODERN SKIN CARE: ETHNOMEDICINAL VALUE AND DERMATOLOGICAL POTENTIAL OF *ALCHEMILLA SPECIOSA* BUSER

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Species of the genus *Alchemilla* (lady's mantle) have a long tradition of use in traditional medicine, particularly for treating skin disorders. Topical preparations from *Alchemilla* species, such as *A. vulgaris* L., have been applied to wounds, rashes, eczema, and acne because of their astringent and anti-inflammatory properties, highlighting their relevance in ethnodermatology. Despite this rich ethnopharmacological background, *Alchemilla speciosa* has remained virtually unexplored from phytochemical and biological perspectives. This study aimed to characterize the phytochemical composition of the aerial parts of Alchemilla speciosa Buser and evaluate their in vitro biological activities, focusing on potential dermatological applications.

The chemical profiles of *A. speciosa* extracts and fractions were analyzed using LC-MS/MS and GC-MS. The total phenolic, flavonoid, and phenolic acid contents were determined spectrophotometrically. Antioxidant activity was assessed using $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radical scavenging and metal chelation assays. Enzyme inhibition studies against collagenase, elastase, and tyrosinase were conducted to assess the anti-aging and skin-lightening potential of the extracts. The antibacterial activity was evaluated against key skin-associated pathogens, including *Cutibacterium acnes*. The cytotoxicity and anti-lipid peroxidation effects were examined in normal human skin fibroblasts. *A. speciosa* was shown to be rich in bioactive compounds, including phenolic acids, flavonoids, and pentacyclic triterpenes. All extracts and fractions exhibited strong antioxidant activity and moderate inhibition of collagenase and tyrosinase, with weaker inhibition of elastase. Notable antibacterial activity against acne-associated bacteria (MIC 12.5–200 µg/mL) was observed, along with low cytotoxicity toward fibroblasts (≥80% viability at 62.5 µg/mL). The most active samples exhibited high therapeutic indices (TI ≥ 10) and effectively inhibited H_2O_2-induced lipid peroxidation, confirming their cellular antioxidant potential. Our findings scientifically validate the traditional use of *Alchemilla* species for skin disorders and identify *A. speciosa* as a promising, underexplored source of natural compounds for dermatological and cosmetic applications.

Key Words: *Alchemilla*, Rosaceae, mass spectrometry, antioxidant activity, enzyme inhibition, cytotoxicity.

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ORAL PRESENTATION

STUDY ON PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF THAI MULBERRY LEAVES

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Mulberry leaves (*Morus alba* L.) are used in many traditional remedies and health products because they contain natural phenolic compounds and have antioxidant activity. In Thailand, mulberry is grown in several regions, but there is not much simple information comparing the leaves from different areas. In this study, we examined the basic phenolic content and antioxidant activity of leaves collected from the northern, central, and northeastern parts of Thailand. We used a hydroalcoholic extraction and simple in-vitro tests. The early results showed that the leaves from different regions and cultivars were not the same, and some samples demonstrated stronger antioxidant properties. From the study, we found that the cultivar from Sakon Nakhon gave an interesting result in terms of antioxidant activity (0.43 mg/mL) in the 2nd round of extraction, while the Khon Kaen cultivar showed a significantly higher total phenolic content of 24.65 mg gallic acid equivalents (GAE) per gram of sample in the 2nd round of extraction. These findings suggest that Thai mulberry leaves may be considered as potential natural ingredients for herbal, health, and cosmetic products. Their in-vitro antioxidant activity indicates a possible relevance for applications related to oxidative stress and skin protection; however, these potential uses were not evaluated in this study and should be investigated in future research. More detailed studies can be conducted in the future.

Keywords: Antioxidant, Mulberry, Phenolic Compounds, Phytochemicals, Thailand

ORAL PRESENTATION

FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) AND ITS ACTIVE COMPOUNDS, DIOSGENIN AND TRIGONELLINE, IN THE PREVENTION OF CANCER AND AGING: A SYSTEMATIC REVIEW OF PRECLINICAL STUDIES

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Objectives: Fenugreek is an herb from the Fabaceae family, rich in phytochemicals like diosgenin and trigonelline, whose antioxidant and anti-inflammatory properties make it a candidate for the current systematic review on preventing cancer and aging, with a focus on preclinical studies.
Methods: Relevant studies were identified from the MEDLINE, Web of Science, and Scopus databases from January 2000 to April 2024. Two independent reviewers assessed the 1280 retrieved articles for English preclinical articles. Clinical articles were added as supporting data. Duplicated articles or non-original ones, as well as irrelevant papers, were excluded.
Results: A total of 231 articles were included in the current study. Significant anticancer effects were shown by fenugreek extracts and isolated compounds, including suppression of NF-κB and Wnt-β-catenin signaling, apoptosis induction, upregulation of p53, Bax, and caspase-3; downregulation of Bcl-2, and G0/G1 or G2/M cell cycle arrest. In vivo, survival increases of up to 60% over controls were observed, with tumor volume reductions ranging from 40% to 78% across models. Antioxidant enzymes, SOD, GPx, and GSH, increased by 20-50%, oxidative stress markers (lipid peroxidation decreased by up to 45% and protein carbonylation by 30%) decreased, skin elasticity increased by 10-20% in clinical trials, and animal models' cognitive performance improved (escape latency decreased by 25-40%).
Conclusions: This study offers insights into the potential application of fenugreek, diosgenin, and trigonelline as complementary therapies for managing cancer and aging. Notable mechanisms include radical scavenging, suppressing apoptosis, and limiting inflammatory pathways. However, the lack of heterogeneous clinical data highlights the essence of conducting more clinical studies in this field.

Key Words: Fenugreek, herbal medicine, nutrition, tumor, Persian medicine, phytopharmacology

ORAL PRESENTATION

GARLIC EXTRACT (*ALLIUM SATIVUM* L.) ATTENUATES NEURODEGENERATION AND MICROVASCULAR DAMAGE IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETIC RETINOPATHY

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Background: Diabetic retinopathy (DR) is a condition that arises as a complication of diabetes. It occurs when the retina gets damaged due to high levels of sugar in the blood, which affects the retina's small blood vessels and neurons. Recent studies have suggested that early treatment with natural remedies might be a viable alternative to treat and prevent the progression of DR. Garlic (*Allium sativum*) belongs to the Alliaceae family, is a common food spice that has been used for both nutritional and medicinal purposes since ancient times. Garlic has numerous health benefits and can aid in preventing several common human diseases. This is due to its antioxidant, anti-inflammatory and antimicrobial properties, as well as its ability to lower glucose, blood pressure, and lipid levels. Moreover, garlic is a cost-effective and globally available remedy. While various researchers investigated the anti-diabetic effect of garlic, very limited studies reported the impact of garlic on DR. Objective: The aim of this study was to test the effectiveness of garlic extract in restoring the retinal cells and the neurovascular unit in DR rat model.
Methods: Male Sprague-Dawley rats were randomly assigned into three groups: normal control (NC), diabetic control (DC), and diabetic treated with garlic extract (DG). Streptozotocin (STZ) was used to induce diabetes in the DC and DG groups. After one to eight weeks of treatment, retinas were enucleated, processed and stained with hematoxylin and Eosin (H&E) and immunostained with Lectin. Images of the retinas were captured and processed for diameter measurement of retinal layers. Each image was counted for cells and analyzed for vascular density, diameter measurement and counting of retinal artery and the branching arterioles using cell counter and vessel analysis. The data obtained were analyzed in GraphPad Prism 9.5.1 using a two-way analysis of variance (ANOVA) followed by Fisher's LSD test to determine statistical significance. Results: Histological results showed that by week eight, diabetic rats had a significant reduction ($p \leq 0.01$) in retinal thickness and layers compared to diabetic rats treated with garlic extract, which exhibited a significantly restored retinal thickness ($p \leq 0.05$) to a level similar to the normal control. This increase is due to the regeneration of some of their neuronal cells such as the ganglion cells, as well as the increase in the sizes of other cells, such as photoreceptor cells that were restored in diameters to levels similar to the control. Immunohistochemical analysis showed that as diabetes proceeded to week eight, a significant reduction is observed in the diabetic rats' retinal vascular density ($p \leq 0.01$) and size of the diameter of their main artery ($p \leq 0.0001$), as well as the numbers of the primary and secondary blood vessels ($p \leq 0.05$ for both) compared to the control. However, the retinas of diabetic rats treated with garlic extract recovered showing a significant increase ($p \leq 0.01$) in retinal vascular density and size of the diameter of their main artery and the numbers of the primary and
secondary blood vessels ($p \leq 0.01$ and $p \leq 0.05$ respectively) compared to diabetic rats.
Conclusion: Garlic extract can potentially enhance DR by promoting the regeneration of retinal neuronal layers and cells and preventing damage to the retina's microvasculature unit. Therefore, garlic extract can be an excellent option to restore the symptoms of DR or to prevent its progression. Healthcare providers and ophthalmologists could consider recommending or incorporating garlic as a supplementary treatment option to enhance retinal health in diabetic patients.

Key Words: Garlic, diabetes, neurodegeneration, microvascular damage

ORAL PRESENTATION

IMMUNE-ENHANCING CAPACITY OF *CALENDULA OFFICINALIS* L. ETHANOLIC EXTRACT DEPENDS ON THE RAISING SYSTEM, SEASON AND STRESS LEVELS IN GOATS

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Objective / Purpose: Marigold is a well-known plant used for its multiple therapeutic effects in ethnomedicine, mainly in dermatology and digestive tract diseases. This study investigated the influence of raising technology and stress levels of goats and also of the sampling season on the effects of an ethanolic *Calendula officinalis* extract on their immune system.
Materials and Methods: Carpathian dairy goats raised either extensively (n=20) or intensively (n=20) were subjected to blood sampling during winter (February) and spring season (May). The N/L ratio was used to measure the stress levels while the *in vitro* leukocyte blast transformation test quantified by glucose consumption was performed to evaluate the effect of an ethanolic *Calendula officinalis* extract on the adaptive immune response and implicitly, antimicrobial protective capacity of the animals.
Results: In extensively raised animals the neutrophils increased from February to May from 27.83±10.92 to 29.25± 7.68, and the lymphocytes from 64.50±10.02 to 65.083±8.43, with a stress index of 0.46±0.25 in February and 0.47±0.19 in May. In the intensively raised animals, there was a decrease of the neutrophils from 38.3±14.88 to 34±10.66, while the lymphocytes increased from 59.7±13.57 to 60.9±9.55, with stress indices of 0.76±0.62 in February and of 0.61±0.38 in May.
The stimulation index induced by the *Calendula officinalis* extract in the extensively raised animals was of 65.40±19.04 in February and 60.77±19.16 in May, while in intensively raised goats 58.00±44.55 February and 64.69±10.85 in May.
Conclusion: The extensive raising technology induced a lesser stress ($p<0.05$) to the goats during both seasons when compared to the intensively raised ones. Nevertheless, the enhancing capacity of the *Calendula officinalis* ethanolic extract on the adaptive cell-mediated response was rather depending on the levels of stress than on the technology, the intensively raised and more stressed animals responding better when the stress levels were higher (winter season).

Key Words: Goats, raising technology, season; stress, *Calendula officinalis*, adaptive immunity

ORAL PRESENTATION

PHYTOCHEMICAL, ANTIOXIDANT, AND CYTOTOXIC ACTIVITY PROFILE OF PARIJOTO ETHANOL EXTRACT (*Medinilla speciosa*) AGAINST TRIPLE-NEGATIVE BREAST CANCER CELLS MDA-MB-231

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Background: Triple-negative breast cancer (TNBC) is the most aggressive subtype of cancer. Based on Globocan 2022, breast cancer occupies the highest position in cancer incidence and mortality in women in Indonesia. Parijoto (*Medinilla speciosa*), a traditional Indonesian plant, is reported to contain bioactive compounds that have the potential to be powerful anticancer and antioxidant agents, the potential cytotoxicity to TNBC cells has not been widely researched.
Objective: This study aims to qualitatively and quantitatively analyze phytochemical profiles, antioxidant capacity, and evaluate the cytotoxic activity of Parijoto ethanol extract against TNBC MDA-MB-231 cells and determine their IC_{50} value.
Method: Extraction is carried out by maceration using 70% ethanol. The cytotoxicity test was carried out by the MTT assay method in the concentration range of 10-500 ppm for 24 hours. Cell viability was calculated based on formazan absorbance at λ 595 nm. Dose-response curve analysis was performed using linear regression to determine the value of IC_{50}.
Results: Phytochemical screening indicated the presence of phenolic compounds, flavonoids, saponins, tannins, and terpenoids. Quantification gave a total phenolic of 8.43 ± 0.70 mg GAE/g, flavonoids 132.63 ± 4.11 mg/kg, and total anthocyanins 109.77 ± 8.58 ppm; antioxidant activity was recorded at 58.94 ± 0.42% inhibition. MTT data showed a decrease in concentration-dependent viability: viability of 100% (0 ppm), 80.9% (10 ppm), 45.0% (50 ppm), 22.6% (100 ppm), 10.0% (200 ppm); anomalies (negative viability values/ >100% toxicity) appeared at ≥300 ppm. Linear regression yields an equation f(x) = 0.2338x + 71.679 (R2 = 0.774) with an IC50 ≈ 47.18 ppm. **Conclusions:** Parijoto extract contains significant phenolics and flavonoids, has moderate antioxidant capacity, and exhibits strong in vitro cytotoxic effects on TNBC MDA-MB-231 cells with an IC50 value ≈ of 47.18 ppm**,** indicating its potential as a source of anticancer compounds for the development of TNBC therapy.

Key Words: Parijoto, Medinilla speciosa, cytotoxic activity, MDA-MB-231, triple-negative breast cancer, IC_{50}

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ORAL PRESENTATION

EFFECT OF LOW-DOSE GAMMA IRRADIATION TREATMENT ON THE MICROBIOLOGICAL QUALITY AND PHYSICO-CHEMICAL CHARACTERISTICS OF *SALVIA ROSMARINUS* L.

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Food irradiation is a preservation process developed in the 1940s; however, its application only became possible after the end of World War II. The main technological effect of this process lies in the reduction of microbial load. Our study is a research work focusing on the effect of low-dose gamma irradiation on *Salvia rosmarinus* (rosemary), specifically on its microbiological quality and physico-chemical characteristics. The samples, vacuum-packed, were exposed to gamma electromagnetic radiation emitted by a radioactive cobalt-60 (^{60}Co) source at the Ionization Station of Boukhalef, part of the Regional Center for Agronomic Research in Tangier, Morocco. In this work, we evaluated the efficiency of the decontamination of *Salvia rosmarinus* leaves using low-dose gamma irradiation, through microbiological and physico-chemical analyses performed before and after the treatment.

The results showed that, after exposure to gamma rays, the overall microbial load of the plant was significantly reduced, particularly that of fecal coliforms, which decreased markedly. We also demonstrated that a 1 kGy dose was sufficient to completely decontaminate rosemary leaves artificially contaminated with *Escherichia coli*, achieving a 100% inhibition rate. The qualitative compositions of essential oils and polyphenols remained similar before and after irradiation. However, the total polyphenol content increased with both the irradiation dose and storage time. The low doses applied for decontamination confirmed the absence of residual radioactivity in *Salvia rosmarinus* leaves and did not alter the color of the plant.

Key Words: *Salvia rosmarinus,* gamma irradiation, microbiological quality, physicochemical characteristics

ORAL PRESENTATION

TIME-DEPENDENT DYNAMICS OF CELL-MEDIATED IMMUNE RESPONSES TO ETHANOLIC NETTLE PLANT (*URTICA DIOICA* L.) EXTRACT IN IMMUNOLOGICALLY MATURE CHICKENS

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Objective/Purpose: *Urtica dioica* proved to be an immune-regulating plant, with beneficial effects in human and veterinary ethnomedicine. This study aimed to investigate the adaptive immunity enhancing potential of the alcoholic nettle plant *Urtica dioica* in antigen stimulated chickens.

Materials and Methods: The 70°ethanolic nettle plant extract was prepared in-house, in the laboratory of the University of Medicine, Cluj. The on-farm investigations were carried out immunologically mature broiler chickens (n=21) aged 47 days, divided into three groups (n=7) and subjected for seven days, once daily to oral administration of 0.5 ml/bird of: ethanolic nettle extract (Group I), 70° ethanol (Group 2) and saline (Group 3). The antigenic priming was carried out injecting once each bird sc with 0.5 ml with a 5% SRBC suspension. Blood samples were collected on heparine on days 0, 7 and 14 and subjected to leukocyte blast transformation test, after mixing with RPMI 1640 (1:4) in a 96-well plate. The experimental variants were: M - control, PHA – 1µl/well phytohemagglutinin, 2.5µl/well Al – alcohol, 2.5µl/well of alcoholic extracts of *Calendula officinalis* and as well as lysate of red blood cells. After 48h of incubation the glucose consumption for cell growth was calculated based on an orto-toluidine test. The statistical significance of the results was calculated by using the Excel program.

Results: There was an increase in the spontaneous blastogenic index in nettle extract treated chickens (30.53±10.91%, versus alcohol - 26.19±4. and environment 14.23±10.15% controls) after 7 days. Nevertheless, the G1 had the lowest spontaneous transformation index after two weeks. Similarly, the *in vitro* response to marigold and cone flower were diminished in nettle treated group.

Conclusion: The nettle plant alcoholic extract improved the short-term spontaneous blastogenic response, supporting the enhanced short term adaptive immune response to the antigenic priming, but without a long-lasting effect in chickens.

Key Words: *Urtica dioica*, chickens, immunologically mature, adaptive cell-mediated immunity, antigenic stimulation

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ORAL PRESENTATION

SSR-BASED MOLECULAR APPROACHES FOR THE SELECTION AND GENETIC CHARACTERIZATION OF MEDICINAL AND AROMATIC PLANTS

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Simple Sequence Repeat (SSR) markers have become indispensable molecular tools for the selection, genetic characterization, and improvement of medicinal and aromatic plants due to their co-dominant inheritance, high polymorphism, reproducibility, and genome-wide distribution. This study synthesizes current advances in SSR-based molecular approaches and highlights their pivotal role in assessing genetic diversity, population structure, and germplasm characterization across a wide range of medicinal and aromatic plant species. Evidence from diverse taxa demonstrates that SSR markers provide high polymorphic information content, enabling precise genetic fingerprinting, cultivar discrimination, and the identification of elite genotypes with desirable bioactive traits. The integration of SSR markers into genetic diversity studies has significantly contributed to the conservation and sustainable utilization of plant genetic resources, particularly for wild and underutilized medicinal species. Recent developments in SSR marker discovery, supported by next-generation sequencing technologies, have further enhanced marker availability and efficiency, facilitating marker-assisted selection and trait mapping in breeding programs. Moreover, the complementary use of SSRs alongside other molecular marker systems, such as SNPs and AFLPs, offers a more comprehensive understanding of the genetic architecture underlying important phytochemical and agronomic traits. Overall, SSR-based molecular approaches represent a robust and versatile framework for advancing genetic research, breeding strategies, and conservation efforts in medicinal and aromatic plants, supporting their long-term sustainability and utilization in pharmaceutical, agricultural, and biotechnological applications.

Key Words: SSR markers, medicinal plants, genetic diversity, molecular analysis, plant breeding

ORAL PRESENTATION

COMPARATIVE EVALUATION OF NANO-CURCUMIN FORMULATIONS: A SYSTEMATIC REVIEW WITH TRANSLATIONAL INSIGHTS AND CLINICAL DOSE RECOMMENDATIONS

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Beyond contributing the characteristic golden color of turmeric, curcumin exerts strong antioxidant and anti-inflammatory effects due to its two phenolic hydroxyl groups, which scavenge free radicals and inhibit oxidative reactions. Curcumin's anti-inflammatory properties have been demonstrated in vitro, in vivo, and in clinical studies. Curcumin's anti-inflammatory properties are widely used in inflammatory bowel disease (IBD), but the major problem with this valuable substance is its absorption and solubility. Due to the low bioavailability of curcumin, several studies have sought to solve this problem using nanotechnology. In a systematic review, we attempted to review these studies to find an appropriate dose for curcumin in nanoform and to compare different nanocurcuminization techniques.

By searching PubMed, Scopus, and Web of Science for the terms “nano,” “curcumin,” “inflammatory bowel disease,” and “colitis,” we found 559 articles up to July 2024. After screening these articles using specific criteria, we finally isolated and reviewed 69 articles. Clinical studies that used nanocurcumin used a wide range of curcumin doses. From 80 mg to 1600 g and more, all of which had therapeutic effects. Also, one study increased the dose to 8 g and concluded that no toxicity was observed at this dose. In a comparison between different formulations, we concluded that Dual-sensitive targeted NPs, PF127-functionalized polymeric NPs, and Macrophage-targeting polymeric systems performed best. The ranking of the remaining formulations will be explained in detail.

Key Words: Curcumin, nanocurcumin, inflammatory bowel disease, nanotechnology, bioavailability, targeted nanoparticles

ORAL PRESENTATION

THE EFFECT OF *AESCULUS HIPPOCASTANUM* L. (HORSE CHESTNUT) EXTRACT ON THE PREVENTION OF PHLEBITIS INDUCED BY PERIPHERAL INTRAVENOUS CATHETERS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL

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The most common complication associated with peripheral intravenous catheter use is phlebitis. This study aimed to investigate the effect of *Aesculus hippocastanum* L. (horse chestnut) extract on preventing phlebitis induced by peripheral intravenous catheters. This study was a randomized, double-blind, placebo-controlled clinical trial involving 100 patients hospitalized at Shariati Hospital in Tehran in 2023. A convenience sampling method was employed, and the 100 patients were divided into intervention and control groups, with 50 individuals in each group, using simple random allocation. After the insertion of an IV catheter, horse chestnut cream was applied to the intervention group, while a placebo cream was applied to the control group. The severity of phlebitis was assessed over 72 hours post-implantation at 0, 24, 48, and 72 hours using the Visual Infusion Phlebitis Scale (VIPS) checklist. Data were analyzed using descriptive and inferential statistics with SPSS version 22 software.

At 24 hours post-intervention, no significant difference was observed between the groups in the incidence of various degrees of phlebitis (P=0.564). However, a significant increase in the incidence of phlebitis was noted in the placebo group after 48 and 72 hours; patients treated with horse chestnut cream demonstrated a significantly lower frequency of phlebitis compared to the placebo group at both 48 hours (P=0.014) and 72 hours (P=0.001) post-intervention. Horse chestnut cream effectively prevented phlebitis induced by peripheral intravenous catheters.

Keywords: Phlebitis, herbal medicine, medicinal plant, complementary and alternative medicine, integrative medicine

ORAL PRESENTATION

IMMUNE-ENHANCING CAPACITY OF *CALENDULA OFFICINALIS* L. ETHANOLIC EXTRACT DEPENDS ON THE RAISING SYSTEM, SEASON AND STRESS LEVELS IN GOATS

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Objective / Purpose: Marigold is a well-known plant used for its multiple therapeutic effects in ethnomedicine, mainly in dermatology and digestive tract diseases. This study investigated the influence of raising technology and stress levels of goats and also of the sampling season on the effects of an ethanolic *Calendula officinalis* extract on their immune system.

Materials and Methods: Carpathian dairy goats raised either extensively (n=20) or intensively (n=20) were subjected to blood sampling during winter (February) and spring season (May). The N/L ratio was used to measure the stress levels while the *in vitro* leukocyte blast transformation test quantified by glucose consumption was performed to evaluate the effect of an ethanolic *Calendula officinalis* extract on the adaptive immune response and implicitly, antimicrobial protective capacity of the animals.

Results: In extensively raised animals the neutrophils increased from February to May from 27.83±10.92 to 29.25± 7.68, and the lymphocytes from 64.50±10.02 to 65.083±8.43, with a stress index of 0.46±0.25 in February and 0.47±0.19 in May. In the intensively raised animals, there was a decrease of the neutrophils from 38.3±14.88 to 34±10.66, while the lymphocytes increased from 59.7±13.57 to 60.9±9.55, with stress indices of 0.76±0.62 in February and of 0.61±0.38 in May.

The stimulation index induced by the *Calendula officinalis* extract in the extensively raised animals was of 65.40±19.04 in February and 60.77±19.16 in May, while in intensively raised goats 58.00±44.55 February and 64.69±10.85 in May.

Conclusion: The extensive raising technology induced a lesser stress ($p<0.05$) to the goats during both seasons when compared to the intensively raised ones. Nevertheless, the enhancing capacity of the *Calendula officinalis* ethanolic extract on the adaptive cell-mediated response was rather depending on the levels of stress than on the technology, the intensively raised and more stressed animals responding better when the stress levels were higher (winter season).

Key Words: Goats, raising technology, season, stress, *Calendula officinalis*, adaptive immunity

ORAL PRESENTATION

INTEGRATED *IN SILICO*, *IN VITRO* EVALUATION AND PHYTO-VESICULAR DELIVERY OF THE ETHANOLIC EXTRACT OF *CONVOLVULUS PLURICAULIS CHOISY FOR* BREAST CANCER THERAPY

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Objective: The research aimed to recognize and separate the key bioactive phytochemicals from the ethanolic extract of the aerial parts of *Convolvulus pluricaulis* Choisy. It sought to assess the *in vitro* antiproliferative impact on MCF-7 breast cancer cell lines via the MTT assay. Phytosomes based formulation was further developed to improve targeted delivery, bioavailability and therapeutic effectiveness. **Methodology:** Gas Chromatography–Mass Spectrometry (GC–MS) and flash chromatography techniques were employed to identify and extract the primary bioactive components. A commercial mass spectral library was utilized to identify individual phytocomponents in GC-MS analysis. Alkaloids, flavonoids, terpenoids, glycosides and plant sterols were detected in *C. pluricaulis*. GC-MS metabolite profiling and molecular docking studies indicated the possible therapeutic effects of phytochemicals such as emicymarin, vitexin, 10-methoxycamptothecin, beta-sitosterol, stigmasterol, seneciphylline, dasycarpidanone and 4, 5-di-epiaristolochene derived from the plant. The research aimed to determine and assess the bioactive properties of the ethanolic extract of the aerial parts of *C. pluricaulis* through *in vitro* bioassay tests on MCF-7 human breast cancer cells utilizing the MTT assay. Phytosomes based formulation of the ethanolic extract was prepared and characterized for particle size, entrapment efficiency and stability to address issues of low bioavailability and poor solubility linked to herbal extracts, aiming for enhanced cellular uptake and controlled release.
Results: The binding energies for emicymarin, vitexin, 10-methoxycamptothecin, beta-sitosterol, stigmasterol, seneciphylline, dasycarpidanone and 4,5-di-epiaristolochene were determined to be -8.55, -4.83, -4.74, -7.96, -6.65, -5.02, -7.49, and -6.77 respectively, reflecting their affinities for the Estrogen (PDB ID: 6CBZ) receptor alpha. The ethanolic extract showed a notable antiproliferative effect on MCF-7 cells ($p < 0.01$) whereas the formulated phytosomes exhibited a much stronger increase in cytotoxic activity ($p < 0.001$), validating its greater effectiveness attributed to enhanced solubility, stability and targeted delivery. **Conclusion:** This study showed that *C. pluricaulis* has strong bioactive compounds that exhibit notable antiproliferative effects on hormone-dependent breast cancer. The integration of the ethanolic extract into a phytosomal carrier system increased both its bioavailability and cellular absorption while also resulting in a significantly stronger cytotoxic effect ($p < 0.001$) in comparison to the crude extract ($p < 0.01$). These results highlight the importance of phytosomes-based delivery as a potential method for creating effective and safe herbal treatments in breast cancer care.

Key Words: *Convolvulus pluricaulis*, GC-MS, Phytocomponents, *in-silico*, MCF-7, Antiproliferative, Bioavailability, Phytosomes

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ORAL PRESENTATION

URTICA PILULIFERA L. SEED AS A TRADITIONAL PERSIAN MEDICINE SUPPLEMENT, IMPROVES FOLLICULOGENESIS IN AN ANIMAL MODEL OF DIMINISHED OVARIAN RESERVE (DOR)

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Urtica pilulifera L. seed (UPS) is a Persian medicine prescription that has positive effects on female infertility by modulating uterine dystemperament. The objective of this study was to examine the therapeutic effects and elucidate the underlying mechanisms of UPS in a Cyclophosphamide (CTX)-induced model of diminished ovarian reserve (DOR) in Balb/c mice. To establish a DOR model, a single intraperitoneal dose of CTX was administered at a dose of 75 mg/kg. Twenty-five mice were randomly allocated into five experimental groups, including control (normal saline), model (DOR), DOR+50 mg/kg UPS, DOR+100 mg/kg UPS, and DOR+200 mg/kg UPS (administered via gavage) for a duration of 14 days. Subsequently, steroidal hormones levels, oxidative stress biomarkers, apoptotic indices, and histopathological alterations were evaluated. GC-MS analysis was performed to characterize the phytochemical constituents of the UPS. Results showed that the UPS extract reduced the malondialdehyde concentration and apoptosis in the DOR model as well as enhanced the superoxide dismutase activity in the ovaries. Moreover, it showed a modulatory effect on steroidal hormones such as FSH, LH, and Estradiol. Histopathological analysis revealed the therapeutic potential of the UPS extract. The main chemical components of UPS were linoleic acid (59.25%), n-hexadecanoic acid (10.36%), and oleic acid (8.29%). The results indicated that the UPS extract has a therapeutic potential in the DOR model, proposing an alternative treatment option. This potential is attributed to the reduction of oxidative stress, modulation of apoptosis, and regulation of steroidal hormones that may be associated with the beneficial effects of fatty acids on fertility improvement.

Keywords: Apoptosis, fatty acids, female infertility, persian medicine, oxidative stress

ORAL PRESENTATION

INTEGRATIVE EXPLORATION OF ENVIRONMENTAL AND DEVELOPMENTAL DRIVERS OF BIFLAVONOID BIOSYNTHESIS IN GINKGO (*GINKGO BILOBA* L.)

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Biflavonoids are a distinct subgroup of flavonoids composed of two identical or non-identical flavonoid monomers linked together. Although the first compound of this class, ginkgetin, was discovered a century ago, biflavonoids remain comparatively poorly understood. Key questions regarding the factors influencing their accumulation, their ecological roles, and the biosynthetic pathways leading to their formation are still largely unanswered. To address these gaps, our project uses the well-known medicinal plant *Ginkgo biloba* L. as a model system. We developed and validated an HPLC–DAD method enabling the simultaneous quantification and identification of five major biflavonoids in ginkgo samples. Using this analytical approach, we performed tissue-specific profiling, which revealed higher biflavonoid accumulation in tissues directly exposed to the environment. This suggests that biflavonoids may play protective or adaptive roles in response to external stimuli. Seasonal profiling further showed that the highest levels occur in yellow, senescent ginkgo leaves, indicating that biflavonoid biosynthesis or redistribution may be linked to leaf aging or seasonal physiological changes. To explore the biosynthetic pathway in more controlled conditions, we also established a ginkgo callus culture system. Elicitation experiments were conducted to investigate whether biflavonoid production could be stimulated *in vitro*, providing additional insights into the regulatory mechanisms underlying their synthesis.

Key Words: Biflavonoids, biosynthesis, ginkgo, environment, biosynthesis

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ORAL PRESENTATION

THE CAPACITY OF *SALVIA OFFICINALIS* L. (SAGE) LEAF EXTRACTS TO ABSORB HEAVY METALS

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The ability of plant extracts to absorb heavy metals plays a crucial role in environmental remediation and the restoration of contaminated ecosystems. This biological process enables plants to sequester harmful metals from soils and water, thereby mitigating their adverse effects on the environment and human health. The significance of developing plants with enhanced heavy metal absorption capacity extends beyond individual species. Furthermore, the propagation of medicinally valuable medicinal and aromatic plants (MAPs), such as *Salvia officinalis*, is hypothesized to not only reduce pollutants but also enhance the concentration of valuable secondary metabolites within them due to the effects of stress exposure. In this study, the interaction of compound *Salvia officinalis* with different transition and heavy metals was examined using UV–Vis spectroscopy in order to understand its binding behavior and selectivity. The study material, which consisted of Salvia *officinalis* leaves, was ground following the drying process and prepared as a powdered sample possessing the homogeneous particle size requisite for the analyses. Subsequently, the samples were extracted for a duration of two hours using an ultrasonic bath method with ethanol employed as the solvent. Clear spectral changes and the appearance of new absorption bands confirmed that complexation occurs, particularly with Hg, Cu, and Pb. Breakpoint analysis indicated approximate stoichiometries of Hg:Ligand ≈ 2:1, Cu:Ligand ≈ 1:1, and Pb:Ligand ≈ 2:1. These results highlight that compound *Salvia officinalis* shows a stronger affinity for Hg (II), Cu (II), and Pb (II), suggesting its promise as a selective chelating agent for environmental and ecological applications.

Key Words: Chelation; heavy metals; metal complexation; phytoremediation; secondary metabolites

ORAL PRESENTATION

MEDICINAL PLANT EXTRACTS FOR SUSTAINABLE PLANT GROWTH AND DEVELOPMENT

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The application of medicinal plant extracts as natural bio-stimulants has emerged as a promising and ecologically sustainable strategy in modern agriculture and horticulture. This review synthesizes current research on how extracts derived from medicinal plants influence key developmental stages including seed germination, vegetative growth, nutrient uptake, stress tolerance, and overall plant productivity. Numerous studies have highlighted the horticultural benefits of specific medicinal plants: neem (*Azadirachta indica*) has been shown to improve seed germination, enhance pest resistance, and suppress pathogen growth during storage; moringa (*Moringa oleifera*) promotes vegetative development due to its high cytokinin content and mitigates abiotic stress effects such as heat and salinity in crops like squash and cucurbits; and aloe vera (*Aloe barbadensis*) supports root development and water retention while also being evaluated in coatings to reduce postharvest ethylene production and decay. Other valuable extracts include garlic (*Allium sativum*) and ginger (*Zingiber officinale*), known for their antimicrobial properties, as well as turmeric (*Curcuma longa*) and holy basil (*Ocimum sanctum*), which contribute antioxidant and immunomodulatory benefits. Seaweed extracts, though not strictly medicinal plants, are also widely used biostimulants that enhance abiotic stress tolerance and nutrient uptake. Bioactive compounds such as phenolics, flavonoids, terpenoids, and alkaloids in these extracts enhance physiological and biochemical processes. This review compiles findings from recent experimental studies, noting variations in effectiveness dependent on plant species, extraction methods, application techniques, and concentration levels. Mechanisms of action include antioxidant activity, hormonal regulation, and antimicrobial effects, all of which contribute to improved plant performance and healthier growth environments. Additionally, postharvest applications of neem and moringa extracts have been reported to extend the shelf life and maintain the quality of horticultural crops such as tomatoes and papayas by reducing pathogen incidence and delaying ripening and decay. Finally, challenges such as extract standardization, dosage optimization, and potential phytotoxicity are addressed. Collectively, the evidence supports the significant potential of medicinal plant extracts as eco-friendly alternatives to synthetic agrochemicals, facilitating the transition toward more sustainable and resilient crop production systems.

Key Words: Medicinal plant extracts, bio-stimulants, sustainable agriculture, bioactive compounds, plant growth promotion

ORAL PRESENTATION

USE OF SUMAC PLANT IN FOODS

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There are 150 known species of sumac *(Rhus spp.)* belonging to the Anacardiaceae (sumac) family worldwide. However, the most widespread and commonly used species is sumac (*Rhus coriaria* L.). Sumac, botanically known as *R. Coriaria* L., is a food/spice commonly used in Mediterranean and Middle Eastern cuisines. Sumac fruits have a purple color close to red, sour taste, sour taste with a distinctive smell. The general composition of dried sumac fruit is mainly composed of moisture, essential oil, protein, fiber, carbohydrates and ash. Sumac spice is obtained by drying and grinding the fruits. This fruit, which is widely used thanks to its sour taste in Türkiye, adds the sauces with the name "Sumak Sour" when it is soaked and filtered in water. The powder of the sumac *(R. coriaria* L.*)* is traditionally used to add sourness and flavor to meat, fish, and vegetable dishes. Furthermore, sumac fruits are consumed as tea in whole form after harvesting, simply by drying them.

Sumac, known for its antioxidant, anti-inflammatory, and antimicrobial properties, is particularly preferred in complementary medicine due to these characteristics. Sumac fruits are a spice that helps fight diabetes, cardiovascular diseases, cancer, inflammatory diseases, colds, and flu. Sumac is used in the treatment of skin inflammation and disorders. Sumac is a diuretic, meaning it helps eliminate toxins from the body through urine. It can be used to treat urinary tract infections and digestive problems. Sumac sour is preferred not only for adding flavor to foods but also for its antimicrobial effect on foodborne pathogenic microorganisms. This allows the foods it is used in to retain their freshness and be preserved for longer. In conclusion, sumac is a versatile food that not only adds a unique flavor and aroma to foods but also provides valuable health benefits to nutrition thanks to its bioactive compounds.

Key Words: *Rhus coriaria* L, aromatic plants, healthy, nutritional supplement, fortification

ORAL PRESENTATION

RHAZES' (865-925 C.E.) CONTRIBUTION TO HERBAL PSYCHOPHARMACOLOGY

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During the Golden Age of Islam (8th to 14th century C.E.), significant advancements were made in the understanding and treatment of neurological and psychological illnesses. Herbal medicines were developed, laying a solid foundation for pharmacological studies on the effects of medicinal plants on psychological and mood disorders. One of the most influential figures in this field was Rhazes. In his two important works, “Al-Hawi” (known as Liber continent in Latin and The Comprehensive Book on Medicine in English) and “Al-Mansouri fi al-Tibb” (Liber Almansoris in Latin), Rhazes described various mental illnesses, their symptoms, and treatment methods. He prescribed medicinal plants to patients suffering from mental disorders, tailoring his approach to the specific conditions presented. He has utilized Saffron, Lemon balm, Opium, Aloe, and Fennel for various applications in the treatment of psychological and mental disorders. By distinguishing between different clinical approaches and treatments for behavioral and mental illnesses, he played a pioneering role in managing neurological, psychological, and cognitive disorders through herbal medicines.
Understanding Rhazes' perspective on the role and mechanism of action of drugs for neurological and mental illnesses provides valuable insight into the roots of psychopharmacology.

Key Words: Persian Medicine, history of pharmacy, psychopharmacology, psychiatry

ORAL PRESENTATION

IS THE IMMUNE EFFECT OF *CALENDULA OFFICINALIS* INFLUENCED BY REGIONAL GEOGRAPHY DURING ACCLIMATIZATION IN ANGORA GOATS?

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Objective / Purpose: Acclimatization of traded farmed animals increases stress levels due to changed geography, climate and raising technology, when the habitat of origin and the site of relocation are higly different. The study aimed at monitoring the potential of an ethanolic *Calendula officinalis* extract to alleviate acclimatization stress in relocated Angora goats.

Materials and Methods: The Angora goats (n=45) were transferred from intensive raising in a Mediterranean climate to extensiv farming in a temperate Romanian climate. The influence of rural (n=28) and urban (n=17) areas, differing by temperature and level of noise was also evaluated. Non-specific and specific immune indicators were investigated as relevant for stress and response to the *Calendula officinalis* ethanolic extract.

Results: Total leukocyte numbers increased non-significantly (from 8,214.04 ±2,124.46 to 9,016.00 ± 2,531.11/mm^3 in the rural area), and decreased from 10,612.5 ±2,533.51 to 9,072.66 ±4,021.84 in the urban area. There was a non-significant decrease in total gammaglobulin levels in both areas during the acclimatization period (from 0.098 ± 0.024 optical density units - ODU to 0.050 ± 0.020 ODU and from 0.111 ± 0.031 to 0.090 ±0.042, respectively). The significant ($p<0.01$) increase in circulating immune complexes' levels from 0.3 ± 0.02 to 125 ± 63 and from 1.01±0.2 to 120±60.02 conventional units proved the acclimatization attempt to a microbiologically different environment. The *Calendula officinalis* extract exerted a positive influence on phagocytosis in the urban group. The cell-mediated immunity was more influenced by the *C. officinalis* extract in the urban than in the rural area, (-5.98 ± 48.1 to 4.81 ± 15.12% and from - 0.87 ± 34.93 to 3.96 ± 17.07%, respectively).

Conclusion: The acclimatization to a new habitat was more strongly influenced by the relocation to the urban than the rural area, while the *Calendula officinalis* extract showed stress alleviating potential for the species.

Key Words: Angora goats, acclimatization stress, geographic region, *Calendula officinalis*, immunological profile

ORAL PRESENTATION

ANTIFUNGAL ACTIVITY OF SOLID RESIDUE FROM HYDRODISTILLATION OF *SALVIA ROSMARINUS* SPENN.

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The hydrodistillation process generates high amounts of by-product, namely solid and water residues. The present study aims to evaluate the chemical composition of solid residue extract from the hydrodistillation of *Salvia rosmarinus* Speen. *(SRE)* using UHPLC-DAD-ESI/MS and its antifungal effect against five phytopathogenic fungi. Results showed that the polyphenol and flavonoid content of SRE are 0.44 mg GAE/g DW and 0.12 mg QE/g DW, respectively. UHPLC-DAD-ESI/MS analysis revealed that the predominant compounds in SRE are Rosmanol (28,31%), rosmarinic acid (7.95%), carnosol (7.90%),), and rosmadial (7.59%),). The antifungal activity results showed that the SRE revealed a variable activity depending on the tested species. Thus, results showed a reduction in mycelial growth of *Fusarium moniliforme, Fusarium oxysporum*, and *Penicillium* at all concentrations tested and that of *A. ochraceus* at high concentrations. However, *Rhizopus* sp. was the most resistant even at high concentrations. These findings suggest that SRE may be a more effective natural alternative for controlling the growth of phytopathogenic fungi, especially in agriculture and food industries.

Keywords: *Salvia rosmarinus* Speen., hydrodistillation, by-products, chemical composition, antifungal activity

ORAL PRESENTATION

THE IMPORTANCE OF ROSEHIP CULTIVATION IN THE YOZGAT PROVINCE OF TÜRKİYE

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Türkiye is in a position to enable growth due to its geographical and ecological regions, and has a large amount of genetic diversity and many fruits, vegetables, vineyards and ornamental plants. The genus *Rosa* covers more than 100 species in the Northern Hemisphere, including the temperate and subtropical zones, and Anatolia is home to 27 of these native species. Rosehip grows naturally in almost every region of Türkiye, particularly in the central and northern Anatolian regions, such as Kastamonu, Çorum, Amasya, Tokat, Sivas, Gümüşhane, Erzincan and Erzurum. In Yozgat, 254 species belonging to 47 families have been identified, 62 of which are endemic. In Yozgat, summers are generally hot and dry, winters are cold and rainy, and there are significant temperature differences between night and day, as well as between summer and winter. In Yozgat, 254 species belonging to 47 families have been identified, 62 of which are endemic. Rosehip is a species that may have economic importance in Yozgat. It has a high nutritional value and grows naturally in all of Yozgat's provinces. However, rosehip is not suitable for fresh consumption or as a table fruit; it is an industrial fruit that can only be consumed after processing. It is used to make products such as rosehip marmalade, fruit juice and rosehip tea, which are produced in 20 different facilities across the country. As well as being used for their nutritional value, rosehips are used for medicinal purposes, as rootstock for roses, as landscape plants and hedgerows, to prevent erosion, and the waste fruits and their seeds are used as animal feed. Increasing cultivation will be possible by cultivating cultivars detected through selection in naturally grown populations and by establishing gardens. This will lead to the cultivation of productive rosehip varieties with economic importance.

Key Words: Rose hips, Yozgat, Türkiye, nutritive value, usage patterns

ORAL PRESENTATION

COMPLEMENTARY AND ALTERNATIVE MEDICINE FOR SEXUAL DYSFUNCTION: AN UMBRELLA REVIEW OF THE SYSTEMATIC REVIEWS AND META-ANALYSES

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Introduction: Sexual dysfunction can affect self-confidence, quality of life, and relationships. Due to side effects from conventional medicine, many clinicians seek complementary and alternative medicine solutions. Our aim is to provide comprehensive evidence on the effectiveness of these options for addressing sexual dysfunction.
Method: This umbrella review followed a registered protocol (PROSPERO: CRD420251141964) and PRISMA guidelines, searching databases including PubMed, Web of Science, Scopus, and Google Scholar. The search terms used were (Sexual dysfunction OR Dyspareunia OR Premature ejaculation OR Retrograde ejaculation OR Erectile dysfunction OR Impotence, vasculogenic OR Vaginismus OR Libido) AND (Plant OR Ethno OR Pharmacognosy OR Plant Preparations OR Herb OR Extract OR Phyto OR Complementary OR Traditional OR Natural OR Alternative medicine). Irrelevant, preclinical, or non-English studies were excluded. Of 1,081 articles, 30 were included in the final analysis. The quality and validity of the included meta-analyses were assessed using the AMSTAR-2 and GRADE frameworks.
Result: We examined complementary and alternative medicine, focusing on herbal medicine and acupuncture. We found significant correlations between sexual function and factors like the Female Sexual Function Index (FSFI) for women and the Intravaginal Ejaculatory Latency Time (IELT) and International Index of Erectile Function (IIEF) for men. Various herbs, including Tribulus terrestris, Maca, Yohimbine, Saffron, Rosa damascena, and Ginkgo biloba, showed potential benefits for sexual function in both genders.
Conclusion: Tribulus terrestris, ginseng, and saffron positively impact IIEF-15 and IIEF-5 scores, which evaluate sexual function. Rosa damascena enhances sexual desire, orgasms, and satisfaction, while saffron improves female arousal, lubrication, and pain symptoms. Maca root also boosts sexual function and libido, though its effects depend on dosage. Most studies on these substances are of low quality, indicating a need for further clinical trials to better understand their effects and potential side effects in complementary medicine.

Key Words: Sexual dysfunction, complementary and alternative medicine, umbrella review, herbal medicine, acupuncture, Persian medicine

ORAL PRESENTATION

EFFECTS OF GRAPES AND GRAPE PRODUCTS ON HUMAN HEALTH

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Grapes, with approximately 75 million tons of fresh production on 67 million hectares worldwide, are among the most widely cultivated fruit species. The reason for their extensive production is not only their consumption as table, raisin, and wine grapes, but also their suitability for a wide range of processed products including molasses, vinegar, jam, compote, fruit leather, köfter, and bastık. In addition, grape seeds and seed oil are valuable components used in the pharmaceutical and cosmetic industries.

Grapes and grape-derived products have numerous health-promoting properties. Studies have demonstrated that not only the flesh, but also the seeds, skins, and stems contain highly valuable compounds. Due to the presence of secondary metabolites in grapes, they exhibit antioxidant, anticancer, antibacterial, antidiabetic, cardioprotective, hepatoprotective, and neuroprotective effects. Grapes are rich in resveratrol as well as other polyphenols in their chemical composition. It is known that grape juice, seeds, and seed oil each contain high levels of bioactive compounds at varying proportions. White and red grape juices are significant sources of minerals (Mg, Ca, Mn, Fe, Cu, Zn, Si, S, Cl), and exhibit remarkable antioxidant and antimutagenic activity. Grape seeds are also rich in fiber (40%), essential oils (16%), protein (11%), and tannins (7%). Grape seed oil contains oleic, palmitic, stearic acids, and trace amounts of other fatty acids. Owing to its phenolic components such as phytosterols, tannins, tocopherols, proanthocyanidins, flavonoids, resveratrol, phenolic acids, and carotenoids, grape seed oil presents notable antioxidant activity. Due to this rich composition, interest in grapes and grape products has been steadily increasing, and the use of grape extracts as dietary supplements is becoming more widespread. This review discusses the effects of grapes and grape products on human health.

Key Words: Grape, secondary metabolite, polyphenol, resveratrol, anthocyanin

ORAL PRESENTATION

ARGANIA SPINOSA FRUIT SHELL EXTRACT SUPPRESSES CHEMICALLY INDUCED SKIN CANCER IN A MOUSE MODEL

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Argania spinosa is a Moroccan endemic tree well-known for its oil (argan oil), which is extracted from the almonds of its fruits. The argan fruit shell, a by-product of argan oil extraction, has demonstrated a melanogenesis-promoting effect and antioxidant properties due to its rich chemical composition. Despite its many protective effects on the skin, the in vivo effects of argan fruit shell extract (AFSEE) on skin cancer have not been reported so far. This study was designed to investigate the chemo-preventive effect of AFSEE on chemically induced skin cancer in mice. A two-stage model of skin papillomagenesis, using DMBA plus croton oil, was employed for the study, and the chemo-preventive effect was evaluated using morphological, histopathological, and biochemical features. AFSEE inhibited the development of papillomagenesis, reduced lipid peroxidation levels in skin and liver tissues, and increased antioxidant enzyme activities. These findings highlight the chemo-preventive effect of argan fruit shell extract against the development of skin cancer without causing any signs of toxicity in animals.

Keywords: *Argania spinosa*, fruit shell, skin tumor, antioxidant enzymes, lipid peroxidation, carcinogenesis, hyperplasia

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ORAL PRESENTATION

MAPPING TRADITIONAL PHARMACEUTICAL PRODUCTS IN IRAN: A DESCRIPTIVE CROSS-SECTIONAL STUDY

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Traditional Persian Medicine is one of the oldest medical systems all over the world which originated in ancient Persia (Modern Iran). Traditional Pharmaceutical Products (TPP) in Iran are defined as products produced based on the Traditional Persian Medicine text-books. This descriptive cross-sectional study reports the plant diversity and frequency of use, dosage forms, non-herbal medicinal materials, domestic vs. imported species, and related topics. For this purpose, the official list of TPP was obtained from Iran Food and Drug Organization in March 2025. The list of ingredients of each product was manually extracted. The results of this evaluation showed that total of 531 TPP are nationally available which are made from 247 medicinal ingredients, including 216 herbs, 16 minerals, 14 animals/ animal materials, and 1 fungus. The most popular dosage forms are medicinal oils (150), followed by syrups (116), capsules (111), tablets (44) and ointments (42). The most abundant plants in TPP were *Rosa damascena* (70), *Pistacia lentiscus* (49), *Viola odorata* (48), *Olea europaea* (41, mostly as oil), and *Terminalia chebula* (40). The most popular plant parts used were fruits (55), followed by seeds (40), roots (23), leaves (19), and flowers (18). Amongst non-herbal materials, honey (81) and beeswax (27) were the most popular ingredients. The only fungal species used in these products is *Laricifomes officinalis*. Lack of scientific names on the products' labels, ambiguity in ingredients listing and standardization, and high proportion of imported plants are among the challenges which need to be addressed in future policy-making in this field.

Key Words: Traditional medicine, phytotherapy, Persian medicine, Iran

ORAL PRESENTATION

QUANTITATIVE HPTLC ANALYSIS OF B-SITOSTEROL FROM *VANDA ROXBURGHII* AND DEVELOPMENT OF A BIOACTIVE FILM-FORMING SPRAY

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Vanda roxburghii (Orchidaceae) is a traditionally valued medicinal plant known for its anti-inflammatory, antimicrobial, analgesic, antifungal, antiulcer, and antioxidant activities, attributed to bioactive constituents such as β-sitosterol, flavonoids, and glycosides. The present study reports the development and validation of a robust HPTLC method for the quantification of β-sitosterol in *V. roxburghii* and its incorporation into a bioactive film-forming spray formulation. Chromatographic separation was achieved on silica gel 60 F254 plates using a mobile phase consisting of toluene:ethyl acetate:n-hexane:formic acid (7:2:1:0.2, v/v/v/v), with densitometric detection after derivatization using anisaldehyde–sulfuric acid reagent. The method demonstrated good linearity over the range of 50–250 ng/spot with a correlation coefficient (r^2) of 0.9985. The limits of detection and quantification were found to be 62.62 ng/spot and 155.40 ng/spot, respectively, confirming the sensitivity and reliability of the method in accordance with ICH guidelines. The quantified β-sitosterol extract was subsequently incorporated into a film-forming spray system optimized using Design of Experiments (DoE). Excipients such as Eudragit® S-100, HPMC, PEG-400, glycerine, and an ethanol–acetone solvent system were selected to achieve desirable film-forming and delivery characteristics. The optimized formulation exhibited suitable viscosity (188.41 cps), density (3.7319 g/cm³), and rapid drying time (106.12 s), indicating its potential as an effective topical delivery system. The study highlights the successful integration of validated phytochemical quantification with formulation development, supporting the therapeutic applicability of *V. roxburghii*-based bioactive spray systems.

Key Words: β-sitosterol, HPTLC, *Vanda roxburghii*, film-forming spray, method validation, design of experiments

ORAL PRESENTATION

MICROWAVE-ASSISTED CARBON QUANTUM DOTS FROM *URTICA DIOICA* SUBSP. *DIOICA* L.: RESEARCH INTO THEIR ANTIOXIDANT, ANTI-INFLAMMATORY, AND ANTI-CANCER POTENTIAL

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Cancer continues to represent a major global health concern, maintaining high mortality rates despite substantial progress in diagnostic and therapeutic approaches. In recent years, research has increasingly focused on the development of innovative nanocarrier systems designed to improve drug delivery, bioavailability, and therapeutic efficacy. Among these, zero-dimensional quantum dots (QDs) have emerged as promising nanomaterials due to their tunable physicochemical properties, ultra-small size (1–10 nm), and unique photoluminescent behavior. Since the discovery of carbon-based quantum dots (CQDs) in 2004, their synthesis from renewable natural sources, including medicinal plants, has attracted considerable attention [1]. Given the well-established pharmacological profile of *Urtica dioica* L., which exhibits antioxidant, anti-inflammatory, cardioprotective, antiproliferative, and cytotoxic activities [2], it was selected as the source material for CQD synthesis in the present cancer-oriented study. The ethanol extract (UDE) and its derived CQDs (UDEQ) were prepared from the aerial parts of *U. dioica* subsp. *dioica* L, which was collected from Kocaeli-Türkiye. Total phenolic, flavonoid, and triterpenoid contents were quantified, while antioxidant and anti-inflammatory activities were evaluated using DPPH radical scavenging and *in vitro* iNOX inhibition assays. Cytotoxic effects were assessed against A549, B16-F10, MCF-7, MDA-MB-231, and HT-29 cancer cell lines, as well as healthy dental fibroblast and J774 macrophage cells. Microwave-assisted synthesis produced UDEQ with an average particle size of 10.39 ± 0.55 nm, a zeta potential of –3.33 ± 2.59 mV, and a quantum yield of 11.66%. UDE exhibited higher phenolic and flavonoid contents than UDEQ, consistent with stronger DPPH radical scavenging activity. Both preparations were selectively cytotoxic toward malignant cells while remaining non-toxic to normal lines at 320 µg/mL. Regarding anti-inflammatory activity, UDEQ exerted stronger iNOX inhibition than UDE.

Key Words: *Urtica dioica* subsp. *dioica* L, carbon quantum dots, anti-inflammatory, cancer

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ORAL PRESENTATION

ANTIMICROBIAL EFFICACY AND CYTOTOXICITY OF *PELARGONIUM GRAVEOLENS* AND *PINUS SYLVESTRIS* ESSENTIAL OILS AGAINST ANTIBIOTIC-RESISTANT BACTERIA FROM *SCIURUS VULGARIS*

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Objective: This study evaluated the antimicrobial activity and cytotoxicity of *Pelargonium graveolens (citronellol 36.4%, geraniol 13.4%) and Pinus sylvestris (α-pinene, ~40%)* essential oils against bacterial isolates from *Sciurus vulgaris*, including both Gram-positive (*Staphylococcus*, *Bacillus*, *Enterococcus*) and Gram-negative (*Escherichia*, *Enterobacter*, *Pseudomonas*) genera.
Materials and methods: Pure 24-hour cultures of 20 bacterial strains exhibiting a multiple antibiotic resistance (MAR) index ≥ 0.2 were used for the assessment. Essential oils were tested using both the agar diffusion (aromatogram) and broth microdilution methods to determine minimum inhibitory (MIC) and bactericidal (MBC) concentrations. In parallel, cytotoxicity was assessed on squirrel fibroblasts by measuring optical density and calculating cell viability.
Results: *Pelargonium graveolens* demonstrated moderate to strong antimicrobial activity, particularly against Gram-positive bacteria, with MIC values ranging from 0.25% to 4% and bactericidal effects at higher concentrations. Its activity against resistant isolates was comparable to, and in some cases exceeded, that of several standard antibiotics tested in parallel. *Pinus sylvestris* exhibited weak antimicrobial effects, with MIC values between 1% and 8%, and no consistent bactericidal activity, although minor inhibition was observed for certain strains. Both oils preserved high fibroblast viability (≥83%) at the highest concentrations tested, indicating a favorable safety profile.
Conclusion: Overall, *Pelargonium graveolens* showed selective and effective antimicrobial behaviour against antibiotic-resistant bacterial communities from *S. vulgaris*, while *Pinus sylvestris* displayed limited efficacy. Both disk diffusion and microdilution assays reflected similar susceptibility trends, supporting the reproducibility of the antimicrobial effects. The safety of these oils at bioactive concentrations supports their prospective application for managing bacterial infections in wildlife and encourages further *in vivo* investigations.

Key Words: *Pelargonium graveolens*, *Pinus sylvestris*, essential oils, antimicrobial activity, antibiotic-resistant bacteria, cytotoxicity

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ORAL PRESENTATION

IN VITRO ANTIBACTERIAL PROPERTIES OF PEPPERMINT, *SALVIA FRUTICOSA*, *SALVIA OFFICINALIS*, AND *OCIMUM BASILICUM* ESSENTIAL OILS AGAINST CLINICALLY RELEVANT PATHOGENS

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Essential oils obtained from medicinal plants have long been examined for their antimicrobial potential, particularly as interest grows in novel strategies to address rising antibiotic resistance. This study investigated the in vitro antibacterial activity of four widely used essential oils—peppermint (Mentha piperita), *Salvia fruticosa*, *Salvia officinalis*, and basil (*Ocimum basilicum*)—against a panel of clinically relevant bacterial strains. The tested organisms included Escherichia coli NCTC, E. coli ATCC, Staphylococcus aureus, Enterococcus faecalis, Acinetobacter baumannii, and Pseudomonas aeruginosa. Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method in 96-well microplates. Essential oils were prepared in twofold serial dilutions in Mueller–Hinton broth, and standardized bacterial inocula were added to each well following CLSI/EUCAST recommendations. After incubation at 37°C for 18–24 hours, the lowest concentration that fully inhibited visible bacterial growth was recorded as the MIC value. The essential oils exhibited varying levels of antibacterial activity, with more pronounced effects against Gram-positive species. These findings provide comparative baseline data on the antibacterial properties of selected medicinal essential oils and contribute to the growing interest in plant-derived agents as potential supportive options alongside conventional antimicrobials.

Key Words: *Salvia fruticosa, Salvia officinalis, Ocimum basilicum*, antibacterial, essential oil

ORAL PRESENTATION

ESSENTIAL OILS FROM OMANI MEDICINAL PLANTS: NATURAL ANTIFUNGAL AGENTS FOR PLANT DISEASE AND AFLATOXIN MANAGEMENT

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Essential oils (EOs) extracted from Omani medicinal and aromatic plants (MAPs) possess strong bioactive properties that make them promising natural tools for plant disease management and food safety enhancement. Comprehensive studies on *Zataria multiflora* (Shirazi thyme), *Heliotropium bacciferum*, *Ocimum dhofarense*, *Kleinia odora*, and *Myrtus communis* revealed pronounced antifungal and antimicrobial effects against major phytopathogens including *Aspergillus flavus*, *Pythium aphanidermatum*, *Fusarium* spp., and *Botrytis cinerea*. Among these, *Z. multiflora* EO showed exceptional efficacy by inhibiting *A. flavus* growth and suppressing aflatoxin B_1 biosynthesis, highlighting its potential as a safe alternative for mycotoxin management in stored produce. EOs from *H. bacciferum* and *O. dhofarense* demonstrated notable AFB_1 detoxification capacity, while *K. odora* oil exhibited strong inhibitory effects on toxigenic fungi. GC–MS profiling confirmed that the antifungal activity correlates with high concentrations of phenolic monoterpenes such as thymol, carvacrol, and eugenol, along with oxygenated sesquiterpenes and other volatile metabolites. Greenhouse and postharvest trials validated these EOs as effective treatments for damping-off control in cucumber and reduction of fruit rots in tomato and strawberry. The accumulated evidence positions Omani MAP-derived essential oils as sustainable, low-residue alternatives to synthetic fungicides, offering environmentally friendly components for integrated pest and toxin management systems. Advancing these bioactive oils toward standardized formulations could significantly strengthen clean-label plant protection and mycotoxin control strategies across arid-region agriculture.

Keywords: Essential oils, antifungal activity, aflatoxin detoxification

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ORAL PRESENTATION

FROM FOLK REMEDY TO DERMOCOSMETIC POTENTIAL: PHENOLIC-RICH EXTRACTS OF *PSIDIUM CATTLEYANUM* AS MULTIFUNCTIONAL AGENTS FOR SKIN HEALTH

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Psidium cattleyanum Sabine (F. Myrtaceae), commonly known as strawberry guava or araça, is traditionally used in folk medicine for the treatment of infections, gastrointestinal disorders, and inflammatory conditions. Despite its ethnopharmacological relevance, comprehensive studies on its dermatological potential remain limited. The present study aimed to characterize the phytochemical composition of *P. cattleyanum* leaves and fruits and to evaluate their *in vitro* biological activities with particular emphasis on skin-related applications. Leaves and fruits collected on Santo Antão Island (Cape Verde) were extracted using methanol–acetone–water (3:1:1, v/v/v) and 70% ethanol, followed by fractionation. Total phenolic, flavonoid, and phenolic acid contents were determined spectrophotometrically. UHPLC-DAD-ESI-IT-MS profiling identified 42 compounds in the extract. Antioxidant capacity was assessed using $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assays. The potential anti-aging and skin-lightening properties were evaluated through inhibition of collagenase, elastase, and tyrosinase. Antibacterial activity was examined against skin-associated pathogens, including *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. Cytotoxicity was assessed on GMK cells using the MTT assay.

The methanol–acetone–water extract and its butanolic fraction exhibited the highest polyphenol content and strongest antioxidant activity. Butanolic and ethyl acetate fractions showed the most pronounced inhibitory effects against collagenase, elastase, and tyrosinase. All tested extracts demonstrated bacteriostatic activity against acne-associated bacteria and showed synergistic or neutral interactions with selected antibiotics, without antagonism. No significant cytotoxicity was observed at biologically active concentrations. Overall, *P. cattleyanum* leaf extracts represent a rich source of bioactive phenolic compounds with promising antioxidant, anti-aging, and antimicrobial properties, highlighting their potential application in dermocosmetic and phytopharmaceutical formulations.

Key Words: *Psidium*, Myrtaceae, anti-aging, antioxidant, antibacterial

Acknowledgements
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ORAL PRESENTATION

AN ETHNOBOTANICAL STUDY OF MEDICINAL PLANTS IN SEBEN AND KIBRISCIK (BOLU-TURKIYE)

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This study presents significant ethnobotanical information on folk medicinal plants and their ethnopharmacological uses in the Seben and Kıbrıscık districts of Bolu Province. The aim of this study is to collect and identify the plants used by the local people for therapeutic purposes and to reveal information about traditional herbal medicine. The research was conducted between 2024 and 2025 and is based on plant specimens collected during field study. A total of 77 taxa of plants used in folk medicine, belonging to 28 families, were identifiedin this study. The most common families were Rosaceae (17%), Lamiaceae (11.7%), Boraginaceae (5.2%), Fabaceae (5.2%), and Papaveraceae (5.2%). In addition, a cultural importance index (CI) and use report (UR) were calculated for each species. Based on the CI, the most important plants were *Pinus sylvestris* L., *Rosa canina* L., *Juglans regia* L., *Hypericum perforatum* L., and *Urtica dioica* L. Analysis of preparation methods revealed that infusion (47.59%) was the most preferred technique, followed by direct use (21.69%), decoction (15.06%), other methods (9.02%) and poultice (6.63%). Ethnopharmacological data demonstrated that medicinal plants were primarily used to treat respiratory system, skin, circulatory system and digestive system diseases. Overall, the findings highlight the persistence of traditional healing practices in rural communities of Northwestern Anatolia and emphasize the importance of preserving this local knowledge, which continues to play a vital role in primary healthcare in the region.

Key Words: Ethnobotanical study, ethnopharmacology, medicinal plants, traditional knowledge, Türkiye

ORAL PRESENTATION

CONSERVATION AND VALORIZATION OF SALT TOLERANT PLANTS IN SOUTHERN PORTUGAL

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The saline coastal habitats of southern Portugal harbour a rich and underexplored salt tolerant flora, including halophytes, with significant medicinal potential. Within the scope of the Biodiversa+ project SaltyBEATS, we conducted an in-depth botanical survey of salt-affected ecosystems across the Algarve, focusing on the identification of native, invasive, and endangered vascular plants with recognized or emerging ethnomedicinal value. Our fieldwork revealed a diverse community of salt-tolerant species, including native halophytes such as *Sarcocornia perennis* and *Arthrocaulon macrostachyum*, invasive species like *Carpobrotus edulis*, and rare endemics such as *Limonium algarvense*, all of which have documented bioactivities and are traditionally or experimentally associated with antioxidant, anti-inflammatory, neuroprotective, or antimicrobial effects. To address the urgent need for conservation of *L. algarvense*, a "Near Threatened" Algarve endemic, we developed and optimized an *in vitro* propagation protocol using shoot tip explants. The protocol achieved high multiplication efficiency under kinetin- and auxin-supplemented conditions, followed by successful rooting with calcium-enriched media. Despite low acclimatization success, this methodology offers a promising *ex situ* strategy for preserving this species and sustainably supplying biomass for research and valorisation. These findings highlight the medicinal relevance of salt-tolerant plants from marginal habitats and reinforce the importance of conserving its biodiversity in Mediterranean coastal ecosystems. Integrating field-based biodiversity assessment with biotechnological conservation tools contributes to sustainable resource use and supports the development of Nature-Based Solutions (NbS) rooted in regional plant heritage.

Key Words: Halophytes, Invasive species, Endemic flora, In vitro conservation, Salt-affected habitats

Acknowledgements
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ORAL PRESENTATION

CALENDULA OFFICINALIS AND *ECHINACEA ANGUSTIFOLIA IN VIVO* TREATMENT INDUCED CHANGES OF IMMUNE ORGANS IN CHICKENS

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Objective/Purpose: *Calendula officinalis* and *Echinacea angustifolia* are well known for their medicinal uses, including veterinary medicine. The aim of this study was to identify the impact of oral administration of alcoholic extracts of each of the plants on the growth and metabolism of the immune organs (thymus, bursa, spleen) of immunologically immature/mature chickens.

Materials and Methods: Twenty-eight of each 47 (G1-4) and 19 days (G5-8) old chickens were divided into four equal groups and subjected to 7-day oral administration of 0.5 ml of: *C. officinalis* extract (G1,5), *E. angustifolia* extract (both from Natex, Romania) (G2,6), 70° alcohol (G3,7) and saline (G4,8). At the end of the experiment the birds were euthanized and the immune organs were collected, wighed and processed for investigation of metabolic parameters (cellular breath Warburg method, glycogen – Montgomery technique, DNA and RNA quantification – Ogur and Rosen method). The statistical significance of the differences between groups and versus control was estimated.

Results: In 47-days-old chickens, the C. officinalis alcoholic extract negatively influenced the weight of all immune organs, while the E. angustifolia extract negatively influenced only the bursa with positive effects on the other organs. Both extracts positively influenced the thymus and negatively the spleen and the burs in 19 days old chickens. The highest increase was observed in glycogen concentrations in 47 days old chickens and lesser in the 19 days old ones for both extracts. Both nucleic acids significantly ($p<0.05$-0.01) decreased in the older versus younger chickens, where the impact was positive on DNA and RNA except for the marigold extract on DNA (-30.33% versus control, NS).

Conclusion: Both the *Calendula officinalis* and *Echinacea angustifolia* ethanolic extracts exerted variable effects on both the weight and metabolic parameters of primary and secondary immune organs, related to the age of the individuals.

Key Words: *Calendula officinalis, Echinacea angustifolia*, chickens, *thymus*, spleen, bursa Fabricii

ORAL PRESENTATION

THE IMPORTANCE OF SUMAC (*RHUS CORIARIA* L.) BREEDING

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Sumac (*Rhus coriaria* L.) belongs to the *Anacardiaceae* family, comprising 77 genera and approximately 600 species. *Rhus* L. is a species naturally distributed throughout the Canary Islands, countries bordering the Mediterranean, and eastward to Tajikistan, Afghanistan, and Iran, as well as Bulgaria and Russia in the Balkans. Sumac is used in spice and dye production, and is rich in antioxidants, antimicrobials, and phenolic compounds. As a result, sumac is a very important medicinal and aromatic plant used for centuries in traditional medicine to treat various diseases and consumed as a spice. Because of its natural tolerance to low water and nutrient conditions, sumac is an important plant species that can be used in the rehabilitation of arid lands and in the fight against erosion. This study was conducted to emphasize the importance of breeding sumac, a plant species crucial for human nutrition, ecosystem conservation, and the sustainability of rural life. Breeding and developing species/varieties resistant to environmental stress factors, especially adaptation to climate change, is crucial for sustainable plant/food production. Sumac (*Rhus* spp.) is an ecologically and economically important species due to its high adaptability, drought resistance, and soil remediation potential. Because sumac is generally propagated by seed, there is a high degree of genetic variation. Existing sumac populations exhibit heterogeneity in terms of plant and fruit quality traits due to their genetic diversity. This can limit its commercial value and ecological use. Identifying genotypes superior to natural populations in terms of yield and quality traits and expanding their cultivation clearly demonstrates the necessity and importance of sumac breeding. Despite its superior botanical nutritional and adaptability, sumac has been neglected in breeding research. It is urgent and important to conduct breeding studies to conduct morphological, phenotypic, and genotypic analyses of existing sumac populations, fill the gap in the literature on sumac breeding worldwide, and to conduct breeding studies to identify sumac genotypes for high-yielding, high-quality sumac varieties. Increasing environmental stress factors associated with climate change make breeding species adapted to semiarid areas, such as sumac, even more crucial. Utilizing waste and dry land will make a sustainable contribution to rural development. For these reasons, sumac breeding is strategically important for both the conservation of biodiversity and the sustainability of high-value-added products.

Key Words: *Rhus coriaria* L, breeding, climate change, yield, quality, aromatic plants, healthy

ORAL PRESENTATION

CROCETIN-LOADED NANOGEL FROM *NYCTANTHES ARBORTRISTIS*: A TARGETED PHYTOACTIVE APPROACH FOR IMPROVED TREATMENT OF SKIN CANCER

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The present study develops a crocetin-loaded nanogel formulation from *Nyctanthes arbortristis* (Night Jasmine) and determines its anticancer efficacy and targeted drug delivery potential. This plant is mentioned in traditional medicine for its diverse phytochemical profile and promising therapeutic properties, especially in the prevention and management of cancers. Herein, the carotenoid crocetin was isolated from the seeds of *N. arbortristis* using advanced chromatographic and spectrometric techniques, and its anticancer activity was confirmed by molecular docking against key cancer-associated proteins displaying strong binding affinities. The crocetin isolate was encapsulated within a chitosan-based nanogel prepared by ionic gelation, which yielded a stable biocompatible semi-solid gel optimized for dermal application. Comprehensive physicochemical evaluation demonstrated high drug entrapment, favorable particle size, and non-Newtonian rheology supporting its suitability for topical delivery. *In vitro* studies showed sustained and controlled release of crocetin from the nanogel under skin-relevant pH conditions and significant cytotoxic effects against skin cancer cell lines (B16F10) with lower IC50 value compared to crude extracts. *Ex vivo* skin permeation assays confirmed enhanced epidermal penetration, while *in vivo* studies using a DMBA-induced skin carcinogenesis mouse model demonstrated a marked reduction in tumor size, lesion thickness, and histopathological markers of malignancy with the nanogel treatment. There were no significant adverse effects on liver and kidney function, indicating a favorable safety profile. These findings endorse the crocetin-loaded nanogel as a potent and innovative phyto-nanotechnological strategy for localized, effective, and patient-friendly management of skin cancer.

Keywords: *Nyctanthes arbortristis*, carotenoid, chitosan nanoparticles, skin cancer, DMBA

ORAL PRESENTATION

HERBAL TREATMENT AND ALTERNATIVE APPLICATIONS IN ANIMAL PRODUCTION (HOMEOPATHY)

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In livestock production, the adoption of management and feeding practices that align with the natural needs of animals, the restriction of chemically synthesized drugs, and the growing interest in alternative solutions have become increasingly important in recent years. The rise of microorganisms resistant to antibiotics and similar chemicals widely used in conventional systems, along with higher residue risks in animal-derived foods and their negative implications for human health, has strengthened the interest in plant-based applications. Many aromatic and medicinal plants possess properties such as appetite regulation, support for wound healing, inhibition of microbial growth, and protection against external parasites, making them valuable components in complementary approaches for various animal species. For this reason, plant-derived solutions can serve as alternatives to synthetic growth-promoting substances in both conventional and organic livestock systems. Organic livestock production represents a system in which natural animal requirements are prioritized through appropriate management and feeding, and the use of chemically synthesized pharmaceuticals is markedly restricted.

The wide diversity of botanical resources allows for the modern re-evaluation of natural substances historically used in animal health. Reassessing traditional practices in light of current scientific knowledge is essential for identifying modes of action, potential risks, and practical outcomes. Scientific investigation of plant-, animal-, or mineral-derived healing agents contributes to minimizing undesirable effects and developing safer natural applications. Furthermore, the scientific assessment of certain traditional methods, such as homeopathy, may support the emergence of new alternative options in livestock production. Overall, the systematic evaluation of natural treatment approaches holds considerable potential to enhance sustainable livestock systems and foster new research opportunities in the future.

Key Words: Herbal treatments; alternative applications; organic livestock; sustainable animal production; homeopathy

ORAL PRESENTATION

ANTIMICROBIAL ACTIVITY OF STARCH-BASED ORAL STRIPS CONTAINING PLANT ESSENTIAL OILS AND FRUIT EXTRACTS AGAINST *STREPTOCOCCUS MUTANS*

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Dental caries is a biofilm-associated pathology in which the bacteria *Streptococcus mutans* has a significant role. This preliminary in vitro study evaluated starch-based edible films, designed as oral strips, incorporating cinnamon and clove essential oils, cranberry and pomegranate peel extracts against *S. mutans* using broth microdilution (MIC/MBC) and agar disk diffusion methods. Starch based films were formulated with individual active components: cinnamon essential oil (CIN, 1% w/w), clove essential oil (CLO, 1% w/w), cranberry extract (CRN, 1% w/w), and pomegranate peel extract (POM, 2% w/w), and a combination (COMB: 0.5% cinnamon, 0.25% clove, 1% cranberry, 1% pomegranate; 2.75% total). The starch–glycerol control dispersion showed no inhibition up to the highest tested level. MIC values (µg/mL of active) were: CLO 125, CIN 250, CRN 250, POM 1000, while COMB exhibited the lowest MIC of 62.5 µg/mL. MBC values followed the same pattern (CLO 250, CIN 500, CRN 500, POM >1000, COMB 125). MIC and MBC for COMB were significantly lower than those of all single-active dispersions ($p < 0.05$), indicating enhanced antimicrobial potency at reduced individual component levels. CLO also showed a significantly lower MIC than CIN, CRN, and POM ($p < 0.05$). In disk diffusion, the control discs showed only their intrinsic diameter (6 mm) being signicantly lower than all ($p < 0.05$). Among single-actives, CLO (20.5 ± 1.3 mm) and CIN (18.2 ± 1.1 mm) generated significantly larger zones than CRN (13.4 ± 0.9 mm) and POM (11.5 ± 0.8 mm; $p < 0.05$). COMB yielded the largest zones (23.0 ± 1.4 mm), significantly exceeding all single-active formulations ($p < 0.05$). The results indicate that starch-based oral strips incorporating these plant-derived actives, especially the combination formulation, demonstrate inhibitory and bactericidal activity against *S. mutans* in vitro and require further investigation in multi-species biofilm and in situ oral models.

Key Words: *Streptococcus mutans*, plant antimicrobials, edible films, essential oils, fruit extracts.

ORAL PRESENTATION

COMPARATIVE EFFECTS OF PURE CURCUMIN AND TURMERIC EXTRACT ON LIFESPAN EXTENSION IN *CAENORHABDITIS ELEGANS*

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Curcumin is a major bioactive molecule of *Curcuma longa* L. (turmeric) [1], yet it remains unclear whether its biological effects on aging are driven primarily by the pure compound or enhanced through the phytochemical complexity of turmeric extract. This study compares the lifespan-extending potential of pure curcumin and turmeric extract in *Caenorhabditis elegans* using curcumin-equivalent doses of 10, 25, 50, and 100 µM [2]. The curcumin content of the extract was quantified by HPLC to ensure accurate dose matching. Age-synchronized N2 worms were exposed to each treatment at 35 °C, and survival was recorded at each 20 minutes. Lifespan data were analyzed using Kaplan–Meier curves and log-rank statistics. This work aims to determine whether turmeric constituents other than curcumin contribute synergistically to longevity, thereby providing insight into the relative efficacy of isolated natural products versus whole-extract preparations in aging research.

Key Words: Curcumin; *Curcuma longa*; *Caenorhabditis elegans*; lifespan assay; aging; HPLC standardization

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ORAL PRESENTATION

HAWTHORN (*CRATAEGUS* SPP.) AS A MEDICINAL AND AROMATIC PLANT: BIOACTIVE PROFILE

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Hawthorn (*Crataegus* spp.) is a wild fruit species with a wide geographical distribution and a long-standing history of use within medicinal and aromatic plant traditions. In recent years, scientific interest in hawthorn has increasingly focused on its bioactive composition, particularly in relation to its value as a natural source of functional compounds. This paper aims to present an overview of the bioactive profile of hawthorn fruits based on previously published studies, within the context of medicinal and aromatic plant research. The available literature indicates that hawthorn fruits contain a diverse range of bioactive compounds, including phenolic acids, flavonoids, proanthocyanidins, and other secondary metabolites. The qualitative and quantitative composition of these compounds varies considerably among *Crataegus* species and genotypes and is strongly influenced by ecological and environmental factors such as altitude, climate, and growing conditions. This variability highlights the importance of genetic resources and habitat characteristics in determining the bioactive profile of hawthorn.

Rather than emphasizing pharmacological or clinical effects, this review approaches hawthorn from a phytochemical and botanical perspective. The bioactive profile of *Crataegus* species is discussed in relation to their relevance as raw material for medicinal and aromatic plant applications. In addition, gaps in the current literature are highlighted, and future research directions are proposed, particularly from the perspectives of horticulture, plant biochemistry, and the sustainable utilization of wild fruit resources.

Key Words: Hawthorn, *Crataegus* spp., bioactive compounds

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ORAL PRESENTATION

PHENOLIC COMPOSITION OF *RHUS CORIARIA* L. (SUMAC) FROM DIFFERENT ECOLOGICAL SITES IN THE EASTERN MEDITERRANEAN REGION OF TÜRKİYE

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Rhus coriaria L. (sumac) is a widely used medicinal and aromatic plant in the Mediterranean region, valued for its culinary applications and high phenolic content. Owing to the pronounced ecological heterogeneity of Türkiye, environmental factors such as altitude may play a key role in shaping the phenolic composition of sumac. This study aimed to evaluate altitude-related variations in the phenolic profiles of *R. coriaria* leaves and fruit outer pericarps collected from different ecological sites in Hatay Province, Türkiye. Samples were obtained from thirteen natural populations distributed across five districts at altitudes ranging from 172 to 800 m. Dried leaf tissues and fruit outer pericarps were extracted using a methanol–water (70:30, v/v) solvent system and analyzed by LC–MS/MS. Major phenolic compounds, including gallic acid, catechin, myricetin, quercetin, kaempferol, and resveratrol, were quantified, and principal component analysis (PCA) was applied to assess relationships between phenolic composition and altitude.

The results demonstrated clear altitude-associated differences in both tissues. Samples from mid-altitudes (300–600 m) formed distinct clusters in PCA plots and were characterized by higher phenolic abundance and greater chemical diversity. Low-altitude samples showed more homogeneous phenolic profiles, whereas high-altitude samples exhibited distinct compositional patterns, likely reflecting adaptive responses to environmental stress. Sample separation was primarily driven by catechin, gallic acid, myricetin, and quercetin. Overall, mid-altitude environments appear to provide favorable conditions for enhanced phenolic accumulation in *R. coriaria*. While fruit samples exhibited relatively consistent phenolic profiles, leaf samples showed greater variability. These findings contribute to understanding ecological influences on sumac phytochemistry and offer valuable insights for quality-oriented raw material selection and future breeding strategies.

Keywords: *Rhus coriaria* L. phenolic compounds, altitude effect, LC–MS/MS principal component analysis (PCA)

Acknowledgements: This study was supported by Hatay Mustafa Kemal University Scientific Research Projects under the project number 25.YL.018.

ORAL PRESENTATION

UNRAVELLING THE ANTIOXIDANT AND ANTIPROLIFERATIVE POTENTIAL OF *MORINGA OLEIFERA:* AN *INVIVO* & *IN VITRO* STUDY

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Cancer is acknowledged as a significant cause of mortality throughout the globe. With the rise in cancer cases worldwide, alternative treatments are being taken to mitigate the side effects of conventional therapies. *Moringa oleifera* can be effective against more than 300 diseases due to the presence of numerous phytochemicals within it. Every part of the Moringa plant, including leaves, roots, stems, and even bark, has useful medicinal properties and can be used to treat respiratory problems, skin infections, cardiovascular problems, diabetes, liver and kidney diseases, nervous diseases, joint pain, and cancer. Due to its diverse applications, the present study was designed to investigate its anticancerous and antioxidant efficacies against cancer including both *in vitro* and *in vivo* studies. Phytoconstituents of aqueous extract of *Moringa oleifera* (AEMO) were identified by LC-MS analysis and quantified by TPC and TFC. A DPPH Assay was carried out to determine AEMO's antioxidant activity. MTT Assay was employed to evaluate the *in-vitro* antiproliferative efficacy of AEMO against Hepatocellular carcinoma cell line (Hep-G2) and squamous cell carcinoma cell line (CAL27). The main bioactive compounds identified by LC-MS were quinic acid, octadecanoic acid, hexadecenoic acid, and α-sitosterol. The administration of AEMO prolonged the life span of tumor-bearing mice by reducing tumor weight. Liver and kidney function were not significantly altered by AEMO treatment. Additionally, treatment with AEMO on Hep-G2 and CAL27 cell lines showed dose and time-dependent antiproliferative efficacy *in-vitro* through MTT assay. The current study's results show that AEMO has a great deal of potential to stop the growth of tumors without interfering with the body's regular physiology or function, making it a viable treatment option for cancer.

Key Words: *Moringa oleifera*, phytochemical analysis, antioxidant efficacy, anticancer evaluation, biochemical parameters

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ORAL PRESENTATION

INFLUENCE OF FLOWERING STAGE ON CARNOSIC ACID CONTENT AND BIOACTIVE PROPERTIES OF *SALVIA ROSMARINUS* L.

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Salvia rosmarinus L. (rosemary) is a medicinal and aromatic plant widely recognized for its rich content of phenolic diterpenes, particularly carnosic acid, which contributes significantly to its antioxidant and antimicrobial properties. This study investigated the influence of the flowering stage of *Salvia rosmarinus* L. on the accumulation of carnosic acid and its associated biological activities. Plants were collected at different stages of flowering between October 2021 and March 2022, and their phenolic extracts were analyzed using UHPLC–DAD/ESI/MS. The results revealed that the concentration of carnosic acid varied significantly throughout the flowering period, reaching its maximum in mid-December (17.74%). This stage coincided with the highest antioxidant and antibacterial activities, demonstrating a strong relationship between the phenological development of the plant and its bioactive potential. The extracts obtained in mid-December exhibited the strongest antioxidant capacity (IC_{50} values of 38.37 ± 0.47 µg/mL for DPPH, 81.93 ± 0.45 µg/mL for FRAP, and 274.73 ± 1.04 µg/mL for TAC assays). Similarly, the antibacterial assays revealed maximum inhibition against *S. aureus*, *E. coli*, and *P. aeruginosa* (MIC = 31.25 mg/mL) during this same stage. These results indicate that the mid-flowering phase represents the optimal harvest period for obtaining rosemary extracts rich in carnosic acid and exhibiting potent biological activities. Understanding this temporal variation provides valuable insight for optimizing the use of *S. officinalis* in pharmaceutical and nutraceutical applications.

Keywords: *Salvia rosmarinus*, carnosic acid, flowering stage, antioxidant activity, antibacterial activity

ORAL PRESENTATION

NATURAL ANTI-QUORUM SENSING AND DERMAL-PROTECTIVE PHYTOCHEMICALS FROM *PAEDERIA FOETIDA* LINN.: AN INTEGRATED *IN SILICO* AND *IN VITRO* INVESTIGATION AGAINST MULTIDRUG-RESISTANT *ACINETOBACTER BAUMANNII*

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Acinetobacter baumannii is a multidrug-resistant (MDR) pathogen recognized for its robust biofilm formation, environmental persistence, and resistance to conventional therapies. Its virulence is tightly modulated by quorum sensing (QS) pathways, making QS interference an attractive strategy not only for combating infection but also for supporting skin health, reducing inflammation, and maintaining microbial balance. Plant-derived phytochemicals offer a natural, biocompatible alternative to harsh antimicrobial agents commonly used in medical and cosmetic applications. This study explored the antibiofilm, anti-QS, antimicrobial, and dermal-protective potential of *Paederia foetida* Linn. ethanolic extract against MDR *A. baumannii*, integrating GC–MS profiling, advanced in silico modeling, and in vitro assays. Ethanolic extracts of *P. foetida* were analyzed by GC–MS, revealing 30 phytochemicals including 5-hydroxymethyl-2-furaldehyde, 4H-pyran-4-one derivatives, loliolide, and eugenol—compounds known for antimicrobial, antioxidant, and anti-inflammatory properties. Molecular docking against five QS/biofilm-regulatory *A. baumannii* proteins (AF-A0A7S8WE28-F1-v4, AF-A0A059ZL64-F1-v4, AF-Q2VSW6-F1-v4, AF-A0A2P1B9S4-F1-v4, and AF-A0A5P9VY74-F1-v4) revealed strong binding affinities, with eugenol demonstrating the highest interaction energy (–6.3 kcal/mol). ADMET predictions indicated favorable absorption, low toxicity, and good drug-likeness, suggesting suitability for topical and health-related applications. In vitro testing confirmed antimicrobial activity (MIC = 7.81 mg/mL; MBC = 31.25 mg/mL) and a significant dose-dependent reduction in biofilm biomass ($p < 0.001$). FE-SEM imaging demonstrated membrane disruption and collapse of biofilm architecture. Beyond antimicrobial effects, the presence of antioxidant and anti-inflammatory phytochemicals such as loliolide and eugenol suggests additional roles in soothing irritated skin, mitigating oxidative stress, and preventing biofilm-associated skin disorders—making *P. foetida* a promising ingredient for natural therapeutic and beauty formulations. In conclusion, *P. foetida* extract displays potent antibacterial, anti-QS, antibiofilm, and dermal-protective properties against MDR *A. baumannii*. These findings position *P. foetida* as a valuable Thai medicinal plant with strong potential for development into natural antimicrobials, skincare bioactives, and multifunctional phytopharmaceuticals for future health and beauty innovations.

Keywords: *Acinetobacter baumannii*, biofilm disruption, dermal-protection, molecular docking, *Paederia foetida*, quorum sensing inhibition

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ORAL PRESENTATION

INTEGRATION OF EXPERIMENTAL AND MOLECULAR DOCKING APPROACHES TO EVALUATE THE ANTIMICROBIAL POTENTIAL OF A POLYHERBAL GEL FROM *ARGEMONE MEXICANA* AND *CARICA PAPAYA*

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Introduction/Objective: The need for safe and efficient natural antimicrobial agents is global need due to arising antibiotic resistance. Computational studies were conducted on phytoconstituents of *Carica papaya* and *Argemone mexicana* and thereafter, a novel polyherbal antibacterial gel of leaf extracts of *Carica papaya* and *Argemone mexicana*, was made with an emphasis on its antimicrobial activity and its safety.

Methods: Molecular docking was performed using Schrodinger (2023-1), 3 major bacterial target enzymes DNA Gyrase B (PDB ID: 4URO), MurA (PDB ID: 1UAE), and FabI (PDB ID: 4NR0) were used. The polyherbal gel was made, its physicochemical characteristics, including pH, spreadability, homogeneity, and stability, were assessed. Using the cup-plate diffusion method using tetracycline and erythromycin as standards, antimicrobial activity was evaluated against Staphylococcus aureus and Escherichia coli. The HET-CAM (Hen's Egg Test–Chorioallantoic Membrane) assay was used to evaluate the formulation's safety.

Results: The key phytoconstituents quercetin, protopine, sanguinarine, and protocatechuic acid were predicted to interact with bacterial target enzymes DNA Gyrase B (PDB ID: 4URO), MurA (PDB ID: 1UAE), and FabI (PDB ID: 4NR0) with higher docking scores. The results of the experiment were supported by docking analysis, which showed substantial binding affinities and hydrogen bond interactions, especially between quercetin, protopine, and sanguinarine with FabI and protocatechuic acid with MurA.The produced gel showed promising antibacterial activity with a significant zone of inhibition of 25 ± 1 mm and good physicochemical stability. The results of the experiment were supported by docking analysis, which showed substantial binding affinities and hydrogen bond interactions, especially between quercetin, protopine, and sanguinarine with FabI and protocatechuic acid with MurA.

Discussion: The study conduction computational studies with successful scores and binding, the assay results show synergistic phytoconstituents in the polyherbal formulation improve antibacterial activity, confirming its mode of action, according to the combined experimental and in silico results.

Conclusion: The *Argemone mexicana–Carica papaya* polyherbal gel is a topical antimicrobial treatment that is safe, stable, and effective. It is a viable natural substitute for treating antibiotic

Key Words: *Argemone Mexicana, Carica Papaya*, polyherbal gel, antibacterial activity, molecular docking, dna gyrase b, mura enzyme, fabi reductase, phytoconstituents, topical formulation

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ORAL PRESENTATION

EVALUATION OF BLOOD BRAIN BARRIER PERMEABILITY OF FLAVOKAWAIN A IN RATS USING LC-MS/MS

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Neurological conditions such as neurodegeneration, schizophrenia, epilepsy, stroke, depression, anxiety and traumatic brain injury represent a substantial global health burden, emphasizing the urgent need for safe, plant-derived neuroprotective therapeutics. Flavokawain A (FKA), a naturally occurring chalcone isolated from *Piper methysticum,* has demonstrated diverse pharmacological activities including anti-inflammatory, anti-cancer and neuroprotective effects in various *in vitro* and *in vivo* studies. Despite these promising properties, FKA's ability to cross blood brain barrier (BBB), a critical factor for central nervous system efficacy, remains unreported. This study aimed to develop and validate a robust, sensitive LC-MS/MS method for quantifying FKA in brain homogenate to evaluate its BBB permeability. Following oral administration of FKA (50 mg/kg) to Sprague-Dawley rats, plasma and brain samples were collected at multiple time points. Carbamazepine was used as an internal standard (IS) and samples were processed by protein precipitation using methanol. Chromatographic separation of FKA and IS was achieved using a Symmetry C18 column (150 x 3.9 mm, 5µm) with gradient elution (methanol and 0.1% formic acid) at flow rate of 0.5 ml/min. The quantitation employed positive electrospray ionization with multiple reaction monitoring (MRM) mode, offering high sensitivity and specificity. The method exhibited excellent linearity ($r^2 > 0.99$) across the range of 0.1-100 ng/ml in both plasma and brain homogenate, with limit of detection of 0.05 ng/ml. Accuracy and precision were found within acceptance criteria. FKA was detected in brain homogenate at both 2-hour and 4-hour time points, demonstrating its ability to cross BBB. In conclusion, these results provide the first experimental data on the BBB permeability of FKA, supporting its potential in neuroprotective and therapeutic applications.

Key Words: Flavokawain A, Blood brain barrier, LC-MS/MS

ORAL PRESENTATION

COMPREHENSIVE PHYTOCHEMICAL MAPPING OF *ABIES WEBBIANA*: UNLOCKING ITS BIOACTIVE AND THERAPEUTIC POTENTIAL

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Objective: This study aimed to systematically extract, profile, and quantify the phytochemical constituents of *Talishpatra* (*Abies webbiana*) to establish a comprehensive phytochemical baseline and assess its therapeutic potential.

Methods: Fresh plant materials of *Abies webbiana* were accurately identified and collected from the Himachal Pradesh ecological regions (Himachal Pradesh State Biodiversity Board), shade-dried, and pulverized to a uniform powder. Methanolic extraction was performed to maximize the yield of bioactive compounds. The concentrated extracts were qualitatively screened for major classes of secondary metabolites, including alkaloids, flavonoids, tannins, phenolics, saponins, glycosides, terpenoids, and steroids. Quantitative analyses were conducted to determine the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity using DPPH and FRAP assays.

Results: Qualitative phytochemical screening confirmed the presence of multiple secondary metabolites, indicating the chemical richness of *Abies webbiana*. Quantitative evaluations revealed substantial levels of phenolic and flavonoid compounds, along with notable antioxidant activity, as demonstrated by DPPH and FRAP assays. These findings highlight the presence of potent bioactive molecules that may contribute to the therapeutic effects of the plant.

Conclusion: This study successfully established a detailed phytochemical profile of *Abies webbiana*, supporting its relevance in traditional medicine and its potential for nutraceutical and pharmacological applications. The baseline data generated in this study can guide future research on the biological activity and therapeutic utilization of this medicinal plant.

Key Words: *Abies webbiana*, phytochemical extraction, qualitative and quantitative analysis, secondary metabolites, bioactive compounds.

ORAL PRESENTATION

CONJUGATION-BASED NANOCARRIER DRUG DELIVERY FOR CANCER THERAPY

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Objective: This study aimed to develop a novel, actively targeted drug delivery system to enhance the therapeutic efficacy of doxorubicin (DOX) and mitigate its systemic toxicity and lack of tumor selectivity, which are significant challenges in current cancer treatments. We hypothesized that DOX-loaded pegylated liposomal systems (LDM), surface-functionalized with the $(CR)_4$ peptide, would improve tumor targeting.
Methods: The liposomal system was prepared using a remote loading technique and optimized via Box-Behnken design (BBD) within the Response Surface Methodology (RSM). Molecular docking was used to assess the binding affinity of the $(CR)_4$ peptide to cancer-related proteins, specifically HER3 and tubulin β colchicine. The optimized LDM was characterized for its physicochemical properties (size, PDI, zeta potential, entrapment efficiency), *in vitro* drug release, and hemolysis/biocompatibility. *In vitro* cytotoxicity was evaluated against various cancer cell lines (MCF-7, HCT-116, MiaPaca-2, A549) using the SRB assay, examining parameters like nuclear fragmentation, reactive oxygen species (ROS) generation, and mitochondrial membrane potential. *In vivo* studies assessed the biodistribution and histopathological effects of LDM.
Results: Molecular docking indicated that the $(CR)_4$ peptide binds strongly to HER3 (score: -11.906) and tubulin β colchicine (score: -9.582). The optimized LDM exhibited a spherical shape with a vesicle size of 118.5 ±1.28 nm, PDI of 0.291 ± 0.006, zeta potential of 12.35± 0.91 mV, and entrapment efficiency of 67.344 ± 1.27%. *In vitro* release showed approximately 66.66 ±1.98% drug release over 72 hours at pH 6.8. LDM demonstrated significantly superior cytotoxicity, particularly against the MCF-7 (breast cancer) cell line, with an IC50 of 4.9 ± 0.91µM, following the cytotoxicity hierarchy: HCT-116 < MiaPaca-2 < A549 < MCF-7. Furthermore, LDM showed enhanced nuclear fragmentation, ROS generation, and altered mitochondrial potential. It also exhibited excellent clinical safety with significantly low hemolysis (4.23 ±0.17%) compared to free DOX (44.5 ± 0.23%). *In vivo* studies confirmed sustained drug release, the highest retention in tumor tissue, and minimal organ toxicity.
Conclusion: The developed $(CR)_4$ peptide-functionalized DOX-loaded pegylated liposomes (LDM) demonstrate outstanding safety and biocompatibility while exhibiting potent anti-tumor effects, particularly against breast cancer. This novel targeted delivery system is a promising, well-tolerated treatment option that effectively enhances the therapeutic index of doxorubicin.

Key Words: Nanodrug delivery, doxorubicin, pegylated liposomes, $(CR)_4$ peptide, cancer therapy, targeted delivery

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ORAL PRESENTATION

PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT PROFILING OF *TAMARINDUS INDICA* AQUEOUS/METHANOL LEAVES AND PULP EXTRACTS

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Antioxidant potentials have been demonstrated to play important role in management and therapy against many disease conditions. Other associated role of this property was also found to be important in the field of nutrition and environmental ecotoxicology. Biological and environmental relevance has been reported on one of the most commonly abundant and under-utilized plant materials such as tamarind. The purpose of this work is to explore the phytochemical and antioxidant profile of methanol and aqueous tamarind leaves and pulp extract. Both the aqueous and methanol extract demonstrated appreciable content of total phenolics, flavonoids, anthocyanidins, and flavonols. Methanol leaves extract shows greater antioxidant capacity when compared with aqueous leaves/pulp and methanol pulp extract. The compounds found to be present in the extracted have been reported to confer antioxidant potentials. *Tamarindus indica* extract demonstrated high content of antioxidant phytochemicals and antioxidant capacity which was fully backed by the individual compound contained. Antioxidant activity is higher in methanol extract than aqueous evidence by the phytochemical contents of the two extracts. Thus, this property of tamarind extract could make it useful in the so many fields including medicinal chemistry, nutrition and environmental science.

Key Words: Antioxidant, phytochemicals, *Tamarindus indica,* and under-utilized plant

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ORAL PRESENTATION

AGITATED *IN VITRO* CULTURES OF HOUTTUYNIA CORDATA AS A SOURCE OF PHENOLIC COMPOUNDS WITH ANTIOXIDANT ACTIVITY

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Houttuynia cordata Thunb. is a plant native to Southeast Asia with a long-standing history of use in traditional medicine and Asian cuisine. It contains numerous bioactive compounds that exhibit anti-inflammatory, anticancer, antiviral, antibacterial, and antioxidant properties [1, 2]. *H. cordata* was introduced into official medicine in Europe and received a monograph in the European Pharmacopoeia in 2017 [3]. Currently, there is growing interest in utilising the plant in biotechnology as a source of natural products with therapeutic potential. This study investigated the impact of different combinations of plant growth regulators (kinetin - Kin, indole-3-acetic acid - IAA, naphthalene-1-acetic acid - NAA, 6-benzylaminopurine - BAP, as well as the absence of growth regulators) on the production of secondary metabolites in *Houttuynia cordata* agitated *in vitro* cultures. The cultures were maintained in Murashige and Skoog medium for 4 weeks. Qualitative and quantitative analyses of active compounds in the plant extracts were performed using high-performance liquid chromatography (HPLC-DAD).

All analyzed extracts contained the following flavonoids: quercetin, isoquercitrin, quercimetrin, hyperoside, rutoside, quercitrin, avicularin, and luteolin, as well as phenolic acids: protocatechuic, chlorogenic, neochlorogenic, cryptochlorogenic, 4-O-caffeoylquinic, and *p*-coumaric acid. The dominant metabolites were 4-O-caffeoylquinic acid and avicularin. The best growth was observed in cultures supplemented with 0.5 mg/L BAP + 0.2 mg/L IAA. Nevertheless, the highest total content of the tested compounds was observed in cultures maintained without growth regulators (196.57 mg/100 g of dry weight). The antioxidant potential of the extracts varied depending on the test method used (DPPH, FRAP and Folin-Ciocalteu). To conclude, *in vitro* cultures offer a promising alternative for obtaining active compounds from plants compared to harvesting them from their natural environment. Further research in this field may lead to the development of new, efficient, and environmentally friendly methods for producing essential medicinal compounds.

Key Words: Fish mint, *in vitro* culture, antioxidant potential, phenolic compounds

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ORAL PRESENTATION

COMPARATIVE STUDY OF ESSENTIAL OIL COMPOSITION AND BIOLOGICAL ACTIVITIES IN SPONTANEOUS AND CULTIVATED *LAVANDULA DENTATA*

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Introduction The Moroccan flora, which includes more than 800 species of medicinal and aromatic plants (MAP), represents an important reservoir of bioactive molecules (phenolic acids, flavonoids, and terpenoids). These biomolecules, synthesized by plants as part of their natural defense against enemies [1], are used in various medical, cosmetic, and culinary fields, offering significant opportunities for local development. Lavender (*Lavandula dentata*), of the Lamiaceae family, is one of the most economically important MAP, thanks to its numerous biological properties [2]. It's naturally widespread in the Mediterranean region, Southwest Asia, and the Arabian Peninsula [3]. In Morocco, *L. dentata* grows spontaneously in the Mediterranean coast in the north, in the High Atlas regions, and in the Atlantic coast (Essaouira-Agadir) in the center of country [4]. This MAP species has long been used by locale local populations for therapeutic purposes [5, 6]. During the last years, it is increasingly cultivated for its EO, which is highly sought after in the perfumery, cosmetics, and pharmaceutical industries.
Objective The objective of this study is to perform a comparative analysis of the composition and biological activities of the EO extracted from the leaves of spontaneous and cultivated pants of *L. dentata* plant, thus evaluating the impact of agroecological practices during its cultivation.
Material & methods Samples of aerial parts were collected at the flowering stage from *L. dentata* plants growing spontaneously or cultivated in Ouirgane, region of Marrakech-Safi. Essential Oils Extraction and GC-MS Analysis as well as their biological (antibacterial, antioxidant and nematocidal) activities were performed on EO of both wild and cultivated *L. dentata.*
Results Obtained results showed that EO of the spontaneous and cultivated plants contained respectively 21 and 23 components, accounting for over 98% of the respective total compositions. Borneol (49.47% and 32.83%), eucalyptol (23.01% and 14.71%), and β-pinene (3.95% and 5.83%) were the major compounds of wild and cultivated EO respectively. Other compounds were also highly present in wild (β-eudesmol, 3.79% and myrtenol, 3.61%) and cultivated (isobornyl acetate, 24.45%) plants. On the other hand, the biological properties of the essential oils varied between wild and cultivated plants. Indeed, *L. dentata* growing spontaneously in Ouirgane region exhibited slightly higher antibacterial, antifungal, antioxidant, and nematocidal activities than *L. dentata* plants cultivated in the same region. These small differences in biological properties between the wild and cultivated plants demonstrate that domestication may be considered a promising alternative for producing *L. dentata*, thus helping to preserve of these natural resources.

Key Words: *Lavandula dentata*, essential oils, gas chromatography, antibacterial, antifungal, antioxidant and nematocidal activities

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ORAL PRESENTATION

GREEN HOT EXTRACTION APPROACH OLIVE LEAF INDUSTRIAL WASTE: BOX-BEHNKEN OPTIMIZATION AND BIOACTIVITY ASSESSMENT FOR SKIN ANTI-AGING POTENTIAL

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Olive leaves, produced in large quantities as by-products of olive processing, represent a major agricultural waste. Their rich phytochemical profile makes them a valuable bioresource within supporting environmental sustainability and resource efficiency [1,2]. Elastase inhibition preserves skin elasticity and aids in anti-aging [3]. Olive leaf extracts exhibit antioxidant, anti-inflammatory, and photoprotective activities, suggesting anti-aging potential partly mediated by metalloproteinase inhibition [4,5]. Accordingly, this study aimed to optimize the extraction process of industrial olive leaf waste and investigate its suitability for skin anti-aging applications. Olive leaf waste was obtained from Gökçe Olive Oil Factory (Erzin/Hatay/Türkiye). Extraction was performed using *n*-hexane in the Buchi E-816 hot extraction system, employing heating power, extraction time, and raw material/solvent ratio as independent variables within a Box-Behnken Design (BBD). Elastase inhibition served as the response variable for optimization. The cytotoxicity of the optimized extract (0.5-500 µg/mL) was evaluated on L929 mouse fibroblast cells using the MTT assay. BBD analysis showed that the quadratic model provided the best fit ($p<0.0001$). The raw material/solvent ratio was the most influential factor, with linear, and selected interaction terms contributing significantly ($p<0.05$). The optimized extraction conditions were 79.37% heating power, 88.01 minutes extraction time, and 2.43 g/100 mL raw material/solvent ratio, yielding an extract with strong elastase inhibitory activity (IC_{50}=10.59±0.9698 µg/mL). MTT results demonstrated that the extract did not diminish cell viability across the tested concentrations and markedly enhanced fibroblast proliferation at 100 µg/mL, suggesting meaningful implications for skin regeneration and anti-aging applications. In conclusion, olive leaf industrial waste extracted under optimized conditions exhibits potent elastase inhibition and fibroblast-proliferative activity, highlighting its promise as a natural agent for skin aging prevention. These findings highlight the importance of sustainable extraction strategies in pharmacognosy and offer preliminary evidence supporting further mechanistic and *in vivo* studies on optimized olive leaf waste extracts.

Key Words: Box-Behnken Design, hot extraction, green extraction, olive leaf waste, skin anti-aging

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ORAL PRESENTATION

DETERMINATION OF THE EFFECT OF ONTOGENETIC VARIABILITY ON SOME MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF THE IMMORTELLE (*HELICHRYSUM ITALICUM* (ROTH) G. DON FIL.) PLANT

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Helichrysum italicum (Roth) G. Don fil. (family Asteraceae) has been used since Ancient Greek and Roman times due to its medicinal and cosmetic properties, and it continues to play an important role in the traditional medicine of Mediterranean countries today. Determining the factors that influence yield and quality in the cultivation of this commercially important plant is of great significance. In this study, plant height, fresh and dry herbage yield were determined during the pre-flowering, flowering, and post-flowering stages of *Helichrysum italicum*. The Nitrogen Balance Index (NBI) and flavonoid values were measured using a Dualex Scientific device immediately before harvest. Among the Dualex measurements, NBI and flavonoid values were found to be statistically significant at the 1% level. The highest values were obtained during the flowering period, with 11.25 dx for NBI and 1.87 dx for flavonoid, while the lowest values were recorded during the post-flowering stage, with 3.35 dx for NBI and 1.03 dx for flavonoid. The highest values for plant height, fresh herb yield, and dry herb yield were determined as 47 cm, 1798.9 g and 602.0 g, respectively. Thus, the flowering period was determined to be the most suitable harvest period for achieving maximum yield and quality from this widely commercially used plant, considering all the parameters examined.

Key Words: Dualex, flavonoid, Van, variability, yield

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ORAL PRESENTATION

DEVELOPMENT AND CHARACTERIZATION OF DRUG BEARING SURFACE MODIFIED NANOCARRIERS FOR THE EFFECTIVE MANAGEMENT OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by cognitive decline and memory impairment, primarily driven by amyloid-β plaque accumulation and tau pathology. Despite the clinical use of cholinesterase inhibitors such as rivastigmine tartrate (RVG), their therapeutic efficacy remains limited due to poor stability, low bioavailability, and restricted delivery across the blood–brain barrier (BBB). The present study aimed to enhance RVG delivery and therapeutic performance by developing D-glucosamine and DSPE-PEG2000-amine modified liposomes designed for targeted transport through the BBB. Preformulation studies confirmed RVG's physicochemical suitability for encapsulation. A 3^3 factorial Box–Behnken design was employed to optimize formulation variables, including the molar ratio of SPC:DSPC, CHEMS concentration, and sonication time. The optimized RVG-loaded liposomes (pRVG-LPs) exhibited an average vesicle size of 133.0 ± 2.69 nm, whereas D-glucosamine-modified RVG liposomes (Dg-RVG-LPs) showed a slightly larger size of 179.9 ± 2.31 nm. Both formulations demonstrated high entrapment efficiency and controlled drug release, with accelerated RVG release under acidic conditions. The therapeutic performance of the optimized formulation was evaluated through *in-vitro, ex-vivo,* and *in-vivo* studies. SH-SY-5Y cell assays revealed significantly enhanced cellular uptake and biocompatibility of Dg-RVG-LPs compared with unmodified liposomes. Ex-vivo studies in rat pups showed improved neuronal viability with D-glucosamine functionalization. *In-vivo* investigations in mice demonstrated that Dg-RVG-LPs enhanced cognitive function and reduced amyloid-β deposition more effectively than conventional RVG formulations. Overall, the findings highlight D-glucosamine-modified liposomes as a promising nanocarrier system for targeted RVG delivery in Alzheimer's disease, offering improved stability, BBB transport, and therapeutic efficacy.

Key Words: Rivastigmine tartrate, D-glucosamine, DSPE-PEG2000-amine liposomes, Blood–brain barrier targeting, Alzheimer's disease therapy, Box–Behnken design.

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ORAL PRESENTATION

BIOPHYSICAL CHARACTERIZATION OF *SALVIA OFFICINALIS* AND OCIMUM BASILICUM ESSENTIAL OILS USING MODEL LIPID BILAYERS AND MICROFLUIDIC SYSTEMS

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Essential oils from *Salvia officinalis* (sage) and *Ocimum basilicum* (basil) are known for their antimicrobial properties, yet the biophysical mechanisms underlying their membrane-targeted effects remain insufficiently understood. This study comparatively evaluated the disruptive actions of these oils on synthetic phospholipid bilayers and bacterial cells using an integrated biophysical–microfluidic approach. Small unilamellar vesicles composed of phosphatidylcholine and phosphatidylglycerol were prepared and analyzed for membrane fluidity using DPH/TMA-DPH fluorescence anisotropy, permeabilization through calcein leakage, and structural destabilization via dynamic light scattering and zeta potential measurements. A PDMS Y-channel microfluidic chip enabled real-time visualization of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 following exposures to essential oils, while antimicrobial potency was determined by CLSI-standard MIC assays. S. officinalis induced pronounced decreases in anisotropy (up to 45%), extensive calcein leakage (70–85%), 2–3-fold vesicle enlargement, and strong surface-charge neutralization, correlating with >80% bacterial cell death within 20 minutes. In contrast, *O. basilicum* produced moderate membrane perturbation and slower bactericidal activity. MIC values further confirmed the superior antimicrobial potency of sage (0.25–0.5 mg/mL) compared to basil (0.5–1 mg/mL). Overall, sage essential oil displayed stronger and faster membrane-disruptive effects, and the combined biophysical and microfluidic analysis provides clear mechanistic insight supporting the potential use of essential oils, particularly *S. officinalis*, in natural antimicrobial formulations.

Key Words: *Salvia officinalis*, *Ocimum basilicum*, essential oils, membrane biophysics, lipid bilayers, fluorescence anisotropy, calcein leakage, dynamic light scattering (DLS)

ORAL PRESENTATION

A POLYHERBAL APPROACH TO WOUND CARE: DEVELOPMENT OF A METERED-DOSE FILM-FORMING SPRAY USING MODIFIED PANCHAVALKAL EXTRACT

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A polyherbal film-forming metered-dose spray incorporating a modified Panchavalkal extract was developed to enhance wound healing using a plant-based therapeutic approach. The methanolic polyherbal extract was formulated with Eudragit® S100 and suitable excipients, and sixteen formulations were designed and optimized using Design of Experiments with STATEASE® software. The formulations were evaluated for physicochemical characteristics, phytochemical stability, and film-forming ability, and the optimized formulation was filled into a metered-dose spray container. The selected formulation produced a uniform, stable film with favorable viscosity (4–13 cps), rapid drying time (40–70 seconds), good washability, and strong antimicrobial activity. In vivo excision wound studies demonstrated significant enhancement in wound healing, evidenced by faster wound contraction and reduced epithelialization period (20 days and 11 days, respectively; $p < 0.001$) compared to the disease control group. Complete wound closure (100%) was achieved within 24 days, indicating superior wound-healing efficacy of the modified Panchavalkal formulation. The developed metered-dose film-forming spray demonstrates promising antimicrobial and wound-healing potential and represents a viable polyherbal wound-care system.

Key Words: Film-forming spray, modified panchavalkal, polyherbal formulation, wound healing, antimicrobial activity, medicinal plants

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ORAL PRESENTATION

EFFECTS OF MULCHING PRACTICES ON ESSENTIAL OIL COMPOSITION OF ZAHTER (*Thymbra spicata* var. *spicata* L.)

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Mulching is a commonly used agronomic practice in the cultivation of medicinal and aromatic plants to improve soil moisture conservation, suppress weed growth, and regulate soil temperature. However, its effects on the qualitative characteristics of essential oils have not been sufficiently clarified. This study aimed to evaluate the influence of mulching application on the essential oil composition of zahter (*Thymbra spicata* var. *spicata* L.), with particular emphasis on changes in major volatile constituents. The experiment was conducted under field conditions using black polyethylene mulch as an inorganic mulching material, along with a non-mulched control treatment. Aerial parts of zahter plants were harvested at the full flowering stage, and essential oils were extracted by hydrodistillation. The chemical composition of the essential oils was determined using gas chromatography–mass spectrometry (GC–MS), and the relative abundance of individual compounds was compared among treatments.

The results revealed that mulching significantly affected the essential oil composition of zahter. Although total essential oil yield exhibited slight variations among treatments, substantial differences were observed in the proportions of major components such as carvacrol, thymol, p-cymene, and γ-terpinene. Mulched plots generally showed higher concentrations of phenolic monoterpenes, which are responsible for the characteristic aroma and strong biological activity of zahter essential oil. These variations are likely associated with improved soil microclimatic conditions and enhanced nutrient availability under mulching practices. In conclusion, mulching application can be considered an effective cultural practice not only for improving soil conditions but also for enhancing essential oil quality in zahter cultivation. Selecting appropriate mulching materials may serve as a practical approach to optimize the chemical composition of zahter essential oil, thereby increasing its economic value and suitability for pharmaceutical, food, and aromatic applications.

Key Words: Zahter, *Thymbra spicata* var. *spicata* L., mulching, essential oil composition, phenolic monoterpenes, GC–MS analysis

ORAL PRESENTATION

DESERT MEDICINAL-PLANT ENDOPHYTES AS DUAL-FUNCTION BIOPROTECTANTS AND STRESS MODULATORS: A TRANSLATIONAL PIPELINE FOR ARID AGRICULTURE

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Arid and saline agroecosystems demand sustainable solutions that simultaneously control diseases and enhance crop stress resilience. Our long-term research has focused on isolating and characterizing endophytic fungi and bacteria from desert medicinal and aromatic plants (MAPs) capable of serving as dual-function bioinoculants. Representative fungal strains including *Talaromyces, Cladosporium,* and *Trichoderma,* and bacterial strains belonging to *Bacillus* and *Pseudomonas* species were identified through morphological and multigene analyses and screened for antagonism against *Pythium aphanidermatum*, volatile metabolite production, and plant-growth-promoting traits. Greenhouse and field assays on tomato, cucumber, cabbage, and okra under drought and saline irrigation confirmed their capacity to suppress damping-off disease and enhance growth, yield, and physiological performance. For example, *Talaromyces* sp. improved drought tolerance and fruit quality in tomato and cucumber, while *Trichoderma* and *Bacillus* isolates produced antifungal volatiles and strengthened root architecture. Scalable production was achieved using locally available grass-biomass carriers, offering a low-cost substrate for commercial bioformulations. These findings establish a translational pipeline that converts desert MAP endophytes into standardized inoculants and seed/soil treatments for arid-climate horticulture.

Key Words: Fungi; oomycetes; bacteria; drought tolerance; salinity stress; bioinoculants; sustainable horticulture

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ORAL PRESENTATION

DETERMINATION OF SOME MORPHOLOGICAL, PHYSIOLOGICAL, AND COLOR VALUES OF CASTOR BEAN (*RICINUS COMMUNIS* L.) GROWN IN VAN PROVINCE

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Castor bean (*Ricinus communis* L.) is an important industrial crop cultivated commercially in many countries and regions around the world. The plant is predominantly grown in India, China, Brazil, and the United States. It has wide applications in medicine, the plastics industry, dye production, and textiles. The oil extracted from its seeds, due to its ricinolein content, is also utilized in biodiesel production. This study was conducted in the experimental fields of Van Yüzüncü Yıl University, Faculty of Agriculture. The castor bean plants used as study material were first grown into seedlings under controlled conditions in a growth chamber with a 16/8-hour light/dark photoperiod, 250±10 µmol m^{-2} light intensity, 25°C temperature, and 65% relative humidity, after which they were transplanted into the experimental area. Dualex measurements were taken immediately before harvest. The obtained Dualex values were as follows: NBI 11.5 dx, chlorophyll 21.8 dx, flavonoid 1.9 dx, and anthocyanin 0.16 dx. The plant height was measured as 37 cm, and the leaf area was determined to be 31.82 cm^2. Color parameters were measured separately for leaves and stems. The L*, a*, b*, chroma, and hue values were 41.25, -1.57, 13.22, 13.32, and 96.5 for the leaves, and 27.72, 16.10, 2.16, 16.25, and 7.6 for the stems, respectively. It was observed that the growth of castor bean, which is primarily cultivated in tropical and warm regions, was limited under the ecological conditions of Van.

Key Words: Dualex, NBI, Color, Van

ORAL PRESENTATION

AROMATIC DIVERSITY AMONG WILD AND CULTIVATED STRAWBERRIES STRAWBERRY FRUITS

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Strawberries are one of the most important fruit species in the berry group. They are cultivated both in greenhouses and open fields on every continent. Interest in strawberries is increasing year by year due to their attractive and aromatic fruits. The strawberry is renowned for its distinctive fragrance and is regarded as one of the most popular fruits globally. While, strawberries are predominantly consumed fresh, they are also frequently utilized in the production of juices, jams, and ice cream. With the expansion of the strawberry economy and evolving consumer expectations regarding fruit quality, researchers have shifted their focus beyond basic quality metrics, such as yield and general fruit quality, to include the aroma of strawberries. Strawberries exhibit one of the richest diversities of aromatic substances among fruits, making aroma a critical component of strawberry fruit. Compared to cultivated strawberries, wild strawberries are characterized by low yields and small berries. Another negative characteristic of wild strawberries is their very soft fruit. On the other hand, wild strawberries are valued for their higher bioactive content and aroma diversity, which is mainly determined by volatile organic compounds (VOCs). Wild strawberries, with broader and more intense VOC profiles, are especially important in breeding programs. This study compared wild and cultivated strawberry fruits in terms of aroma compounds. The primary aroma compounds in wild strawberry, include γ-Decalactone (31.10%), 2-Undecanone (17.20%) and 1-Pentadecone (12.40%) while cv. Monterey include γ-Decalactone (39.22%) and Nerolidol (30.15%), respectively.

Key Words: Strawberry, aromatic diversity, wild, cultivated, VOC profile

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ORAL PRESENTATION

VEGETATIVE GROWTH RESPONSE OF LAVENDER TO MULCHING IN OLIVE–LAVENDER INTERCROPPING SYSTEMS

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Intercropping lavender with olive trees has gained increasing attention as a sustainable land-use strategy in Mediterranean agroecosystems, providing both economic diversification and ecological benefits. However, competition for water and nutrients may limit the vegetative development of lavender under olive orchard conditions. Mulching is a widely adopted agronomic practice known to improve soil moisture conservation, suppress weed growth, and regulate soil temperature. This study aimed to evaluate the effects of mulch application on the vegetative growth of lavender cultivated between olive rows. The experiment was conducted under field conditions in an established olive orchard, where lavender plants were grown as an intercrop. Black polyethylene mulch was used as an inorganic mulching material, along with a non-mulched control. Vegetative growth parameters such as shoot length, plant height, canopy diameter, fresh biomass, dry biomass, peduncle length and spike length were measured during the growing season.

The results demonstrated that mulch application significantly enhanced vegetative growth of lavender compared to the control treatment. Mulched plots showed increased plant height, greater shoot proliferation, and higher biomass accumulation. These improvements were more pronounced under mulch treatments, likely due to improved soil moisture availability, reduced weed competition, and enhanced soil physical conditions. In contrast, non-mulched plots exhibited lower growth performance, possibly as a result of increased water stress and competition within the olive orchard system. In conclusion, mulching represents an effective management practice for promoting vegetative growth of lavender in olive–lavender intercropping systems. The integration of mulch application can improve lavender establishment and growth performance, thereby increasing the sustainability and productivity of olive orchards with aromatic plant intercropping. These findings highlight the potential of mulching to optimize resource use and support diversified cropping systems in semi-arid Mediterranean environments.

Key Words: Lavender, olive orchard, intercropping system, mulching, vegetative growth

ORAL PRESENTATION

LaMYC7-MEDIATED TRANSCRIPTIONAL REGULATION OF β-CARYOPHYLLENE BIOSYNTHESIS IN *LAVANDULA ANGUSTIFOLIA*

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Caryophyllene is the main sesquiterpene compound in lavender; however, the genes regulating its biosynthesis remain largely unknown. Here, we identified LaMYC7, a positive regulator of caryophyllene biosynthesis, which confers plant resistance to *Pseudomonas syringae*. LaMYC7 was highly expressed in glandular trichomes, and overexpression of LaMYC7 significantly increased caryophyllene content and reduced susceptibility to *P. syringae* in *Nicotiana*. Further analysis demonstrated that LaMYC7 directly bound to the promoter region of *LaTPS76*, which encodes the terpene synthase (TPS) for caryophyllene biosynthesis—and that *LaTPS76* was also highly expressed in glandular trichomes. Notably, co-expression of *LaMYC7* and *LaTPS76* in *Nicotiana* was found to further enhance the plant's resistance to *P. syringae*. Additionally, the *LaMYC7* promoter contains hormone- and stress-responsive regulatory elements, and it responds to various treatments, including ultraviolet radiation, low temperature, salt stress, drought, methyl jasmonate application, and *P. syringae* infection. Under these treatments, changes in caryophyllene content were consistent with alterations in *LaMYC7* transcript abundance. Based on these results, LaMYC7 is not only involved in caryophyllene biosynthesis but also responds to *P. syringae* infection. Thus, the MYC transcription factor gene LaMYC7 can be utilized for breeding lavender varieties with high caryophyllene yields and pathogen resistance.

Key Words: *Lavandula angustifolia*, LaMYC7, caryophyllene biosynthesis, *Pseudomonas syringae*

ORAL PRESENTATION

PROTECTIVE ROLE OF *CYNARA CARDUNCULUS* L. LEAF EXTRACT ON GUT BARRIER AND INFLAMMATION: INSIGHTS FROM *IN VITRO* MODELS

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Intestinal epithelial cells form a highly specialized barrier that protects the body from harmful substances present in the gut lumen through tight junctions (TJ), which connect adjacent cells, regulate paracellular transport, and maintain epithelial polarity. When TJ integrity is compromised, intestinal permeability increases, a condition commonly referred to as "leaky gut". This phenomenon is frequently associated with gastrointestinal disorders such as inflammatory bowel disease (IBD), characterized by chronic immune-mediated inflammation and impaired intestinal function. Polyphenols, naturally occurring compounds in plants, have gained attention for their ability to support gut health by preserving barrier integrity and modulating inflammatory responses. In this context, the present study investigated the in vitro effects of a standardized polyphenol-rich extract obtained from *Cynara cardunculus* L. subsp. *scolymus* leaves (CCLE) on intestinal epithelial barrier function and acute inflammation induced by TNF-α in Caco-2 cells.

Treatment with CCLE during cell differentiation improved barrier properties, as evidenced by increased transepithelial electrical resistance (TEER), reduced fluorescein permeability, and enhanced expression of TJ proteins (occludin, claudin-1, ZO-1). CCLE also accelerated TJ reassembly in the Ca^{2+} switch assay, effects linked to activation of the AMPK/SIRT1 signaling pathway. Furthermore, in the TNF-α-induced inflammation model, CCLE inhibited NF-κB activation, downregulated pro-inflammatory mediators (IL-8, COX-2), and reduced oxidative stress by stimulating the Nrf2 pathway, thereby improving cellular redox balance. Overall, these findings highlight the potential of CCLE to reinforce intestinal barrier integrity and counteract inflammatory damage. Considering that *Cynara* leaves are currently an agro-industrial byproduct, their valorization as a source of bioactive polyphenols represents a promising strategy for promoting gut health and supporting the prevention or management of IBD.

Key Words: *Cynara cardunculus* L., intestinal epithelial barrier function, AMPK/SIRT1 pathway, inflammatory bowel diseases, NF-κB, Nrf2

ORAL PRESENTATION

NANOCURCUMIN IN PRECLINICAL RESEARCH: A SYSTEMATIC REVIEW OF IN VITRO AND IN VIVO EVIDENCE

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Curcumin is a well-known phytochemical derived from the species *Curcuma longa*. Many benefits have been demonstrated in studies for curcumin, the most important and proven of which are its antioxidant and anti-inflammatory properties. However, these benefits come with a major downside: its extremely low bioavailability. Several studies have been conducted to increase the solubility, stability, and gastrointestinal absorption of curcumin using nanotechnology. In this systematic review, we aimed to examine various strategies and nanocurcumins for inflammatory bowel disease (IBD). A comprehensive literature search was conducted in PubMed, Scopus, and Web of Science up to July 2024, using the terms "nano," "curcumin," "inflammatory bowel disease," and "colitis." Out of 559 retrieved records, 69 eligible studies were analyzed. The formulations identified included lipid-based systems, polymeric nanoparticles, micelles, niosomes, dendrimers, and hybrid nanocarriers. The results showed that nanocurcumins reduced proinflammatory mediators TNF-α, IL-1β, and IL-6, among others, while increasing anti-inflammatory mediators IL-4 and IL-10. Our study provides a closer look at the advancement of nanotechnology in the optimal clinical use of the world of medicinal plants.

Key Words: Curcumin, nanocurcumin, inflammatory bowel disease, nanotechnology, anti-inflammatory activity, bioavailability

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ORAL PRESENTATION

COMPARISON OF THE MORPHOLOGICAL AND KARYOLOGICAL CHARACTERISTICS OF *CENTAUREA ANTHEMIFOLIA* AND *C. SIPYLEA* (COMPOSITAE)

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This study presents a comparative evaluation of the morphological and karyological characteristics of *Centaurea anthemifolia* Hub.-Mor. and *C. sipylea* Wagenitz, two closely related species belonging to the family Compositae. Although the morphological features of both taxa have been previously documented, their karyological properties are reported here for the first time. Detailed morphological examinations revealed clear diagnostic differences between the species, particularly in stem indumentum, leaf segmentation, involucre size and shape, appendage morphology, and pappus length. *C. anthemifolia* is characterized by its dwarf habit, dense white-tomentose stems, narrower involucres, more numerous cilia on the appendages, and shorter floral and pappus structures, whereas *C. sipylea* exhibits a taller stature, slightly arachnoid indumentum, larger ovoid involucres, fewer cilia, conspicuous hyaline auricles, and longer florets and pappus. Karyological analyses demonstrated that both species share the same diploid chromosome number (2n = 18, x = 9). However, notable differences were identified in chromosome morphology and karyotype asymmetry indices. *C. anthemifolia* exhibits a more heterogeneous and asymmetric karyotype (7m + 2sm; Stebbins 4B), while *C. sipylea* shows a more symmetrical chromosomal structure (8m + 1sm; Stebbins 4A). Additional asymmetry parameters (TF%, AsK%, A1–A2, CVcl, CVci) consistently indicate greater chromosomal variability in *C. anthemifolia*. Overall, the combined morphological and cytogenetic evidence clearly differentiates the two species. These findings contribute to a better understanding of species delimitation within *Centaurea* and provide valuable cytotaxonomic data for future evolutionary and systematic studies.

Key Words: Asteraceae, chromosome, endemic, knapweed

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ORAL PRESENTATION

NUTRITIONAL CHARACTERIZATION, FATTY ACID PROFILE AND ANTIOXIDANT PROPERTIES OF SUPERCRITICAL CO_2 EXTRACTS OF *CUCURBITA MOSCHATA* DUCHESNE EX POIRET SEEDS

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Cucurbita moschata Duchesne ex Poiret is known to harbor a wide array of bioactive compounds exhibiting antioxidant activities, including flavonoids, phenolic compounds, lipids, saponins, terpenoids expecially. Due to the presence of bioactive lipids, and proteins in its capsules and seeds, this species has found extensive applications across pharmaceutical, nutraceutical and cosmetic industries. In supercritical fluids, the diffusion coefficients of substances such as lipids and waxes are significantly higher than in liquids, which accelerates the extraction process. In our study, *C. moschata* seed oil was extracted by supercritical carbon dioxide extraction and extraction kinetics were modeled with fixed control method. Extraction efficiency and nutritional valueswere investigated in process parameters such as pressure (37 MPa), temperature (50 ^{0}C), 180 minutes and CO_2 flow rate (1 L CO_2 /min).

The carbohydrate, protein, and dietary fiber contents of pumpkin seed oil were determined to be 0, which is attributable to its lipid-based matrix, indicating the absence of these components at detectable levels. Total fatty acid contents of *C. moschata* seed oil were calculated from GC–MS temperature–distribution profiles. The determination of the total fat content of *C. moschata* seed oil as 99.86% indicates a very high degree of purity, with impurity levels being negligible. The study investigated the fixed oil's fatty acid content from the *C. moschata* seeds; linoleic acid (45.6%), oleic acid (33.7%), palmitic acid (12%), and stearic acid (6%) were the main constituents identified in the samples' composition. The nutritional energy value of *C. moschata* seed oil was determined to be 898.74 kcal/100 mL. ABTS•$^+$ radical scavenging activity (IC_{50}: 30.26±0.73 µg/mL), metal chelating activity (IC_{50}: 32.63±0.22 µg/mL) and CUPRAC activity ($A_{0.50}$: 149.74±0.75 µg/mL) were determined in *C. moschata* supercritical extract. DPPH• scavenging assay did not show any significant activity in the extract. The *C. moschata* seed oil although numerous studies have been conducted on *C. moschata* in the existing literature, research involving supercritical carbon dioxide extraction remains quite limited. These findings give support to the ethnopharmacological use of the plant in the treatment of several inflammatory ailments and food industry. Therefore, we believe that this study can constitute an important step in understanding the health benefits of *C. moschata* seed oil.

Key Words: *Cucurbita moschata*, CO_2 extract, dietary fiber, oil, nutritional protein, supercritical, total fatty acid.

ORAL PRESENTATION

EFFECT OF A MEDICINAL PLANT PHYTOCOMPOSITION ON CORTISOL AND CYTOKINE LEVELS IN DEXTRAN-INDUCED INFLAMMATION

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The objectives of study were a comparative investigation of the mix of dry extracts from medicinal plants, such as *Lycopus europaeus, Aerva lanata, Equisetum arvense, Tribulus terrestris, Achillea millefolium, Cucumis sativus seeds, Zea mays stigmas with styles,* and *Glycyrrhiza glabra* conditionally named Ecustim on the dynamics of cortisol and some cytokins (IL-10, IL-1β, TNF-α) level in the blood during dextran-induced aseptic inflammation model. The effect of phytocomposition was studied on white rats. Experimental model of aseptic inflammation was reproduced by injecting 0.1 ml of a 6% dextran solution into the hind paw of rats. The preventive effects of Ecustim (100, 200 mg/kg), as well as sodium diclofenac (10 mg/kg) and Articur (100 mg/kg), were studied. Peripheral blood serum samples from the rats were collected after administration of the studied doses of Ekustim, diclofenac sodium, and Articur. One hour after dextran injection, a significant increase in peripheral blood cortisol levels was observed in all experimental groups studied. At the same time, cortisol levels increased by 3.7- and 4.1-fold in the groups treated with Ekustim and diclofenac sodium, respectively, compared with the control group. Two hours after the start of the experiment, Ekustim led to an 81.9% increase in peripheral blood hormone levels compared with the control group, and three hours after the administration of the phylogogenic agent, this difference amounted to 78.2%. It was established that the investigated phytocomposition also increased the level of IL-10 by 4.6 times and decreased the level of IL-1β by 5.4 times in the blood in dextran-induced inflammatory process. Thus, cortisol exerts an effect in reducing inflammatory reactions, and an increase in cortisol and IL-10 production and decrease in IL-1β level during inflammation undoubtedly has a positive impact on enhancing the anti-inflammatory effects of the studied phytocomposition.

Key Words: Phytocomposition, cortisol, cytokine, dextran-induced inflammation

ORAL PRESENTATION

EFFECTS OF CHENOPODIUM ALBUM L. ON ENZYME INHIBITION MECHANISMS

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Chenopodium album L. is traditionally used in Türkiye to alleviate bloating and gas pain [1]. Based on this data, the inhibitory effect of the plant on key enzymes involved in gastric disorders — chymotrypsin, trypsin, lipase, α-glucosidase, xanthine oxidase, and urease —was investigated. For this purpose, aqueous and ethanol extracts were prepared from the aerial parts of the plant. Subsequent liquid-liquid fractionation of the ethanol extracts yielded dichloromethane, ethyl acetate, *n*-butanol, and remaining water fractions. The aqueous extract was similarly processed to obtain ethyl acetate, *n*-butanol, and remaining water fractions. The dichloromethane fraction of the ethanol extract demonstrated notable inhibitory activity against trypsin and chymotrypsin (IC_{50} values of 35.41 ± 1.66 µg/mL and 28.17 ± 0.94 µg/mL, respectively). The ethyl acetate fraction of the ethanol extract significantly inhibited α-glucosidase, lipase, and urease (IC_{50} values of 90.73 ± 2.05 µg/mL, 21.65 ± 1.52 µg/mL, and 75.32 ± 1.07 µg/mL, respectively). All tested extracts and fractions exhibited low to moderate inhibitory activity against xanthine oxidase. The results validate the traditional use of *Chenopodium album* for gastrointestinal complaints by demonstrating its significant inhibitory potential against several digestive and metabolic enzymes. Further studies are planned to identify the specific bioactive compounds responsible for these observed effects.

Key Words: *Chenopodium album*, chymotrypsin, trypsin, lipase, α-glucosidase, urease

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ORAL PRESENTATION

ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS ON ORAL MICROORGANISMS IN INFLAMMATORY PERIODONTAL DISEASES

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Objective / Purpose: The continuously increasing rate of antimicrobial resistance development among microorganisms necessitates the search for novel substances with antimicrobial activity. Medicinal plants, widely used in both folk and conventional medicine, represent a promising source of biologically active compounds with antimicrobial properties. The aim of this study was to investigate the spectrum of antibacterial activity of essential oils against obligate anaerobic microorganisms of the oral cavity isolated from periodontal pockets in the context of periodontitis.

Methods: Essential oils obtained from the following wild-growing plants were used in this study: *Eucalyptus globulus* Labill., *Lavandula angustifolia* Mill., *Origanum vulgare* L., *Mentha piperita* L., *Matricaria recutita* L., *Salvia officinalis* L., *Anethum graveolens* L., *Thymus serpyllum* L., *Pimpinella anisum* L., *Carum carvi* L., *Coriandrum sativum* L., and *Pinus sylvestris* L. Microbial susceptibility to essential oils was determined using the agar well diffusion method.

Results: Analysis of the chemical composition of the essential oils of *Pinus sylvestris* L., *Eucalyptus globulus* Labill., and *Anethum graveolens* L. revealed several common components with potential significance in growth inhibition and bactericidal activity against periodontopathogenic bacteria, namely monoterpenes (α-pinene, β-pinene, and limonene). In addition, strains of three species—*Porphyromonas asaccharolytica*, *Slackia exigua*, and *Schaalia odontolytica*—collectively exhibited the highest persistence rate, accounting for 51.72% of the total sample (17.24% each).

Conclusions: Thus, the results of this *in vitro* study indicate that all investigated essential oils demonstrated antimicrobial activity against numerous strains of obligate anaerobic periodontopathogens isolated from periodontal pockets of patients with periodontitis. In particular, the highest activity was observed for essential oils of *Pinus sylvestris* L., which inhibited the growth of 91.37% of strains, *Eucalyptus globulus* Labill., with 87.93% of sensitive strains, and *Anethum graveolens* L., with 81.03% of sensitive strains. Other essential oils were also effective but exhibited slightly lower antimicrobial activity compared to the aforementioned oils.

Key Words: antimicrobial activity, anaerobic bacteria, essential oils

ORAL PRESENTATION

CUSCUTA EPITHYMUM (L.) L. IN ENDOMETRIOSIS: TRANSLATIONAL INSIGHTS BRIDGING PERSIAN MEDICINE AND MOLECULAR MECHANISMS

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Endometriosis is a chronic inflammatory disorder affecting female reproductive health. *Cuscuta epithymum* (CE), an herb traditionally associated with spleen-related functions in Persian Medicine, has shown pharmacological potential relevant to this condition. CE contains bioactive flavonoids, terpenoids, and alkaloids with anti-inflammatory, antioxidant, and neuroimmune-modulating effects. These compounds interact with pathways central to endometriosis pathophysiology, including oxidative stress, cytokine signaling, immune cell modulation, angiogenesis, and pain regulation. This multi-mechanistic profile aligns with the complex nature of the disease and highlights CE as a promising candidate for further research. Direct studies of CE in endometriosis are lacking. Key next steps include standardized extract preparation, dose optimization, and safety evaluation. In vitro studies can examine effects on endometrial stromal cells, angiogenic pathways, oxidative markers, and immune modulation, followed by in vivo models to clarify the impact on lesions and pain. Positive mechanistic evidence could support early-phase clinical trials evaluating safety and biomarker responses. Integrating traditional insights from Persian Medicine with modern molecular and immunological data provides a novel translational perspective. *Cuscuta epithymum* emerges as a biologically credible candidate for further research in endometriosis.

Key Words: Endometriosis, *Cuscuta epithymum*, infertility, immune modulation, Persian Medicine

ORAL PRESENTATION

PHYSICOCHEMICAL CHARACTERIZATION AND BIOACTIVE COMPOUNDS OF CRAFT PALE ALE BEER WITH ADDITION OF *OPUNTIA APURIMACENSIS* (TUNA AYRAMPO) EXTRACTS AND AGRO-INDUSTRIAL RESIDUES

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Craft beers infused with the distinctive flavors and aromas of native Andean fruits have sparked interest in enhancing the bioactive compounds and sensory qualities of beer. This research centered on creating a pale ale using the juice of Ayrampo prickly pear and agro-industrial byproducts. The objective - to analyze physicochemical properties, phenolic compounds, particularly betalains antioxidant capacity, taste and acceptability of craft beer produced using Ayrampo prickly pear juice (*Opuntia apurimacensis*) and agro-industrial waste (AIW) from the extrusion of cereals. The methodology included malting the barley (MB), macerating, cooking the wort together with the AIW, hops, and the addition of Ayrampo prickly pear juice followed by inoculation of *Saccharomyces cerevisiae* and fermented for 15 days. In the mashing process, MB and AIW were added at various proportions. Results of pH and ºBrix measurements at the end of the maceration stage and the alcoholic degrees at the end of fermentation are presented. The acceptability was tested with 30 semi-trained panelists, reporting significantly different scores for general appearance, color, clarity, aroma and flavor, the highest score was obtained by the sample with 90% MB and 10% AIW. The appropriate percentage when adding Ayrampo prickly pear juice and AIW in the preparation of ale beer is 10%. In this winning treatment, the phenolic compound of 7,990 mg/g of sample was determined, its antioxidant capacity was 32,655 mMol TE/L by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, and total betalains of 8,535 BET mg/g of sample.

Key Words: craft beer, betalains, phenolic compounds, agricultural industrial waste, prickly pear juice

ORAL PRESENTATION

PLANT-INSPIRED RATIONAL DESIGN AND SYNTHESIS OF NOVEL HYBRID SMALL MOLECULES TARGETING SOD1 AND TDP-43 PROTEINS

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Neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS), are closely linked to pathological protein aggregation processes involving superoxide dismutase-1 (SOD1) and TAR DNA-binding protein-43 (TDP-43). Natural products and plant-derived bioactive compounds have long provided structurally diverse scaffolds and pharmacophoric motifs for drug discovery against such complex targets. In this study, a plant-inspired medicinal chemistry approach was applied to the rational design and development of novel hybrid small molecules capable of simultaneously targeting both SOD1 and TDP-43 proteins. Guided by heterocyclic and aromatic frameworks frequently encountered in plant-derived bioactive compounds, a focused virtual library of approximately 100 natural product-like hybrid structures was constructed. These compounds were evaluated by molecular docking studies against SOD1 (PDB ID: 5YTO) and TDP-43 (PDB ID: 4Y00) binding sites using Glide/XP protocols. Computational screening identified three pyrazoline-based lead candidates exhibiting the highest binding affinities toward both protein targets. Detailed interaction analyses revealed that these hybrids form favorable hydrogen bonding, π-π stacking, and electrostatic interactions with key residues involved in SOD1 and TDP-43 recognition. Following *in silico* validation, the synthesis of selected hybrid molecules was initiated. Pyrazoline-based starting materials were successfully prepared using facile synthetic routes and were structurally confirmed by ^{1}H NMR, ^{13}C NMR and HRMS analyses. This work highlights the potential of plant-inspired scaffold hybridization combined with rational molecular design as an effective strategy for discovering new dual-target ligands relevant to anti-ALS research.

Key Words: ALS, SOD1, TDP-43, plant-derived bioactive compounds, pyrazoline

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ORAL PRESENTATION

KARYOMORPHOLOGICAL STUDIES OF THREE ENDEMIC *AETHIONEMA* (BRASSICACEAE) SPECIES IN TURKIYE

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The genus *Aethionema* W.T.Aiton, belonging to the Aethionemeae tribe and known as rock rose in our country, originates from the Irano-Turanian Phytogeographical region and is represented by approximately 60-70 species worldwide. The tribe originated from the Anatolian Cross during the Pliocene and subsequently spread from Southwest Asia and the Mediterranean regions to Northwest Africa. Türkiye is the most important center of gene and differentiation for the *Aethionema* genus. It is represented by 51 species in Türkiye. A member of the Brassicaceae family, which includes many taxa used in traditional medicine and cuisine, some taxa belonging to the *Aethionema* genus are also used by the public against certain diseases. *Aethionema* is a taxonomically problematic genus. Karyological information is important in solving taxonomic problems. This study aims to characterize three endemic species (*Aethionema alanyae* H.Duman, *Aethionema adiyamanense* Yıld. & Kılıç, and *Aethionema lepidioides* Hub.-Mor.) of the *Aethionema* genus chromosomally. Determining the relationships between karyomorphological features among taxa that are clearly different or very closely related is of taxonomic importance. In this study, chromosome counting and morphology-based analyses were performed on three species that serve as models for the genus *Aethionema*, whose chromosomes are so small that counting them is often impossible. Chromosome counts were determined as $2n$=24 for *A. lepidioides* and *A. adiyamanense*, and $2n$=36 for *A. alanyae*. All chromosomes of the species are metacentric. The chromosomes of the taxa are quite small, averaging less than 2 micrometers. According to the chromosomal indices used, although *A. alanyae*, *A. adiyamanense*, and *A. lepidioides* show partially similar asymmetry indices [CV_{CI} (3.535; 4.314; 3.029), AI (0.59; 0.569; 0.537) and M_{CA} (9.74; 10; 9.38)], we can say that *A. lepidioides* has the most primitives among these three species.

Key Words: Chromosome number, Cruciferae, Karyomorphology, Karyotype

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ORAL PRESENTATION

ASSESSMENT OF THE CYTOTOXIC EFFECTS OF *MUSCARI MICROSTOMUM* P.H. DAVIS & D.C. STUART EXTRACTS

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Türkiye, with over 3,000 documented species in its flora, ranks among the world's leading countries in terms of aromatic and medicinal plant diversity. While this rich biodiversity offers significant potential, studies investigating the chemical composition and biological activities of many plant species are limited. The genus *Muscari*, commonly known as grape hyacinth, is an important group of bulbous plants widely found throughout Türkiye. However, the biological characteristics of various *Muscari* species have not yet been sufficiently investigated. In this study, the cytotoxic effects of extracts obtained from different parts of *Muscari microstomum* were investigated using two different extraction methods. The extracts were applied to colorectal cancer cell lines at five different concentrations and incubated for two different durations. Cytotoxic activity was evaluated via MTT test. The findings showed that the tested extracts caused cytotoxic effects in a dose/time-dependent manner. Additionally, the magnitude of the cytotoxic response varied depending on both the plant part used for extraction and the extraction method applied. These results demonstrate that the biological activity of *Muscari microstomum* is largely influenced by both extraction-related factors and the origin of the plant tissue. Overall, the data suggest that *Muscari microstomum* exhibits considerable cytotoxic potential against colorectal cancer cells and could be a promising natural resource for the discovery of novel anticancer agents. To better understand this potential, further studies focusing on the isolation, characterization, and elucidation of the mechanisms of action of the responsible bioactive compounds are needed.

Key Words: Çayır müşkürümü, MTT, Türkiye

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ORAL PRESENTATION

SYNERGY OF PHYTOTHERAPY AND ACUPUNCTURE FOR VITALITY AND UROGENITAL HEALTH IN PERIMENOPAUSAL AND MENOPAUSAL WOMEN

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Objective / Purpose: Perimenopause and menopause are characterized by a decline in estrogen levels, leading to vaginal mucosal atrophy, disturbances in the vaginal microbiota, and impaired pelvic floor function. Stress urinary incontinence is one of the most clinically relevant symptoms, resulting from reduced muscle tone and connective tissue changes. This paper explores a complementary therapeutic approach integrating honey, herbal macerates, a plant-based medical product, acupuncture, and Kegel exercises for women in perimenopause and menopause. Honey and herbal macerates exhibit antimicrobial and anti-inflammatory properties and promote epithelial repair, contributing to mucosal regeneration and microbiota balance. Bioapigyn® vaginal ointment for pelvic muscle tonus, based on bee-derived components, alleviates vaginal dryness and irritation while supporting epithelial healing and optimal urogenital moisture. Pelvic floor rehabilitation plays a central role, and in addition to Kegel exercises, acupuncture is increasingly used as supportive therapy. Evidence suggests that acupuncture may modulate neuroendocrine balance, improve vascularization, and enhance neuromuscular coordination, helping reduce incontinence episodes.

Materials and Methods: The study included patients from a gynecological clinic who, following complete gynecological examination, breast ultrasound, and laboratory assessment, opted for a complementary therapy approach due to vasomotor symptoms, dyspareunia, and incontinence.

Results: This therapeutic combination is suitable for patients who cannot or prefer not to use hormone replacement therapy (HRT), but can also complement HRT. Findings suggest that a multidisciplinary approach improves quality of life during perimenopause and menopause. No adverse effects, worsening of symptoms, irritation, or allergic reactions were reported during treatment or follow-up.

Conclusion: The combination of these therapeutic approaches represents an option for patients who cannot or do not wish to use hormone replacement therapy. Further randomized controlled studies are needed to establish standardized guidelines for their use in clinical practice**.**

Key Words: Honey, herbal macerate, stress urinary incontinence, urogenital atrophy, menopause, acupuncture

POSTER PRESENTATION

Poster Presentations

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POSTER PRESENTATION

EVALUATION OF PHENOLIC CONTENT, ANTIOXIDANT CAPACITY, AND ENZYME INHIBITION ACTIVITIES OF *NEPETA ITALICA* L. ETHANOL EXTRACT

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The genus *Nepeta* (Lamiaceae) is represented by more than 250 species worldwide, with 41 taxa found in Turkey, 18 of which are endemic (1). *Nepeta* species, traditionally used as antitussive, sedative, diuretic, antispasmodic, and antipyretic agents, are rich in terpenoids, flavonoids, and phenolic compounds (1). Their essential oils are utilized in the perfume, flavoring, and food industries, and exhibit antibacterial, antifungal, antioxidant, and anti-inflammatory activities (1). Due to the potential harmful effects of synthetic antioxidants, *Nepeta* species have attracted increasing interest as natural antioxidant sources (2). In this study, the antioxidant capacity and cholinesterase, urease, and tyrosinase enzyme inhibition activities of the ethanol extract of *Nepeta italica* L. were determined. The analyses revealed that the biological potential of the ethanol extract was generally low to moderate. The total phenolic (11.13 µg PEs/mg) and flavonoid (5.48 µg QEs/mg) contents were limited, suggesting relatively weak antioxidant activity. Indeed, the ABTS (IC_{50} = 179.46 µg/mL) and DPPH (IC_{50} = 232.58 µg/mL) results were lower than those of standard antioxidants such as BHA and α-tocopherol, yet still meaningful for a naturally derived plant extract. In the anticholinesterase assays, the extract was inactive against AChE, while the BChE inhibition rate was found to be 47.05%, indicating potential for use as a supportive agent in the management of neurodegenerative diseases. Additionally, the urease inhibition activity was moderate at 30.93%, whereas no activity was detected against the tyrosinase enzyme. Overall, the findings demonstrate that although *Nepeta italica* extract is not particularly rich in phenolic content, it exhibits measurable biological activity on certain enzymes, suggesting its pharmacological potential for further investigation.

Key Words: *Nepeta italica L.*, antioxidant activity, enzyme inhibition, phenolic compounds

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POSTER PRESENTATION

THE INFLUENCE OF CULTIVATION PRACTICES ON THE CHEMICAL COMPOSITION AND BIOLOGICAL POTENTIAL OF *THYMUS SATUREIODES*

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Introduction: The Lamiaceae family is one of the most diverse and extensive plant families within the medicinal, aromatic and pharmaceutical domains. It holds significant socio-economic importance due to its various applications in the food, cosmetic, and pharmaceutical industries. Within the Lamiaceae family, the genus Thymus is predominantly found in the Mediterranean region, particularly in Morocco, where 13 species are endemic. One noteworthy species is *Thymus satureioides* which primarily thrives in the arid and semi-arid regions of the Anti-Atlas, Middle Atlas, and High Atlas Mountains, though it is rarely found in colder environments. Economically, thyme is particularly significant in the market, as highlighted by export analyses showing thyme as the dominant species with an average export volume of about 1,973.49 tons. However, this medicinal and aromatic plant faces major challenges in sustainable management practices, including anarchistic plant collection often conducted without proper management strategies. Additionally, climate-related pressures, such as low rainfall, have contributed to the decline of wild plant populations in Morocco. Notably, over 90% of the country's aromatic and medicinal plant (AMP) production still relies on wild harvesting, a practice that poses serious risks to biodiversity and the long-term availability of these plants.
Objective: This study aims to ensure the preservation and sustainable use of *Thymus satureioides* L., we examined the impact of growth conditions on the composition and biological activities of EO extracted from the leaves of both cultivated and wild *Thymus satureioides*.
Material & methods: Samples of aerial parts of wild and cultivated *Thymus satureioides* were collected during the flowering stage from the rural commune of Ouirgane, region of Marrakech-Safi. The wild plants were collected at the coordinates. Essential Oils Extraction and GC-MS Analysisas as well as biological (antibacterial, antioxidant and nematicidal) activities were carried out on both wild and cultivated L. dentata.
Results: The gas chromatography/mass spectrometry (GC/MS) analysis of EOs led to the identification of 18 and 24 compounds representing more than 83.53% of the total oils. The major compounds of wild *T. satureoides* EOs were thymol (25.01%), endo-borneol (16.44 %), L alpha terpineol (7.60%), and gamma-terpinene (7.45 %). Whereas EOs obtained from cultivated plants were dominated by thymol (31.72 %), endo-borneol (15.31 %), gamma-terpinene (7.53 %), and camphene (6.25 %). Qualitatively, wild EO was distinguished by the presence of some minor compounds such as gamma muurolene, t-muurolol, camphor, p-cimene, and borneol. Regarding the biological activities, the wild EO sample exhibited higher antioxidant, anti-microbial and nematicidal activity compared to the cultivated EO. These findings highlight the crucial role of wild environments in shaping the chemical profile of *T. satureoides*, thereby influencing the plant's biological activities. However, cultivation remains a promising approach for conserving of this diversity under managed conditions.

Key Words: *Thymus satureioides*, essential oils, gas chromatography analysis, antibacterial, antifungal, antioxidant and nematicidal properties.

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POSTER PRESENTATION

PICROCROCIN FROM *CROCUS SATIVUS* AS A POTENTIAL PHOSPHOLIPASE A_2 INHIBITOR: AN INTEGRATED *IN SILICO* ADMET, DOCKING AND MOLECULAR DYNAMICS STUDY

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Picrocrocin is one of the major apocarotenoids of *Crocus sativus* L. (saffron) and is primarily responsible for its characteristic bitter taste. Beyond its sensory role, picrocrocin has attracted increasing scientific interest due to its potential pharmacological properties, particularly in the modulation of inflammatory processes. Phospholipase A_2 (PLA_2) is a key enzyme in the inflammatory cascade, catalyzing the release of arachidonic acid and triggering the biosynthesis of pro-inflammatory mediators. In this study, an integrated *in silico* strategy was employed to investigate the pharmacokinetic profile and molecular interactions of picrocrocin toward PLA_2 through ADMET prediction, molecular docking, and molecular dynamics (MD) simulations. ADMET analysis indicated that picrocrocin exhibits favorable pharmacokinetic characteristics, including good aqueous solubility, acceptable intestinal absorption, absence of major cytochrome P450 inhibition, and low predicted toxicity, supporting its drug-likeness and safety profile. Molecular docking against PLA_2 revealed stable binding affinity, with picrocrocin occupying the enzyme active site and establishing multiple hydrogen bonds and polar interactions with key catalytic residues, suggesting a potential inhibitory effect on PLA_2 activity. To further validate the docking results, molecular dynamics simulations were performed to evaluate the stability and behavior of the picrocrocin–PLA_2 complex under physiological conditions. MD trajectory analyses, including root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration, and hydrogen bond persistence, demonstrated a stable ligand–enzyme complex with limited conformational fluctuations throughout the simulation period. Overall, this comprehensive *in silico* investigation highlights the anti-inflammatory potential of picrocrocin via PLA_2 inhibition and provides mechanistic insights into its molecular interactions and dynamic stability. These findings support the relevance of picrocrocin as a promising natural scaffold for the development of novel anti-inflammatory agents and encourage further *in vitro* and *in vivo* validation.

Key Words: Picrocrocin, Phospholipase A_2, *In silico* analysis, ADMET, Molecular docking, olecular dynamics

Acknowledgment:
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POSTER PRESENTATION

IMPACT OF SILVER NANOPARTICLES ON ANTIOXIDANT ACTIVITY OF *BRASSICA RAPA* VAR. *CHINENSIS* (PAK CHOI) UNDER *IN VITRO* CULTURE CONDITIONS

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The subject of this study was the initiation and optimisation of *in vitro* cultivation of *Brassica rapa* L. var. *chinensis* (pak choi) shoot cultures, with particular emphasis on the effect of supplementing the culture media with non-ionic silver nanoparticles (AgNPs) on the biosynthesis of phenolic compounds, glucosinolates, and silver bioaccumulation in plant biomass. This approach aimed to evaluate the potential of AgNPs in modulating the secondary metabolism of pak choi to enhance the content of biologically valuable substances with antioxidant activity. The culture media were supplemented with AgNPs (dimensions smaller than 5 nm) at concentrations of 0 (control), 5, 10, and 15 ppm. Methanolic extracts from the *in vitro* biomass were analysed for polyphenolic compounds (phenolic acids and flavonoids by HPLC-DAD), glucosinolates (by UHPLC-HRMS), and the bioaccumulation of elements (by ICP-MS). Subsequently, the antioxidant activity of the extracts was evaluated using five methods differing in mechanism and reaction environment (FRAP, CUPRAC, DPPH, ABTS, and total phenolics). Caffeic acid, sinapic acid, and ferulic acid, as well as quercetin and kaempferol, were detected in all extracts. AgNP supplementation significantly increased the content of phenolic acids, nearly doubling their levels. Eight glucosinolates were identified in extracts from supplemented cultures, including the predominant 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin, as well as lower amounts of progoitrin, glucoalyssin, glucobarbarin, glucobrassicin, and gluconasturtiin. At the same time, higher AgNPs concentrations resulted in a significant increase in Ag bioaccumulation in shoot biomass, reaching a maximum of 58.08 ppm. These changes in compound content had a notable impact on the antioxidant potential of all samples, increasing it compared to the control sample, especially at the concentration of 10 ppm of AgNPs. The research highlights the innovative and practical implications of NPs supplementation in *in vitro* culture models to improve their chemical composition and potential applications in the health and food industries.

Key Words: *Brassica rapa* var. *chinensis*, nanoparticles, *in vitro* culture, antioxidant potential, plant secondary metabolites.

Acknowledgements
This study was funded by the Polish National Science Centre, OPUS project no. 2023/49/B/NZ7/02428.

POSTER PRESENTATION

ANTIBACTERIAL AND ANTIINFLAMMATORY ACTIVITIES OF CACAO EXTRACT

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This study aimed to investigate the biological properties of cacao (*Theobroma cacao* L.) extracts prepared from different plant parts, including pod, husk, powder, and seed, using ethanol and water as extraction solvents. The extracts were evaluated for their antioxidant, anti-inflammatory, antimicrobial, and cytotoxic potentials. Antibacterial activity was tested against twelve pathogenic bacterial strains using the disk diffusion method. Total phenolic and flavonoid contents were quantified, and antioxidant activity was assessed using DPPH and ABTS assays. Cytotoxicity was determined by the MTT assay in RAW 264.7 cells, while anti-inflammatory activity was evaluated by measuring nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages. The results showed that none of the cacao extracts inhibited the growth of the tested bacterial strains. However, the ethanolic extracts particularly those from cacao pods contained the highest levels of phenolic and flavonoid compounds and exhibited the strongest antioxidant activity in both DPPH and ABTS assays. Cytotoxicity analysis revealed that the ethanolic powder extract had the lowest LC_{50} value (14.33 µg/mL), indicating high toxicity, whereas extracts from pods and husks demonstrated the lowest cytotoxicity. Regarding anti-inflammatory activity, the ethanolic extracts of pod and powder significantly reduced NO production at concentrations of 50–100 µg/mL ($p < 0.05$). In conclusion, cacao extracts, especially ethanolic extracts from pods and powder, exhibited notable antioxidant and anti-inflammatory properties, although no antibacterial activity was observed under the conditions tested. These findings support the potential of cacao as a natural source of bioactive compounds for future health-related applications.

Key Words: cacao extracts, phenolic compounds, antioxidant activity, anti-inflammatory activity, cytotoxicity, bioactive compounds

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POSTER PRESENTATION

PHYTOCHEMICAL INVESTIGATION OF *NEPETA ITALICA* L. SPECIES BY LC-MS/MS AND GC-MS

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Nepeta L. species, used by the public as a diaphoretic, diuretic, cough suppressant, sedative, antispasmodic, antiasthmatic, febrifuge and for stomach ailments, is the largest member of the Lamiaceae family [1,2]. Thanks to the secondary metabolites they contain, they possess many biological activities such as antioxidant and antibacterial properties. *Nepeta italica*, a member of the *Nepeta* genus, is a species that is widespread in Turkey [2]. In this study, 10 g of the aerial parts of *Nepeta italica* L. species, which were dried and powdered in the shade, were weighed and extracted with ethanol (50 mL each) 3 times under room conditions. After the solvents were evaporated under vacuum, stock solutions of the extracts at a concentration of 4000 µg/mL were prepared and stored at +4ºC. The qualitative and quantitative phytochemical composition of the extracts obtained were analyzed according to an LC-MS/MS method (Shimadzu 8040 model) previously developed and validated by our research group. For aroma analysis, 1 g of dry plant sample was taken, placed in a headspace vial, and kept at 40°C for 15 minutes, and aroma analysis was performed using GC-MS. According to LC-MS/MS results, caffeic acid (526.01 µg/g extract), luteolin-7-glucoside (436.24 µg/g extract) and apigenin 7-O-glucoside (180.74 µg/g extract) were determined as the major components of ethanol extract of *Nepeta italica* species. The results of GC-MS analysis indicate that the major components of the aroma are carvacrol (48.89%), thymol (10.18%), and decan (8.44%).

Keywords: *Nepeta italica*, LC-MS/MS, caffeic acid, aroma, carvacrol

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POSTER PRESENTATION

NEUROPROTECTIVE POTENTIAL OF MEDICINAL SALT TOLERANT PLANTS

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Salt-tolerant plants (STPs), many of which are recognized as medicinal species in various traditional systems, represent an underexploited reservoir of bioactive compounds. Increasing scientific interest in their pharmacological properties reflects the urgent need for novel therapeutic agents, particularly in the field of neuroprotection. This work aimed to evaluate the ethnomedicinal uses of STPs in the treatment of neurological disorders and to assess existing scientific evidence supporting their efficacy. A systematic literature search was conducted across major databases (PubMed, Scopus, ScienceDirect), focusing on *in vitro* and *in vivo* studies reporting neuroprotective activity. Sixteen species from nine plant families were identified, with Chenopodiaceae and Juncaceae being the most frequently represented. Notably, *Salicornia* sp., *Juncus* sp., and *Limonium* sp. exhibited significant cholinesterase inhibitory activity *in vitro*, suggesting potential applications in managing neurodegenerative conditions such as Alzheimer's disease. However, only six of these species have been tested *in vivo*, highlighting a substantial gap between traditional knowledge and experimental validation. These findings emphasize the need for further pharmacological research on salt-tolerant medicinal plants, including *in vivo* assays, mechanism elucidation, and bioassay-guided fractionation for active compound identification. Moreover, saline ecosystems, rich in biodiversity yet still poorly studied, offer untapped potential for discovering plant species with ethnobotanical relevance and pharmacological promise. These insights are particularly relevant in the context of current efforts to valorize medicinal and aromatic plants as sustainable sources of innovative therapeutic agents. Bridging ancestral knowledge with modern pharmacological research, while ensuring environmental and ethical responsibility, could pave the way for the development of novel neuroprotective compounds derived from salt-tolerant plant biodiversity.

Key Words: Halophytes, Neuroprotection, Ethnomedicine, Cholinesterase inhibitors, Medicinal plant biodiversity

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POSTER PRESENTATION

REPURPOSING A MEDICINAL HALOPHYTE: STRESS RESPONSES OF *LIMBARDA CRITHMOIDES* L. DURING ACETAMINOPHEN PHYTOREMEDIATION

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The presence of pharmaceutical residues such as acetaminophen (paracetamol) in aquatic environments presents an increasing challenge for water treatment, especially in saline and estuarine systems. This study explores the potential of *Limbarda crithmoides* L., a medicinal halophyte native to the Mediterranean coast, to act as a phytoremediation agent under pharmaceutical micropollutant stress. While traditionally valued for its antioxidant and medicinal properties, the species' physiological resilience and metabolic plasticity under micropollutant exposure remain poorly understood. Rooted explants of *L. crithmoides* were cultivated *in vitro* in liquid Murashige and Skoog medium containing acetaminophen at 0.1, 1.0, and 2.0 mg/L for 7, 14, and 21 days. Acetaminophen concentrations in the media were quantified by HPLC-DAD and confirmed by GC-MS. The plant's physiological and biochemical responses were evaluated in both aerial and root tissues through measurements of photosynthetic pigments, oxidative stress markers (MDA), osmoprotectants (proline, sugars, proteins), and secondary metabolism indicators (phenolics, flavonoids, PAL activity, and shikimic acid). Results showed high removal efficiency (>98% at all concentrations by day 21), indicating strong phytoremediation capacity. However, this removal was associated with significant physiological trade-offs. Aerial tissues exhibited dose- and time-dependent oxidative stress, pigment fluctuations, and the activation of protective metabolic pathways. Roots remained more stable, suggesting spatial differentiation in stress perception and response. The study highlights the dual identity of *L. crithmoides* as both a bioactive plant and a resilient environmental tool. These findings support its integration into nature-based treatment systems, such as constructed wetlands, designed for saline environments contaminated with pharmaceutical micropollutants.

Key Words: medicinal halophyte; phytoremediation; acetaminophen; oxidative stress; metabolic adaptation; nature-based solutions

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POSTER PRESENTATION

DETERMINATION OF ESSENTIAL OIL AND AROMA CONTENT OF *LIPPIA CITRIODORA* SPECIES

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Medicinal and aromatic plants (MAPs) have gained global importance due to their rich bioactive compounds, particularly essential oils and aromatic substances. These compounds exhibit diverse biological activities and are widely used in the pharmaceutical, food, cosmetics, and perfumery industries. Essential oils serve as natural therapeutic agents, flavorings, preservatives, and fragrance ingredients, meeting the growing demand for natural and health-promoting products [1]. Among them, *Lippia citriodora*, belonging to the Verbenaceae (wild mint/verbena) family, is a perennial shrub native to South America, characterized by its strongly lemon-scented leaves. Extracts and essential oils obtained from its leaves have traditionally been used as herbal teas for digestive relief and relaxation. Modern pharmacological studies have demonstrated that *L. citriodora* possesses significant antioxidant, antimicrobial, anxiolytic (anxiety-reducing), and sleep-improving potential [2-4]. In this study, essential oil and extract of *L. citriodora* species were prepared, essential oil and essential oil content were examined. The essential oil and aroma content of *L. citriodora* were determined by GC-MS/FID device. The major components of the essential oil of the species are trans-citral (24.76%), cis-citral (20.55%) and limonene (10.40%), while the major components of the aroma content are Carvacrol (25.51%), caryophyllene oxide (17.51%) and thymol (7.50%) was found.

Key Words: *Lippia citriodora*, essential oil, essential oil, GC-MS/FID

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POSTER PRESENTATION

USE OF PHYTO-PROTEOLYTIC ENZYMES IN THE CONFIRMATION OF ANTIBODIES TO RED BLOOD CELL ANTIGENS

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Antibodies to RBC antigens is still the problems in transfusion science. Patient with those antibodies will have limited choice of compatible blood for transfusion. Identification and confirmation of antibodies to RBC antigens can be performed based on serological testing. However, different characteristics of RBC antigens required different techniques of inspecting. This study extracted proteolytic enzymes from native fruits including papaya, pineapple, and wild fig. The enzyme papain, bromelain, and ficain was then solubilized in PBS for use in antibody confirmation. Standard antibodies to RBC antigens including IgG, anti-Rh (D, E, c) and IgM, anti- MNS (M, N, Mi^a), were identified and confirmed with phyto-proteolytic enzyme-treated or untreated antibody identification panel cells. Each phyto-proteolytic enzyme digested RBC antigens and surface molecules with time-dependent manner. This reduced the zeta potential and ionic cloud surrounding RBC, making the RBC surface antigens easier to react with antibody molecules. Papain was the universal enzyme for confirmation of both antibodies to enzyme-enhanced Rh antigens (D, E, c) and enzyme-diminished MNS antigens (M, N, Mi^a). It enhanced the hemagglutination score from 8 to 12 and diminished the hemagglutination score from 8 to 5. Bromelain was the most potent enzyme for the eliminating of sialic acid and destroying of protein antigens on RBC surface. The enzyme is suitable for the confirmation of IgM antibody to enzyme-diminished MNS antigens (M, N, Mi^a). Ficain was the lease potent enzyme as it can be used only in the eliminating of RBC sialic acid without any effect on RBC antigens. The use of phyto-proteolytic enzymes in enhancing or diminishing the reactivity of antigen-antibody is one of the confirmation techniques for antibodies to RBC antigens. Combination of other techniques is needed to provide safety selection of blood component for patients.

Key Words: Phytoenzyme, proteolytic enzyme, antibody, RBC Antigen

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POSTER PRESENTATION

CHEMICAL COMPOSITION AND BIOLOGICAL POTENTIAL OF THE COMPONENTS OF VARIOUS POMEGRANATE CULTIVARS FROM UZBEKISTAN

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Objectives: Pomegranate (*Punica granatum* L.) belongs to the Lythraceae family and is native to Central Asia. Besides its nutritional uses, pomegranate has been employed in traditional medicine for treating various diseases. Uzbekistan pomegranate cultivars are known for their unique sweet taste and high productivity. However, there is limited knowledge about their nutritional composition and medicinal value. The current study aimed to quantify the metabolites and evaluate the biological activities of the identified metabolites in five pomegranate varieties (Chust, Dashtobod, Quva, Qizil po'choq, Sherobod).
Methods: Phytochemical analyses were performed using UV-vis spectrophotometry and gas chromatography-mass spectrometry (GC-MS). Additionally, the inhibitory effect of naringenin on xanthine oxidase and its bioactivity in ameliorating uric acid-induced liver injury were investigated. The *in vitro* immunomodulatory activity of punicic acid (a component of pomegranate seed oil) was assessed using RAW264.7 cells. The binding mode of naringenin (which is widely found in pomegranate) to xanthine oxidase was predicted by molecular docking.
Results: The total phenolic, flavonoid, and tannin contents were highest in the Dashtobod cultivar. Among the polyphenols, ellagic and chlorogenic acids were found in substantial concentrations in all extracts. GC-MS analysis identified 18 compounds. High doses of punicic acid significantly restored cyclophosphamide-induced immune injury by enhancing innate and adaptive immunity and stimulating the secretion of immune-related factors. *In vivo* activity studies showed that naringenin improved liver function while inhibiting xanthine oxidase activity. Naringenin alleviated oxidative stress in the liver caused by excess reactive oxygen species through its antioxidant activity. Molecular docking results suggested that naringenin was able to interact with Keap1 and AMPK to exert antioxidant and anti-inflammatory effects.
Conclusion: The findings of this study support the traditional use of pomegranate peels and highlight its potential for further exploration as a source of therapeutic agents.

Key Words: *Punica granatum*, pomegranate, phytochemical, GC-MS, biological activity

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POSTER PRESENTATION

EVALUATION OF ENZYME INHIBITORY ACTIVITIES OF ETHANOLIC EXTRACTS OF *LIPPIA CITRIODORA* KUNTH

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Medicinal plants have been used as natural remedies for centuries due to the biologically active compounds they contain. Among these, the Verbenaceae family includes many species known for their aromatic and pharmacological properties. *Lippia citriodora* Kunth, commonly known as lemon verbena, is a perennial plant native to South America and widely cultivated in the Mediterranean climate. The plant's essential oil contains compounds such as citral, limonene, and verbascoside, which give the plant its characteristic lemon scent and biological activities. In folk medicine, it is used as a digestive aid, mild sedative, and anti-inflammatory. Today, it is valued in the pharmaceutical, cosmetic, and food industries for its antioxidant and antimicrobial properties. In this study, the acetylcholinesterase (AChE), butyrylcholinesterase (BChE), urease, and tyrosinase enzyme inhibition activities of the ethanol extract of *L. citriodora* were determined using an ELISA device. According to the results, the extract exhibited a high level of BChE enzyme inhibitory activity (% inhibition: 87.67±2.21) and a moderate level of urease inhibitory activity (% inhibition: 42.80 ± 1.80), while showing low or no inhibitory activity against AChE and tyrosinase enzymes.

Key Words: *Lippia citriodora,* acetylcholinesterase, butyrylcholinesterase, urease, tyrosinase

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POSTER PRESENTATION

SELECTIVE ANTIPROLIFERATIVE EFFECTS AND BROAD BIOACTIVITY OF *CURCUMA LONGA* L. EXTRACT: ANTIOXIDANT AND ANTIMICROBIAL INSIGHTS

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Scientific research has increasingly contributed to validating the traditional uses of medicinal plants, thereby strengthening the credibility of phytotherapy and supporting its integration into modern medical practice. In this context, the present study investigated the efficacy of the hydroalcoholic extract of *Curcuma longa* L., a species recognized for its high bioactive potential. The antioxidant activity of the extract was first assessed using the DPPH assay, which revealed a significant radical scavenging capacity, with the EC1 sample showing the highest RSA% value (31.11 ± 0.07). Subsequently, the antiproliferative activity was evaluated through the MTT assay applied to a normal pulmonary fibroblast cell line (HFL1) and a murine melanoma cell line (B16F10). The extract demonstrated dose-dependent cytotoxicity toward the B16F10 melanoma cells, while no cytotoxic effects were observed in the HFL1 fibroblast cell line, indicating selective antiproliferative activity. The antimicrobial activity was then examined using disk diffusion and broth microdilution methods against eight reference bacterial strains, representing both Gram-positive and Gram-negative species. The most promising results were obtained for Gram-positive bacteria, where the extract exhibited comparable or even superior efficacy to reference antibiotics, indicating its potential as an alternative antimicrobial agent. Overall, the findings highlight the significant antioxidant, selective antiproliferative, and antimicrobial properties of the *Curcuma longa* L. hydroalcoholic extract, supporting its potential use in the development of natural therapeutic alternatives. Additional studies involving multiple cell lines, synergy assessments with existing treatments, and in vivo evaluations of bioavailability and metabolism are recommended. Long-term safety assessments remain essential to ensure the absence of adverse effects.

Key Words: *Curcuma longa L.*, bioactive potential, antioxidant, cytotoxicity, antioxidant, antimicrobial potential

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POSTER PRESENTATION

LC-HRMS-BASED PHENOLIC PROFILE AND THE ANTIOXIDANT AND ANTI-AGING POTENTIAL OF *Daucus carota* L. FLOWER EXTRACT

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Daucus carota L., known as wild carrot, is a species belonging to the Apiaceae (Umbelliferae) family. It is widely distributed throughout Asia and Europe. Previous studies have demonstrated various biological activities of the plant, including anticancer, antibacterial, antioxidant, and anti-inflammatory effects, attributed to its terpenoids and phenolic compounds [1]. Herein, the plant material was collected from the Çanakkale–Yenice region in July 2024, taxonomically confirmed, and identified as *Daucus carota L. subsp. carota*. A hydroalcoholic extract (EtOH:H_2O, 1:1) was prepared from dried flowers using freeze-drying. Phenolic profiling of the extract was performed via LC–HRMS analysis. The hydroalcoholic extract of *Daucus carota* L. was obtained with a yield of 18.25%. LC–HRMS analysis revealed the presence of 15 phenolic compounds, with fumaric acid (79.65 mg/L), orientin (75.98 mg/L), 6-OH-luteolin-7-*O*-glucoside (65.88 mg/L), caffeic acid (40.28 mg/L), and cynarin (32.98 mg/L) identified as major constituents. Based on this phytochemical profile, *Caenorhabditis elegans* thermotolerance (heat-stress lifespan) assays and antioxidant tests were performed. Heat-stress lifespan assays were conducted at extract concentrations ranging from 50 to 1000 µg/mL to investigate its anti-aging potential [2]. The antioxidant potential of the extract was comprehensively evaluated using multiple *in vitro* assays. The antiradical capacity was determined through DPPH free radical scavenging and ABTS cation radical scavenging methods, while the overall antioxidant capacity was assessed using the CUPRAC reducing antioxidant capacity, and metal chelation assays. Collectively, the findings indicate that the hydroalcoholic extract of *Daucus carota* L. exhibited significant antioxidant activity but did not improve stress resistance in the *Caenorhabditis elegans model.* Nevertheless, the extract represents a rich source of phenolic antioxidants, and further *in vivo* model studies and clinical investigations are required to better understand its potential therapeutic effects.

Key Words: *Daucus carota,* phenolic compound, antioxidant activity, anti-aging, *Caenorhabditis elegans*

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POSTER PRESENTATION

HEAVY METAL ACCUMULATION AND ESSENTIAL OIL COMPOSITION OF LAVENDER (*LAVANDULA VERA* L.) CULTIVATED ON METAL-CONTAMINATED SOILS

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This study aimed to evaluate the accumulation of heavy metals and the chemical composition of essential oils in lavender (Lavandula vera L.) cultivated on soils contaminated with heavy metals near the Non-Ferrous-Metal Works (NFMW) in Plovdiv, Bulgaria. Experimental plots were established at three distances from the pollution source: 0.3 km, 1 km, and 3.5 km. Lavender plants were harvested at full flowering, and heavy metal concentrations in the inflorescences were determined using inductively coupled plasma (ICP) analysis. Essential oils were extracted by steam distillation under laboratory conditions and analyzed for both heavy metal residues and chemical constituents via chromatographic methods. Results indicated that lavender shows considerable tolerance to heavy metal contamination, with no significant negative impact on plant development or essential oil yield and quality. Heavy metal levels in the plant tissues and oils remained within safe limits, suggesting minimal risk of metal transfer into the final product. Chemical analysis of the essential oils revealed a composition consistent with ISO 3515:2002 standards for Bulgarian and French lavender oils. Key aroma compounds such as linalool (22.95–27.18%) and linalyl acetate (23.60–25.54%) were present in high concentrations, while camphor content remained low (0.56–0.65%), enhancing the oil's suitability for perfumery and therapeutic uses. The study demonstrates that lavender can be successfully cultivated on heavy metal–polluted soils, producing high-quality essential oils that meet international standards. Furthermore, the results highlight lavender's potential as a phytoremediation crop, capable of improving soil health while generating economically valuable products. This dual benefit supports the integration of lavender cultivation into remediation strategies for metal-contaminated sites, offering an eco-friendly and sustainable approach to soil restoration and resource utilization.

Key Words: heavy metal accumulation, essential oils, phytoremediation, chemical composition, soil contamination, lavender oil quality

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POSTER PRESENTATION

DESI-MS AS A HYPHENATED TECHNIQUE FOR IDENTIFICATION OF *PELARGONIUM SIDOIDES* AND *PELARGONIUM RENIFORME*

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Objective *Pelargonium sidoides* and *Pelargonium reniforme*, two taxonomically allied South African medicinal plants, are widely used in traditional medicine for treating respiratory and gastrointestinal ailments [1]. Despite their therapeutic significance, differentiation of these species remains challenging due to overlapping morphological traits. While prior phytochemical analyses have identified distinct metabolite profiles between the species, these methods lack the spatial resolution to localize key markers in situ [2].
Method To address this gap, we applied desorption electrospray ionization mass spectrometry imaging (DESI-MSI) coupled with high-performance thin-layer chromatography (HPTLC) for compound detection, identification, and spatial mapping in root extracts of both species.
Results Root methanol extracts (10 mg/mL) were analyzed via HPTLC-DESI-time-of-flight (ToF)-MS, enabling targeted characterization of four marker compounds: umckalin/isofraxidin, isofraxidin sulphite, 5,6,7,8-tetramethoxycoumarin, and 3′,4′-dimethylluteolin. DESI-MSI was further employed to investigate the spatial distribution of these metabolites in fresh root cross-sections (0.08 mm thickness). DESI-MSI revealed species-specific localization patterns: scopoletin and isofraxidin sulphate were concentrated in the cortical regions of *P. sidoides*, while umckalin dominated the vascular bundles of *P. reniforme*.
Conclusions This study demonstrates, for the first time, the utility of HPTLC-DESI-ToF-MS as a rapid, dual-platform approach for the simultaneous separation, identification and spatial resolution of compounds in Pelargonium plants. HPTLC-DESI-MS analysis is cost-effectiveness, adaptability to complex matrices, reducing ion suppression and allowing targeted interrogation of specific bands through visual localization. This technique maintains analytical rigor while addressing several limitations inherent to conventional UPLC-MS methodologies. The distinct spatial distribution of biomarkers provides a novel criterion for species authentication, overcoming limitations of traditional morphological and chromatographic methods. Our findings affirm DESI-MSI as a transformative tool for in situ phytochemical profiling, offering actionable insights for quality control of botanicals and mitigating risks of misidentification in commercial herbal products.

Key words: Desorption electrospray ionization mass spectrometry imaging (DESI-MSI), *Pelargonium sidoides*, *Pelargonium reniforme*

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POSTER PRESENTATION

OPTIMIZING TOMATO SEED GERMINATION USING WATER RESIDUES FROM *SALVIA ROSMARINUS* SPENN. HYDRODISTILLATION

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The use of natural biofertilizers derived from agro-industrial by-products represents a promising approach to sustainable agriculture. In this context, water residues obtained from the hydrodistillation of *Salvia rosmarinus* Spenn., rich in bioactive compounds, were evaluated for their effect on tomato (*Solanum lycopersicum* L.) seed germination. The study aimed to determine how different concentrations of rosemary water residue influence germination dynamics and early seedling development. Five concentrations (12.5%, 25%, 50%, 75%, and 100%) were tested under controlled conditions, with distilled water serving as the negative control. The chemical analysis of the residue revealed high levels of sugars (377.90 ± 23.12 µg/mL), proteins (219.33 ± 9.85 µg/mL), and phenolic compounds, including rosmarinic acid (17.90%) and luteolin-3-glucuronide (13.42%). Germination tests demonstrated a concentration-dependent response: low to moderate concentrations (12.5% and 25%) promoted faster and more uniform germination, while higher concentrations (≥ 50%) exhibited inhibitory or phytotoxic effects. The 12.5% treatment yielded the best germination performance, reducing the germination uniformity index (U80–20) to 7.43 days, compared with longer delays observed at higher doses. These results suggest that diluted rosemary water residue can enhance the metabolic activation of seeds and improve germination synchronization, likely due to the synergistic action of sugars, amino acids, and low levels of phenolic compounds. In contrast, concentrated residues may inhibit germination due to excess phenolics. Therefore, *Salvia rosmarinus* Spenn. hydrodistillation residue at 12.5% can be safely used as a natural seed-priming agent for improving tomato germination and early seedling vigor.

Key Words: *Salvia rosmarinus,* water residue, seed germination, phytotoxicity, sustainable agriculture

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POSTER PRESENTATION

CHEMICAL COMPOSITION AND QUALITY ASSESSMENT OF COMMERCIAL IMMORTELLE HYDROSOLS FROM THE CROATIAN MARKET

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Immortelle (*Helichrysum italicum* (Roth) G. Don) is a Mediterranean species traditionally used in folk medicine for respiratory, digestive, and dermatological ailments. Owing to its medicinal and aromatic properties, commercial interest in this plant has increased within the pharmaceutical and cosmetic industries. The aim of this study was to characterize the volatile compounds present in commercial *H. italicum* hydrosols (HiHY) available on the Croatian market and to evaluate their quality and purity. Hydrosols, by-products of essential oil distillation, have recently gained attention due to their bioactive potential. Samples from the Adriatic (n=2) and continental (n=2) regions were analyzed using gas chromatography–mass spectrometry (GC–MS). Oxygenated monoterpenes dominated in all HiHYs (43.54–69.86%), with neryl acetate being the most abundant constituent (17.66–51.88%), while oxygenated sesquiterpenes were the least represented (1.94–2.87%). For quality assessment, 15 hydrosol samples (9 Adriatic, 6 continental) were evaluated using six physicochemical parameters: relative density (0.957–1.049 and 0.982–1.075), refractive index (1.3327–1.3338 and 1.3328–1.3338), acid value (0.0050–0.0071 and 0.0050–0.0068 mg KOH/g), pH (3.50–5.17 and 3.43–5.80), turbidity (1.35–3.77 and 1.18–5.20 NTU), and essential oil content (0.07–0.13% and 0.02–0.12%) for Adriatic and continental samples, respectively. All HiHYs met the available quality criteria, and no significant regional differences were observed among the evaluated physicochemical parameters. These findings confirm the chemical consistency and high quality of Croatian *H. italicum* hydrosols, supporting their suitability for use in pharmaceutical and cosmetic formulations.

Key Words: *Helichrysum italicum*, hydrosol, GC-MS, oxygenated monoterpenes, physicochemical properties

POSTER PRESENTATION

INTRANASAL ADMINISTRATION OF ASHWAGANDHA AND BRAHMI -LOADED CHITOSAN NANOPARTICLES: A NOVEL APPROACH IN ANXIETY MANAGEMENT

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Anxiety disorders are among the most common mental health conditions worldwide, highlighting the need for novel approaches to treatment. This study describes the development of novel chitosan nanoparticles (CSNPs) loaded with *Withania somnifera* (Ashwagandha) and *Bacopa monnieri* (Brahmi) extracts for intranasal administration as an alternative strategy in anxiety disorders. CSNPs were formulated by ionic gelation method and characterized for size, morphology and encapsulation efficiency. The particle size of the optimized formulation was 200–220 nm and had an entrapment efficiency of 73.68 ± 0.23% and 81.32 ± 0.27% for Ashwagandha and Brahmi respectively. The *in vitro* release studies indicated sustained release of both actives, wherein a maximum of 77.29 ± 1.48% Ashwagandha and 98.45 ± 1.73% Brahmi was released from the nanotech-based spray in 24 hours, compared to faster release from the conventional formulation. *Ex vivo* permeability studies exhibited enhanced permeation, from 1,542.86 µg/cm²/h (control, conventional) to 2,404.78 µg/cm²/h (nanotech-based spray) for Ashwagandha and from 1,633.41 µg/cm²/h (control, conventional) to 1,847.03 µg/cm²/h (nanotech-based spray) for Brahmi. The actives loaded CSNP formulations exhibited a dose-dependent anxiolytic effect in preclinical studies conducted in Wistar rats using Elevated Plus Maze and Light-Dark Transition tests. The intranasal N_2 formulation (0.36 mg/day Ashwagandha plus 0.45 mg/day Brahmi) exhibited significant efficacy, almost comparable to standard oral diazepam. This study established the strength of intranasal delivery of combinatorial Ashwagandha and Brahmi, in anxiety management in comparison to diazepam and oral delivery of the same actives. These results provide sufficient evidence and confidence to conduct clinical trials to ascertain the safety and superior effectiveness of this intranasal combination of Ashwagandha and Brahmi further corroborating their use in clinical AYUSH based medicines.

Keywords: Anxiety, Ashwagandha, Brahmi, nasal delivery, chitosan nanoparticles

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POSTER PRESENTATION

CHARACTERIZATION AND QUALITY EVALUATION OF *HELICHRYSUM ITALICUM* ESSENTIAL OILS AVAILABLE ON THE CROATIAN MARKET

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Helichrysum italicum (Roth) G. Don (immortelle) is an aromatic and medicinal plant widely used in the pharmaceutical, nutraceutical, cosmetic, and fragrance industries due to its diverse biological properties. Extracts and essential oil of *H. italicum* exhibit anti-inflammatory, antioxidant, antidiabetic, insecticidal and repellent, as well as antineoplastic activities. The aim of this study was to characterize the volatile compounds in commercial immortelle essential oils (HiEOs) available on the Croatian market and to evaluate their quality. Essential oil samples originating from Adriatic and continental regions of Croatia were analyzed. Chemical composition was determined using gas chromatography–mass spectrometry (GC–MS). Sesquiterpene hydrocarbons predominated in all samples (43.91–63.15%), with γ-curcumene (27.28%, 23.71%) being the most abundant in Adriatic HiEOs and β-selinene (16.71%, 23.71%) in continental HiEOs. Among oxygenated monoterpenes, neryl acetate was the major constituent (9.22–17.85%). Quality assessment of nine samples (5 Adriatic, 4 continental) included three physicochemical parameters: relative density (0.875–0.900 and 0.897–0.904), refractive index (1.4649–1.4810 and 1.4771–1.4802), and acid value (0.0120–0.0234 and 0.0141–0.0201) for Adriatic and continental oils, respectively. All measured values were within accepted standards, and no significant regional differences were observed. These findings confirm the chemical consistency and high quality of HiEOs on the Croatian market, supporting their potential application in pharmaceutical and cosmetic products.

Key Words: *Helichrysum italicum*, essential oil, GC-MS, sesquiterpene hydrocarbons, physicochemical parameters

POSTER PRESENTATION

DEVELOPMENT AND EVALUATION OF AN HERBAL NANOEMULGEL FOR ACNE AND POST-INFLAMMATORY HYPERPIGMENTATION

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Acne vulgaris is a common dermatological condition that frequently leads to scarring and post-inflammatory hyperpigmentation, thereby affecting patients' quality of life. Conventional treatments such as antibiotics and retinoids often face limitations, including microbial resistance and adverse effects. To overcome these challenges, the present study aimed to develop and compare two topical formulations: a conventional emulgel and a nanoemulgel, both incorporating Resveratrol, Glabridin, Thyme oil, and Rosemary oil, for the treatment of acne and post-inflammatory hyperpigmentation. The nanoemulsion was prepared using high-energy emulsification and subsequently incorporated into a hydrogel matrix to form a nanoemulgel, while the conventional emulgel was prepared without nanonization. Both formulations were characterized and compared for particle size, zeta potential, viscosity, pH, in vitro drug release, and antimicrobial efficacy. Safety evaluation was performed using the Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) assay, and stability studies were conducted under both long-term and accelerated conditions. The nanoemulgel exhibited a significantly smaller globule size and an acceptable zeta potential of -25.2 mV, ensuring enhanced stability. It also demonstrated sustained drug release over 12 hours and superior antimicrobial activity against *Propionibacterium acnes (P. acnes)* when compared to the conventional emulgel. However, anti-inflammatory effects were not assessed in this study. Overall, the comparative evaluation revealed that the nanoemulgel offered better physicochemical stability, controlled drug release, and antimicrobial efficacy, highlighting its potential as a more effective alternative for managing acne and post-inflammatory hyperpigmentation. Further *in vivo* and anti-inflammatory studies are recommended to fully establish its therapeutic potential.

Keywords: *Acne vulgaris*, nanoemulgel, herbal formulation, resveratrol, glabridin, *P. acnes*, dermatology.

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POSTER PRESENTATION

ACMELLA OLERACIA FLOWERS - TRANSFORMING TRADITIONAL TRIBAL KNOWLEDGE TO CONSUMER-FRIENDLY GEL FOR HEALING ORAL ULCERS

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Acmella oleracea (toothache plant) is traditionally used for oral ailments, yet its flowers remain underexplored, particularly for mouth ulcer management. The present study investigates the phytochemistry, antioxidant, antibacterial, and anti-inflammatory activities of ethanol extract of *A. oleracea* flowers and develops a polyherbal gel incorporating *A. oleracea*, *Ocimum sanctum*, and *Psidium guajava* extracts for therapeutic evaluation against mouth ulcers. The flowers of *A. oleracea* were collected, authenticated, extracted with ethanol, and characterized microscopically and phytochemically. The total phenolic and flavonoid content were quantified using Folin–Ciocalteu and aluminum chloride methods, respectively. The antioxidant activity was assessed by DPPH, H_2O_2, NO, and FRAP assays. The antibacterial activity was evaluated using agar well diffusion against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*. The anti-inflammatory potential was determined via albumin denaturation assay. A polyherbal gel was formulated using Ultrez-10 NF and subjected to physicochemical evaluation, in vitro and ex vivo diffusion studies, stability testing, antimicrobial analysis, and HET-CAM irritation assessment study. In vivo anti-ulcer activity was performed on Sprague-Dawley rats for 14 days, evaluating ulcer morphology, wound size, healing rate, and histopathology. *A. oleracea* ethanol extract showed the presence of phenolics, flavonoids, glycosides, alkaloids, and carbohydrates, with high phenolic and flavonoid content. The extract exhibited notable antioxidant activity (IC_{50}: DPPH 89.58 µg/ml; NO 50.86 µg/ml), significant antibacterial activity, particularly against *P. aeruginosa* and anti-inflammatory activity was evident in the albumin denaturation assay. The optimized polyherbal gel demonstrated acceptable physicochemical properties, sustained in vitro and ex vivo release, high stability, and non-irritancy in the HET-CAM assay. In vivo, the polyherbal gel produced significant reduction in ulcer diameter and superior healing ($p < 0.001$), with complete wound closure by day 14 and supportive histopathological recovery. The formulated polyherbal gel showed robust therapeutic efficacy and safety in both in vitro and in vivo models, supporting the traditional use of *A. oleracea* flowers for oral ulcer management. The gel represents a promising topical delivery system for management of mouth ulcers.

Keywords: *Acmella oleracea* flowers, ethanol extract, mouth ulcer, antioxidant, antibacterial, anti-inflammatory, phenolics, flavonoids

POSTER PRESENTATION

PHYTOCHEMICAL INVESTIGATIONS AND CYTOTOXIC ACTIVITY OF *LITSEA GHATICA*, AN ENDEMIC PLANT FROM WESTERN GHATS OF INDIA

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Litsea ghatica C.J. Saldanha belongs to the evergreen family Lauraceae, endemic to the Western Ghats of India. Our laboratory has studied the phytochemistry and biological activity of solvent extracts of this plant since 2018[1]. The present work reports the potential of an endemic plant *L. ghatica* in therapeutics. The objective of this research was to evaluate the cytotoxic activity of *L. ghatica* bark and identify some secondary metabolites in the same. The total phenolic and flavonoid content of the toluene, dichloromethane, methanol extracts and flavonoid rich fraction of the bark of *L. ghatica* was estimated using Folin-Ciocalteu and aluminum chloride spectrophotometric assays respectively. The antioxidant activity of these extracts and the flavonoid rich fraction was evaluated using DPPH and nitric oxide assays. HPTLC fingerprints were developed as a standardization tool for the same. Bioactivity guided fractionation yielded active fractions that were analyzed by LCMS. *In silico* studies like target prediction, molecular docking and ADME prediction were conducted on the identified constituents to identify potential molecular targets. The cytotoxic activity of the extracts and fractions thereof were evaluated in breast cancer (MCF-7, MDA-MB-231), cervical cancer (HeLa), and lymphoma (U-937) cell lines using the Sulforhodamine assay. The methanol extract exhibited promising antioxidant activity versus the standard quercetin. The aqueous methanol fraction of DCM extract exhibited potent cytotoxicity in HeLa and MCF-7 cell lines. The LCMS studies identified the presence of several secondary metabolites some of which could be responsible for the cytotoxic activity. Of these, Apigenin7-(6"-E-pcoumaroylgalactoside),Epicatechin-(4beta-6)-epicatechin-(2beta-7,4beta-8)-epicatechin, Cynaroside and Castillene A emerged to be top four VEGF inhibitors with good binding scores with this target. Phytochemical analysis of *L. ghatica* provides new evidence of the presence of cytotoxic secondary metabolites having potential antioxidant activity. The *in-silico* studies further confirmed that some of these compounds exhibit strong binding affinity to the VEGF receptor, indicating their potential as hits/leads for development as anticancer agents. The ADME studies of the identified compounds supported their utility as lead like molecules for further developmental studies.

Keywords: *Litsea ghatica,* antioxidant activity, flavonoids, phenolics, cytotoxic activity, LCMS, Cynaroside

POSTER PRESENTATION

DISTINCTION OF CHIA GENOTYPES AND ROSMARINIC ACID PRODUCTION *IN VIVO* AND *IN VITRO* USING FLOW CYTOMETRY

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Salvia hispanica L. (chia) is a valuable medicinal and nutritional plant rich in phenolic compounds, including rosmarinic acid (RA) [1,2]. The present study aimed to distinguish two commercial chia genotypes cultivated *in vivo* and *in vitro* using flow cytometry and to evaluate their biosynthetic potential for RA production under elicitation conditions. Flow cytometric analyses were applied for the first time in chia to determine genome size, ploidy level, and base composition (AT/GC ratio), as well as to assess cytoplasmic acidification associated with secondary metabolite accumulation. The results revealed significant genotype-dependent differences in nuclear DNA content and AT/GC ratios, allowing clear discrimination between the studied lines. Additionally, notable differences were observed between *in vivo* plants and *in vitro* shoot cultures, confirming the impact of culture conditions on cellular and metabolic characteristics. To enhance RA biosynthesis, *in vitro* cultures were treated with yeast extract (YeE) and cadmium chloride ($CdCl_2$) as elicitors. YeE significantly increased RA accumulation in a concentration-dependent manner, whereas $CdCl_2$ showed limited effectiveness and negatively affected biomass growth at higher concentrations [3]. Flow cytometric staining with LysoTracker Deep Red demonstrated increased acidic cytoplasmic compartments in elicited cultures, particularly following YeE treatment, which correlated with enhanced secondary metabolite production. These findings indicate that flow cytometry is a powerful and rapid tool not only for genotypic differentiation of closely related *Salvia* varieties but also for monitoring their metabolic activity and response to elicitation strategies. In conclusion, the integration of flow cytometry with *in vitro* culture techniques provides a promising approach for selecting high-performing chia genotypes and optimizing the production of bioactive compounds such as rosmarinic acid for pharmaceutical and biotechnological applications.

Key Words: *Salvia hispanica*, flow cytometry, plant *in vitro* cultures, rosmarinic acid, secondary metabolites, elicitation

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POSTER PRESENTATION

EVALUATION OF THE ADJUVANT QUALITY FOR VACCINES OF WHOLE NETTLE EXTRACT IN IMMUNOLOGICALLY MATURE CHICKENS

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Objective/Purpose: *Urtica dioica* is a natural antioxidant, antimicrobial, and immune-regulating plant, with beneficial effects in human and veterinary ethnomedicine. This study aimed to investigate the vaccine adjuvant potential of the alcoholic nettle plant *Urtica dioica* in antigen stimulated chickens.

Materials and Methods: The 70°ethanolic nettle plant extract was prepared in the laboratory of the University of Medicine, Cluj, by percolation. The investigations were carried out in twenty-one, 47 days old immunologically mature broiler chickens from a commercial farm. On the farm, the chickens were divided into three groups (n=7) and subjected once daily to a seven-day oral administration of 0.5 ml/bird of: ethanolic nettle extract (Group I), 70° ethanol (Group 2) and saline (Group 3). On days 0 and 7, all the birds were antigenically primed with a 5% sheep red blood cell suspension (0.5 ml/bird). The blood was collected on days 0, 7 and 14 (heparine 50IU/ml) and subjected to the carbon particle inclusion test. The dynamics of phagocytosis over time were monitored within the test (0 to 30 min, 30 to 60 min) and during the experimental period (days 0-7 and 7-14). The statistical significance of the results was calculated by using the Excel program.

Results: In the nettle plant extract treated group, on day 0, the in test phagocytosis was increased after 30 min with 30.37% and 41.88% after 60 min of incubation. On day 7 these values were of +58.26% and +50.98%, while after 14 days they reached +76.86% and +81.5%, respectively. Over time, from day 0 to day 7, the increase in phagocytosis was of 27.29% while further there was an increase of 18.6%.

Conclusion: The nettle plant alcoholic extract enhanced phagocytosis dynamics both on short term (in test) and over the experiment, underlining its potential as a vaccine adjuvant, supported by further studies.

Key Words: *Urtica dioica*, chickens, immunologically mature, phagocytosis dynamics, antigenic stimulation

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POSTER PRESENTATION

UHPLC–MS/MS ANALYSIS AND ANTIMICROBIAL PROPERTIES OF STOLONS METHANOLIC EXTRACT AND FRACTIONS FROM THE MOROCCAN ENDEMIC SPECIES *Searsia albida* (SCHOUSB.) MOFFETT

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The growing resistance of microorganisms to conventional antibiotics has driven the search for natural alternatives. This study investigates, for the first time, the chemical composition and antimicrobial potential of *Searsia albida* (Schousb.) Moffett, a Moroccan endemic plant from Essaouira. UHPLC–MS/MS profiling of the methanolic extract and its dichloromethane and ethyl acetate fractions identified 20 phenolic compounds. The dichloromethane fraction, rich in galloylhexose derivatives (30.71%), displayed strong inhibitory effects against Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and *Candida albicans*. In contrast the ethyl acetate fraction, dominated by chlorogenic acid (45.36%), was more effective against Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus hirae*). These results underscore the antimicrobial potential of *S. albida* phenolic fractions as promising candidates for developing natural antimicrobial agents.

Key Words: *Searsia albida*; UHPLC–MS/MS analysis; Antimicrobial activity

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POSTER PRESENTATION

ANTI-AGING PROPERTIES OF *MACLURA POMIFERA* (RAF.) C.K.SCHNEID. GREEN HOT EXTRACT VIA COLLAGENASE INHIBITION

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The genus *Maclura* (Moraceae) has been traditionally used in Asia for diabetes and inflammatory diseases [1, 2]. Human skin consists of epidermis and dermis, the latter being formed by a highly organized extracellular matrix (ECM) composed of fibrous proteins, glycoproteins, and proteoglycans. Chronic exposure to extrinsic stressors, such as UV light, pollution, and chemicals, together with intrinsic determinants, cumulatively initiates biological processes defined as skin aging, a condition in which ECM organization deteriorates, causing wrinkles, dryness, loss of elasticity, and age-related hyperpigmented spots. Both collagen and elastin are core ECM proteins that preserve structural integrity, that is progressively compromised by enzymatic degradation, especially by collagenase, a key wrinkle-accelerating enzyme [3]. In this study, *M. pomifera* fruit peels were processed using a green hot-extraction approach optimized by Box-Behnken design (BBD). Extraction time, heating power, and raw material/solvent ratio were selected as independent variables to maximize collagenase inhibition, quantified spectrophotometrically. The optimal conditions were determined as 75.25°C, 43.85 min, and 2.91 solvent ratio, yielding potent inhibition (IC_{50}=24.18 µg/mL). The findings indicate that BBD-optimized green peel extracts may help preserve ECM integrity by reducing collagenase-mediated collagen degradation, positioning them as promising natural, enzyme-targeted anti-aging actives. Sustainable and cost-efficient processing strengthens their potential for cosmeceutical and pharmaceutical anti-wrinkle applications. Future studies employing aging-relevant mechanistic models, including fibroblast assays and *in vivo* anti-aging assessments, are necessary to validate ECM-protective efficacy and define the underlying biological pathways.

Key Words: Anti-aging, Box-Behnken Design, collagenase enzyme inhibition activity, green extraction, hot extraction, *Maclura pomifera*

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POSTER PRESENTATION

MODULATION OF OXIDATIVE STRESS AND OOCYTE DEVELOPMENTAL COMPETENCE BY *CURCUMA LONGA* L. EXTRACT DURING BOVINE *IN VITRO* MATURATION

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Oxidative stress is a major factor limiting oocyte quality and developmental competence during bovine *in vitro* maturation (IVM). This study evaluated the antioxidant potential of *Curcuma longa* L. extract and its effects on oocyte maturation and viability. Bovine oocytes were cultured in IVM medium supplemented with *Curcuma longa* L. extract, and maturation rate, morphological integrity, viability, and oxidative status were assessed. Supplementation significantly increased the proportion of oocytes reaching the metaphase II stage compared with the control group (78.4 ± 3.1% vs. 64.7 ± 2.8%, $p < 0.05$). Treated oocytes exhibited a marked reduction in intracellular reactive oxygen species levels (−32%, $p < 0.01$) and a significant increase in total antioxidant capacity (+27%, $p < 0.05$). In addition, oocyte viability and cytoplasmic homogeneity were significantly improved, with a higher percentage of morphologically normal oocytes observed after maturation (81.6 ± 2.5% vs. 69.2 ± 3.0%, $p < 0.05$). These findings demonstrate that *Curcuma longa* L. extract effectively modulates oxidative stress during bovine IVM and enhances oocyte developmental competence, supporting its potential application as a natural antioxidant supplement in assisted reproductive technologies.

Key Words: *Curcuma longa* L. extract, oxidative stress, antioxidant activity, bovine oocytes, *in vitro* maturation, oocyte viability, reproductive biotechnology

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POSTER PRESENTATION

EXTRACTION OF CLOVE OIL, ISOLATION OF EUGENOL, AND ANTIOXIDANT AND TOXICOLOGICAL EVALUATIONS

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This study aimed to investigate the bioactive constituents of clove (*Syzygium aromaticum*), focusing on the extraction of essential oil, isolation of eugenol, and evaluation of its biological properties. The extraction of the clove essential oil was carried out by hydrodistillation, with the objective of obtaining the volatile compounds present in the plant, mainly eugenol. This method uses heating in the presence of water to release and condense essential oils, being an efficient and widely used technique in laboratories for the extraction of aromatic substances. After extraction, the isolation of eugenol, the main active constituent of the oil, was performed through an acid-base treatment. This procedure allowed the separation of eugenol from the other substances present in the essential oil, enabling its purification for specific analyses, such as antioxidant activity and toxicological evaluation. The antioxidant activity was qualitatively assessed by thin-layer chromatography (TLC), using the DPPH radical as a revealing agent. The presence of yellow spots on a purple background indicated the ability of the analyzed compounds to act as antioxidants, neutralizing free radicals. Both the isolated eugenol and other fractions of the oil showed positive responses in the test, confirming their antioxidant potential. Finally, the toxic potential was investigated using Artemia salina larvae. This bioassay allowed the assessment of the safety of the tested compounds in living organisms, serving as an important step to predict risks in therapeutic or industrial applications. The LC50 value obtained for the extract, compared with the literature, indicates that both the acetyl + caryophyllene fraction and eugenol are considered highly toxic according to the analysis.

Key Words: Clove, essential oil, antioxidant activity, toxicity

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POSTER PRESENTATION

STRUCTURAL INSIGHTS INTO HIV-1 MATRIX PROTEIN INTERACTIONS WITH PLANT- BASED IP6-DERIVED INHIBITORS

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Human immunodeficiency virus type 1 (HIV-1) is responsible for the development of acquired immunodeficiency syndrome (AIDS) and establishes a persistent infection in the host. This infection cycle is driven by coordinated structural processes that govern viral assembly and entry into host cells. Pr55Gag is central to these processes, and its matrix domain (MA) plays a critical role in membrane targeting, viral morphogenesis, and early stages of infection. Due to its functional importance in viral assembly, MA becomes an attractive target for structure-based antiviral research. Previous studies have demonstrated that inositol hexakisphosphate (IP6) interacts with Gag-derived assemblies and contributes to the stabilization of viral particle formation. However, the direct therapeutic usage of IP6 remains challenging. Building on this established role, plant-based IP6 derivative molecules emerge as promising candidates for for antiviral modulation of HIV-1 assembly. In this study, we aim to elucidate the structural features of HIV-1 MA and its interaction with plant-based IP6-derived hit inhibitors using X-ray crystallography. For this purpose, HIV-1 MA was recombinantly expressed in *E. coli* BL21(DE3) and purified by Ni-NTA affinity chromatography. The affinity tag was removed by TEV protease digestion; the untagged MA was subsequently isolated by reverse chromatography and further purified using size-exclusion chromatography. The resulting protein will be then used in co-crystallization studies with plant-derived IP6 derivative inhibitors, which are currently ongoing. By focusing on MA-ligand complexes, this study investigates how novel plant-based IP6 derivatives may modulate MA structure and function. The structural insights obtained from this approach has potential to deepen our understanding of MA-mediated assembly mechanisms and to support the development of optimized MA-targeting inhibitors for early structural steps in the HIV-1 life cycle.

Key Words: HIV-1 matrix protein, X-ray crystallography, IP6 derivatives, protein-ligand complexes

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POSTER PRESENTATION

LC–MS-BASED PHENOLIC PROFILING OF *CENTAUREA SERICEA* WAGENITZ EXTRACTS

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The genus *Centaurea* has a highly significant position due to the medicinal compounds it contains. Possessing a broad biological activity spectrum to its rich phenolic compounds and sesquiterpene lactones, comprehensive studies demonstrate that this genus besides properties such as antinociceptive, antipyretic, potent anti-inflammatory, broad-spectrum antimicrobial activity, and high antioxidant activity exhibits selective cytotoxic and apoptosis-inducing effects on cancer cell lines, proving it to be a natural medicinal resource with high potential value for modern pharmacotherapy. *Centaurea sericea* Wagenitz. (Saklısarıbaş), a species belonging to this genus, is endemic to the flora of Türkiye and is range across the South Marmara and Upper Sakarya sub-regions. In this study, *C. sericea* samples collected from field studies were shade-dried and powdered, and extracts were obtained using methanol with two different extraction methods (Soxhlet and maceration methods). After complete removal of solvents from the extracts, phytochemical profiling was performed using LC-MS (Liquid Chromatography-Mass Spectrometry) with 35 different standard compounds. According to the analysis results, varying amounts of common compounds were detected in both methods. The dominant compounds in the extracts were identified as quinic acid, chlorogenic acid, and fumaric acid. Trace amounts of ferulic acid, vanillic acid, and gallic acid were detected in both methods. Syringic acid was detected only in the maceration method and not in the Soxhlet extraction. The results show that *C. sericea* may contain important secondary metabolites with biological activity and potential for drug discovery research. This study serves as a precursor for future studies and forms the basis for the pharmaceutical evaluation of *C. sericea* extracts.

Key Words: Bioactivity studies, drug discovery, LC/MS, secondary metabolites

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POSTER PRESENTATION

PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF THE BIOACTIVE POTENTIAL OF EXTRACTABLE FRACTIONS FROM *INONOTUS OBLIQUUS* AND *PHALLUS IMPUDICUS*

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This research investigates the chemical composition, extraction efficiency, and biological properties of two historically valued macrofungi: Chaga mushroom (*Inonotus obliquus*) and the Common Stinkhorn (*Phallus impudicus*), aiming to unlock their potential for value-added industrial applications. The objectives were to determine the proximate chemical composition, evaluate the efficiency of two advanced extraction methods, and assess the antioxidant and antibacterial activities of the resulting extracts derived exclusively from *I. obliquus* and *P. impudicus*. Methods involved proximate analysis, which found that carbohydrates constituted the largest share in both fungi, while *P. impudicus* had the highest protein (20.44%) and fat (1.57%) content. Extracts were obtained using Supercritical Carbon Dioxide Extraction (SCE-CO_2) and Pressurized Liquid Extraction (PLE) with solvents of increasing polarity (hexane, acetone, ethanol). The biological activities were determined using DPPH, FRAP, and Folin-Ciocalteu assays for antioxidant properties, and the agar diffusion method against *Escherichia coli* and *Bacillus subtilis* for antibacterial activity. Efficient Liquid Chromatography (ELC) was used to quantify ergosterol. Results showed that *P. impudicus* yielded the highest amount of non-polar extract in both SCE-CO_2 (0.80%) and PLE with hexane (0.93%). The highest yield of polar extract was achieved using PLE with ethanol. All extracts exhibited antioxidant properties, with the strongest activity observed in the acetone and ethanol extracts of Chaga mushroom (*I. obliquus*). Antibacterial assessment indicated strong selective activity; the SCE-CO_2 extract of *P. impudicus* showed the highest inhibition (14.0 mm) against *E. coli*, while the hexane extract of *P. impudicus* was most effective against *B. subtilis* (14.7 mm). Ergosterol was detected at the highest concentration in the acetone extract of *P. impudicus*, but was absent in the hexane extract of *I. obliquus*. In conclusion, *Inonotus obliquus* and *Phallus impudicus* are rich sources of bioactive compounds. The strong antioxidant capabilities of the polar extracts from Chaga and the significant antibacterial potential of the non-polar extracts from *P. impudicus* underscore their potential as valuable ingredients for the food, cosmetic, and pharmaceutical industries through targeted biorefining.

Key Words: Chaga mushroom, *Inonotus obliquus*, biorefining, antioxidant activity, pressurized liquid extraction, ergosterol

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PHYTOCHEMICAL, ANTIOXIDANT, AND CYTOTOXIC ACTIVITY PROFILE OF PARIJOTO ETHANOL EXTRACT (*Medinilla speciosa*) AGAINST TRIPLE-NEGATIVE BREAST CANCER CELLS MDA-MB-231

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Abstract: Triple-negative breast cancer (TNBC) is the most aggressive subtype of cancer. Based on Globocan 2022, breast cancer occupies the highest position in cancer incidence and mortality in women in Indonesia. Parijoto (*Medinilla speciosa*), a traditional Indonesian plant, is reported to contain bioactive compounds that have the potential to be powerful anticancer and antioxidant agents, the potential cytotoxicity to TNBC cells has not been widely researched. This study aims to qualitatively and quantitatively analyze phytochemical profiles, antioxidant capacity, and evaluate the cytotoxic activity of Parijoto ethanol extract against TNBC MDA-MB-231 cells and determine their IC_{50} value. Extraction is carried out by maceration using 70% ethanol. The cytotoxicity test was carried out by the MTT assay method in the concentration range of 10-500 ppm for 24 hours. Cell viability was calculated based on formazan absorbance at λ 595 nm. Dose-response curve analysis was performed using linear regression to determine the value of IC_{50}. Phytochemical screening indicated the presence of phenolic compounds, flavonoids, saponins, tannins, and terpenoids. Quantification gave a total phenolic of 8.43 ± 0.70 mg GAE/g, flavonoids 132.63 ± 4.11 mg/kg, and total anthocyanins 109.77 ± 8.58 ppm; antioxidant activity was recorded at 58.94 ± 0.42% inhibition. MTT data showed a decrease in concentration-dependent viability: viability of 100% (0 ppm), 80.9% (10 ppm), 45.0% (50 ppm), 22.6% (100 ppm), 10.0% (200 ppm); anomalies (negative viability values/ >100% toxicity) appeared at ≥300 ppm. Linear regression yields an equation f(x) = 0.2338x + 71.679 (R^2 = 0.774) with an IC_{50} ≈ 47.18 ppm. Parijoto extract contains significant phenolics and flavonoids, has moderate antioxidant capacity, and exhibits strong *in vitro* cytotoxic effects on TNBC MDA-MB-231 cells with an IC_{50} value ≈ of 47.18 ppm**,** indicating its potential as a source of anticancer compounds for the development of TNBC therapy.

Key Words: Parijoto, *Medinilla speciosa*, cytotoxic activity, MDA-MB-231, triple-negative breast cancer, IC_{50}

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1. Introduction

Triple Negative Breast Cancer (TNBC), also known as basal-like breast cancer, is an aggressive type of breast cancer (Ghasemi et al., 2021). TNBC is characterized by the absence of expression of the Progesterone Receptor (PR), Estrogen Receptor (ER), and Human Epidermal Growth Receptor 2 (HER2)(Dass et al., 2021). This type of cancer accounts for over 12% to 17% of all breast cancer cases (Singh et al., 2021). According to GLOBOCAN 2022, breast cancer ranks as the number one cancer among females worldwide, with an estimated 2,296,840 new cases(Ferlay et al., 2021). Breast cancer also occupies the highest position in terms of both cancer incidence and mortality among women in Indonesia (Sung et al., 2021). This subtype of breast cancer is characterized by its aggressive nature, distinctive ways it spreads (metastasis), and a poor prognosis when compared to other types (Singh et al., 2021). Triple-negative breast cancer (TNBC) is a heterogeneous malignancy divided into six distinct subtypes: immunomodulatory (IM), luminal androgen receptor (LAR), basal-like 1 (BL-1), basal-like 2 (BL-2), mesenchymal (M), and mesenchymal stem-like (MSL) (Dass et al., 2021). Because the common breast cancer tumor biomarkers are absent, effective molecularly targeted treatment options are currently unavailable (Bao & Prasad, 2019). Representing triple-negative breast cancer (TNBC), the MDA-MB-231 cell line is frequently employed to study the late stages of the disease. These cells are defined by their negative status for key markers: estrogen receptor (ER), progesterone receptor (PR), and HER2. Additionally, they are E-cadherin negative and exhibit an invasive phenotype *in vitro* (Kamra et al., 2025).

Parijoto fruits (*Medinilla speciosa*) are an indigenous plant species of Indonesia extensively employed in traditional medicine. The phytochemical profile of parijoto fruits includes phenolics, flavonoids, saponins, tannins, terpenoids, and anthocyanins (Winanta et al., 2024). The compounds present in the fruit can potentially restrict cancer growth. Specifically, flavonoids exhibit anticancer activity by blocking the multiplication of cancer cells and the enlargement of tumors. Furthermore, saponins have been documented to exert anti-tumor effects against several types of malignancies (Winanta et al., 2024). Severe cellular damage and carcinogenesis result from chronically elevated Reactive Oxygen Species (ROS), which initiate malignancy through the modulation of cell signaling and oncogene activation. Recognizing this link, research has identified many plants with potent ROS scavenger (antioxidant) activity. This activity is often correlated with the ability to inhibit cancer cell growth (cytotoxic activity), positioning these plants as valuable therapeutic and preventive options(Annisaa' et al., 2021). This study first confirmed the presence of key phytochemicals (phenolics, flavonoids, saponins, tannins, terpenoids, and anthocyanins) in parijoto fruits. Secondly, it assessed the fruit's potential for antioxidant activity and its toxicity (cytotoxic activity) against MDA-MB-231 triple-negative breast cancer cells. To the best of our knowledge, this study represents the first integrated investigation combining phytochemical profiling, antioxidant activity, and cytotoxic evaluation of *M. speciosa* fruit ethanol extract against triple-negative breast cancer (TNBC) MDA-MB-231 cells. Unlike previous studies that primarily focused on individual biological activities, different extraction solvents, or alternative cancer cell models, this work provides a comprehensive assessment linking phytochemical composition with functional anticancer effects in a TNBC *in vitro* model.

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Furthermore, this study demonstrates a strong cytotoxic potency of Parijoto ethanol extract, with an IC_{50} value below 50 ppm against MDA-MB-231 cells, highlighting its potential as a promising natural source of anticancer agents for triple-negative breast cancer.

2. Material and Methods

2.1. Materials and Instrumentation

The instrumentation utilized in this study included a modified Ultrasound Bath (model UC-10 SD, 2019) equipped with a fan and pump system for temperature stabilization. Additional equipment included a cabinet dryer (Binder, Germany), grinder, sieve shaker, pH meter (Shimadzu, Japan), UV-Vis spectrophotometer (Shimadzu, Japan), analytical balance (Thermo scientific, USA), vortex mixer (Shimadzu, Japan), micropipette, and standard glassware (Erlenmeyer flasks, test tubes, beakers, volumetric flasks, cuvettes, funnels) (Lemmadi et al., 2025a; Sharma & Hamid, 2025).
The primary material used was Parijoto (*M. speciosa*) fruit. Chemicals and reagents included citric acid (1%), plastic wrap, distilled water, pH 4 and pH 7 buffer solutions, food-grade ethanol (96%), potassium chloride, sodium acetate, gallic acid standard (Sigma), 10% Folin-Ciocalteu reagent (Sigma), and sodium carbonate (Che et al., 2024; Lemmadi et al., 2025a).

2.2. Sample Preparation

Parijoto fruits were separated from stalks and impurities, then thoroughly washed. The fruits were cut into small pieces and sorted. A pre-treatment process was applied involving steam blanching at 100°C for 3 minutes, followed by soaking in 1% citric acid solution for 5 minutes. This pre-treatment approach has been reported to reduce enzymatic degradation while preserving phenolic compounds and anthocyanin stability in plant materials (Patras et al., 2010). The samples were subsequently dried using a cabinet dryer at 60°C for 7 hours. The dried Parijoto was ground into a powder and sieved using 60, 70, and 80 mesh screens. The resulting powder was stored in a sealed container at a room temperature of 4°C prior to extraction (Mrázková et al., 2023).

2.3. Extraction of Parijoto Fruit

Extraction was performed using the Ultrasound-Assisted Extraction method, which has been widely reported as an efficient technique for enhancing the recovery of phenolic, flavonoid, and anthocyanin compounds due to cavitation-induced cell wall disruption and improved mass transfer (Chemat et al., 2017; Vinatoru et al., 2017). Ten grams (10 g) of Parijoto powder was placed in a beaker and mixed with ethanol as the solvent at a ratio of 1:10 (w/v). The extraction was carried out using the modified UAE system, equipped with a fan and pump to maintain temperature stability, at a frequency of 45 kHz for 30 minutes. The extraction was conducted at controlled temperatures of 40°C. The resulting extract was filtered using filter paper for subsequent bioactive component analysis (Lemmadi et al., 2025b).

2.4. Qualitative Phytochemical Screening

The Tanin test was conducted by adding 10 mL of the extract solution to a test tube, followed by the addition of 1% lead (II) acetate solution ($(CH_3\text{-}COO)_2$ Pb). A positive result for tannins was indicated

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by the formation of a yellow precipitate. The determination of terpenoids and steroids was performed using the Liebermann–Burchard reagent (a mixture of acetic anhydride and concentrated sulfuric acid). In this test, 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid (H_2SO_4) were added sequentially to 1 mL of the sample dissolved in acetone. The mixture was shaken and allowed to stand for several minutes. A color change from red to purple indicated a positive result for triterpenoids, while a color change from green to blue indicated a positive result for steroids (Sahira Banu; Cathrine, 2025; Rao et al., 2023).

2.5. Determination of Total Anthocyanin Content

The Parijoto extract was diluted 5 times. The total anthocyanin content was determined using the pH differential method with two buffer systems: potassium chloride buffer (pH 1.0) and sodium acetate buffer (pH 4.5). One milliliter (1 mL) of the diluted extract was mixed with each buffer separately. The samples were incubated in the dark for 15 minutes. The absorbance was measured using a UV-Vis spectrophotometer at wavelengths of 520 nm and 700 nm (Chen et al., 2024; Khoo et al., 2017).

2.6. Analysis of Total Phenolic Content (TPC)

A gallic acid standard solution was prepared at concentrations of 0, 6.25, 12.5, 25, 50, 75, and 100 ppm to construct a calibration curve. The Parijoto extract was diluted 25 times. One milliliter (1 mL) of the diluted extract was mixed with 10 mL of distilled water and 1 mL of 10% Folin-Ciocalteu reagent. The mixture was incubated in the dark for 5 minutes. Subsequently, 2 mL of $Na_2CO_3$was added, and the sample was incubated in the dark for 60 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 750 nm. The total phenolic content was expressed as milligrams of Gallic Acid Equivalents (GAE) per gram (Johnson et al., 2022).

2.7. Antioxidant Activity Assay

One milliliter (1 mL) of the extracted sample was diluted 100 times with methanol in a volumetric flask. A 0.3 mL aliquot of the sample was reacted with 9 mL of DPPH solution and incubated in a dark room for 30 minutes. A blank solution was prepared by mixing 0.3 mL of ethanol with 9 mL of DPPH. The absorbance of the sample and the blank was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm (Alam et al., 2013; Rumpf et al., 2023).

2.8. Determination of Total Flavonoid Content

A quercetin standard solution was prepared at six concentration levels: 0, 5, 10, 20, 30, and 40 ppm to generate a standard curve. The Parijoto extract samples were diluted 5 times. One milliliter (1 mL) of the sample was reacted with 1 mL of $AlCl_3$ and 1 mL of sodium acetate (CH_3COONa). The quercetin standards and samples were incubated in a dark room for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 429 nm. The results were expressed as Quercetin Equivalents (ppm QE) (Rajasekar et al., 2023; Sasadara & Wirawan, 2021).

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2.9. Cytotoxicity Assay against MDA-MB-231 Cancer Cells

The cell line used was the human breast cancer cell line MDA-MB-231. The MTT assay is a colorimetric method for assessing cell viability. MDA-MB-231 cells were cultured in 96-well microplates at a density of 10 × 10^3 cells/well. The cells were incubated for 24 hours in a CO_2 incubator to allow for attachment and proliferation. Following the initial incubation, the medium was replaced with various predetermined concentrations of the extract, and the cells were incubated for another 24 hours. The extract solution was then removed, and the cells were treated with MTT reagent, which is metabolized by viable cells into purple formazan products. The MTT solution was discarded, and the formazan crystals were dissolved using a stopper reagent. Absorbance was measured at 595 nm using an ELISA reader. The intensity of the purple color is directly proportional to the number of viable cells. The absorbance values of the treated groups were compared to the control group to calculate the percentage of cell growth inhibition, allowing for the evaluation of the potential anti-cancer activity of the extract (Kamiloglu et al., 2020; Van Meerloo et al., 2011).

3. Results and Discussion

3.1. Results

Phytochemical Analysis

The phytochemical content of *M. speciosa* that is essential in cytotoxicity is presented in Table 1. Our qualitative analysis shows that *M. speciosa* contains various phytochemicals, including phenolics, flavonoids, saponins, tannins, and terpenoids.

Table 1. Phytochemical Analysis

Phytochemical	Qualitative Screening
Phenolic	+
Flavonoids	+
Saponins	+
Tannins	+
Terpenoids	+

The table 2, illustrates the results of our research, which are the quantitative phytochemical content and antioxidant activity. Our research shows phenolic levels of 8.43 ± 0.70 mg GAE/g, flavonoids 132.63 ± 4.11 mg/kg, and total anthocyanins 109.77 ± 8.58 ppm. Various phytochemical compounds from *M. speciosa* show antioxidant activity of 58.94 ± 0.42 % inhibition.

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Table 2. Quantification Results of Phytochemical Compounds and Antioxidant Activity

Phytochemical	Quantitative	Antioxidant Activity (% inhibition)
Phenolics (mg GAE/g)	8.43 ± 0.70	58.94 ± 0.42
Flavonoids (mg/kg)	132.63 ± 4.11	
Total Anthocyanin (ppm)	109.77 ± 8.58	

Table 3. Cytotoxic Activity of Parijoto Extract on MDA-MB-231 Cancer Cells

Conc (ppm)	Viability (%)	Toxicity (%)	IC50
500	-7.7	107.7	47.18
400	-6.9	106.9	
300	2.2	97.8	
200	10.0	90.0	
100	22.6	77.4	
50	45.0	55.0	
10	80.9	19.1	
0	100.0	0.0	

Our study used various concentrations of parijoto extract to observe cancer cell viability and toxicity levels for each concentration. A concentration of 0 ppm served as the control, where cancer cell viability remained at 100% with a toxicity level of 0%. Our testing on TNBC MDA-MB-231 using the MTT assay showed a very strong effect as the concentration of parijoto extract increased.

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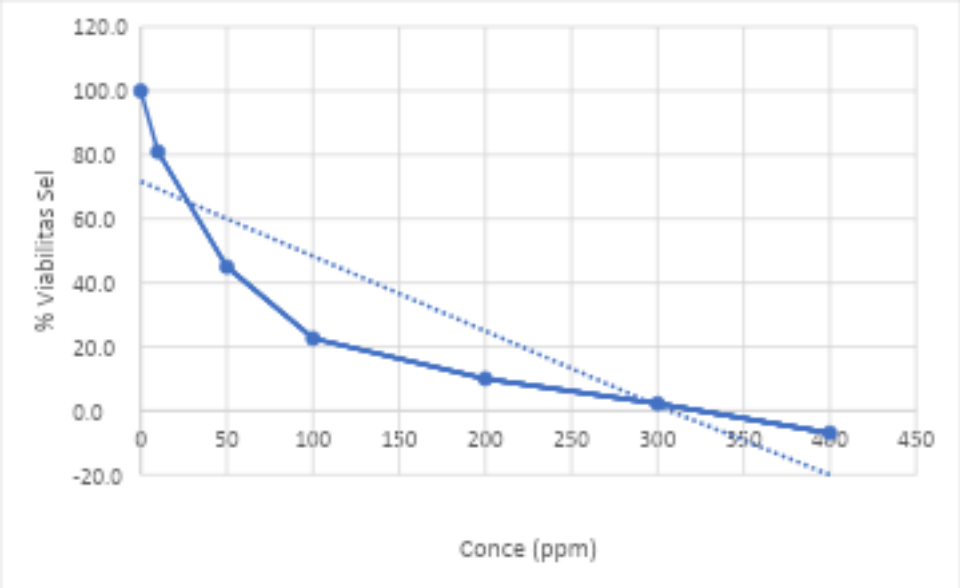

Figure 1. The Relationship Between Extract Concentration and Cell Viability

The correlation between extract concentration and viability showed a negative correlation (Figure 1, showing a negative gradient), which means that the higher the extract concentration, the lower the cell viability level shown in Table 3 from a concentration of 10 ppm with 80.9% viability to a concentration of 500 ppm with -7.7% viability.

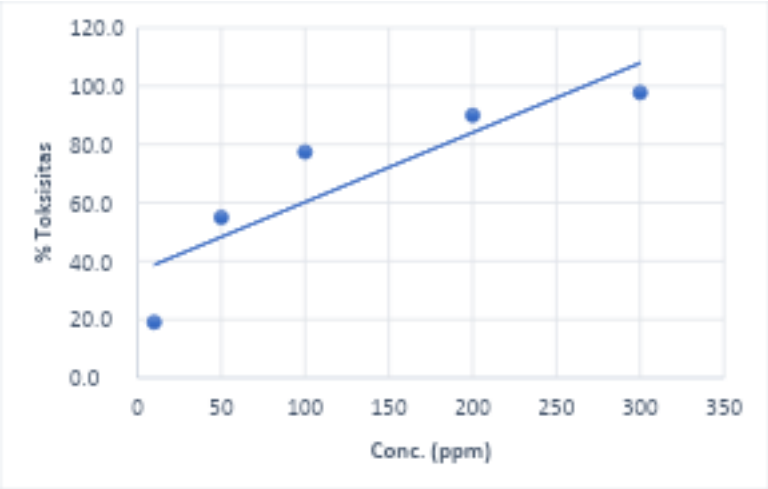

Figure 2. The Relationship Between Extract Concentration and Toxicity Value

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In addition, the concentration of the extract with toxicity shows a positive correlation (Figure 2, showing a positive gradient), which means that the higher the concentration of the extract, the higher the level of toxicity of the extract to cancer cells. Table 3 shows that the concentration of 10 ppm with a toxicity level of 19.1% to a concentration of 500 ppm with a toxicity level of 107.7%. Thus, our linear regression analysis shows an IC50 level of 47.18 ppm with an R2 of 0.774.

3.2. Discussion

The results of this study show that Parijoto contained phenolic, flavonoid, saponin, tannins, and terpenoids. Previous study revealed that parijoto has similar contents with our study, such as polyphenols, flavonoids, tannins, saponins, and terpenoids (Almansour, 2022). The phytochemical content in our study had an antioxidant activity of 58.94 ± 0,42%. Previous research discussing various tropical plants showed that parijoto has antioxidant activity similar to our research of 58.84 ± 0,43(Ratna Shintia Defi; Yohanes Alan Sarsita Putra;Gregorius Yoga Panji Asmara; Victoria Kristina Ananingsih, 2025). Phenolic compounds act as antioxidants that protect human tissues from damage caused by oxidative stress. Antioxidants play a role in eliminating oxidative products such as ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) by inhibiting nuclear factor-κB (NF-κB) or nuclear factor-erythroid factor 2-related factor 2 (Nrf-2) (Rahman et al., 2021). Our research results revealed a decrease in cancer cell viability, and cytotoxicity was concentration-dependent of parijoto extract. This revealed a negative correlation between concentration and viability, meaning that the higher the concentration, the lower the viability of cancer cells, as shown from a concentration of 10 ppm with a viability of 80.9% to a concentration of 500 ppm with a viability of -7.7%. In addition, a positive correlation between concentration and cytotoxicity, as shown from a concentration of 10 ppm with a cytotoxicity of 19.1% to a concentration of 500 ppm with a cytotoxicity of 107.7%. A previous study utilizing *Senecio laetus*, which contains a similar phytochemical profile, also revealed that the number of cancer cell colonies decreased depending on the concentration of the extract used. This phytochemical content plays a role in reducing cancer cell viability through its antiproliferative properties, which disrupt the connections between adjacent cancer cells. That study also revealed that the phytochemical content plays a role in anti-migration, which aims to inhibit cancer cell metastasis (Wadoo et al., 2025)

A comparable study employing *Allium ascalonicum* on TNBC cells, revealing a similar phytochemical content, showed that cell viability depended on the extract concentration. This decrease in cancer cell colony number in the referenced study was concluded to be due to the TNBC cells undergoing apoptosis induced by the phytochemical compounds (Ravindranath & Srinivasan, 2025). Another study utilizing *Artemisia sieberi,* which has a similar phytochemical compound, in this study showed that this phytochemical compound promotes MDA-MB-21 TNBC cells apoptosis through upregulating Bax genes. As the extract concentration increased, the expression of the pro-apoptotic marker Bax gene increased (Albani et al., 2025). Our research encountered a significant methodological artifact at high extract concentrations (≥300 ppm). This was evidenced by the occurrence of negative cellular viability and calculated toxicity values surpassing 100%, which suggests chemical interference with the MTT assay. The MTT assay relies on the reduction of the yellow tetrazolium salt into purple Formazan crystals by metabolically active cells, with the resultant concentration quantified via spectrophotometry. Consistent with existing literature, previous studies investigating the cytotoxicity of phytochemical-rich

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extracts against cancer cells (including MDA-MB-231) have reported similar anomalies at specific concentrations. This artifact is attributed to the presence of reducing phytochemicals, such as phenolic compounds and anthocyanins, which non-enzymatically reduce MTT into Formazan, thereby generating false-positive results for cellular viability(Karakaş et al., 2017). Therefore, IC_{50} determination was focused on the linear concentration range (10-200 ppm) to minimize potential non-enzymatic reduction artifacts associated with polyphenol-rich extracts in tetrazolium-based assays.

The quantitative assessment of cytotoxic potential is generally classified using IC50 values, which allow compounds or extracts to be stratified as having strong (<100), moderate (100 < IC50 <1000), or non-toxic (>1000). Cytotoxicity evaluation via the MTT assay demonstrated that the Parijoto ethanol extract possessed potent inhibitory effects on the MDA-MB-231 cell line, with the IC50 value determined to be 47.18, which meant it was classified as strong cytotoxic. A prior study on the cytotoxicity of Parijoto extract indicated a moderate inhibitory effect, recording an IC50 value of 175±0.962 ppm (Artanti et al., 2022). In contrast to the previous study, where methanol extraction was performed, our IC50 results stemming from the ethanol extract demonstrated superior cytotoxic potency. This variation underscores the influence of the extraction solvent on the final concentration and efficacy of the bioactive constituents. The strong cytotoxic effect observed is likely due to the high measured levels of flavonoids and anthocyanins, compounds well-known for their capacity to induce apoptotic pathways in triple-negative breast cancer (TNBC) cells. Future studies are warranted to further elucidate the molecular mechanisms underlying the cytotoxic effects of *M. speciosa* ethanol extract, particularly its involvement in apoptosis-related pathways such as Bax/Bcl-2 regulation and caspase-3 and caspase-9 activation. In addition, intracellular reactive oxygen species (ROS) modulation should be evaluated to clarify the contribution of redox imbalance to TNBC cell death. Validation using complementary non-tetrazolium-based assays, including sulforhodamine B (SRB), trypan blue exclusion, or flow cytometry, is recommended to strengthen methodological robustness. Further fractionation, standardization of key bioactive constituents, and *in vivo* evaluation will be essential steps toward the translational development of Parijoto-derived anticancer agents.

4. Conclusion

The results of this study demonstrate that the ethanol extract of *M. speciosa* (Parijoto) fruit contains a diverse range of phytochemical constituents, including phenolic compounds, flavonoids, saponins, tannins, terpenoids, and anthocyanins. Quantitative analyses revealed relatively high levels of total phenolics, flavonoids, and anthocyanins, accompanied by moderate antioxidant activity, indicating a significant potential for modulating cellular redox homeostasis (Johnson et al., 2022; Khoo et al., 2017). Biologically, the Parijoto ethanol extract exhibited a clear and concentration-dependent cytotoxic effect against triple-negative breast cancer (CBC) MDA-MB-231 cells, with an IC_{50} value of approximately 47.18 ppm, classifying it as a strongly cytotoxic extract. This cytotoxic potency is notably higher than that reported in several previous studies employing different extraction solvents, highlighting the critical role of extraction methodology in determining the composition and biological efficacy of bioactive compounds (Alam et al., 2013; Rajasekar et al., 2023). Based on the phytochemical profile and supporting literature, the observed anticancer activity is most likely attributable to synergistic interactions between flavonoids and anthocyanins—compounds well documented for their ability to regulate oxidative stress, inhibit tumor cell proliferation, and activate apoptotic pathways in TNBC

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models (Maaz et al., 2025). It is important to note that very high extract concentrations induced interference in the MTT assay, a phenomenon consistent with reports describing interactions between polyphenol-rich extracts and tetrazolium-based reagents. Nevertheless, this methodological limitation does not alter the consistent cytotoxic trend observed within biologically relevant concentration ranges (Ghasemi et al., 2021; Kamiloglu et al., 2020). Overall, this study supports the potential of *M. speciosa* fruit ethanol extract as a natural source of candidate anticancer agents for triple-negative breast cancer and provides a scientific basis for further investigations, including mechanistic studies, isolation of active constituents, and the development of potential therapeutic formulations.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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DESI-MS AS A HYPHENATED TECHNIQUE FOR IDENTIFICATION OF *PELARGONIUM SIDOIDES* AND *PELARGONIUM RENIFORME*

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Abstract: *Pelargonium sidoides* and *Pelargonium reniforme*, two taxonomically allied South African medicinal plants, are widely used in traditional medicine for treating respiratory and gastrointestinal ailments. Despite their therapeutic significance, differentiation of these species remains challenging due to overlapping morphological traits. While prior phytochemical analyses have identified distinct metabolite profiles between the species, these methods lack the spatial resolution to localize key markers *in situ.* To address this gap, we applied desorption electrospray ionization mass spectrometry imaging (DESI-MSI) coupled with high-performance thin-layer chromatography (HPTLC) for compound detection, identification, and spatial mapping in root extracts of both species. Root methanol extracts (10 mg/mL) were analyzed via HPTLC-DESI-time-of-flight (ToF)-MS, enabling targeted characterization of four marker compounds: umckalin/isofraxidin, isofraxidin sulphite, 5,6,7,8-tetramethoxycoumarin, and 3′,4′-dimethylluteolin. DESI-MSI was further employed to investigate the spatial distribution of these metabolites in fresh root cross-sections (0.08 mm thickness). DESI-MSI revealed species-specific localization patterns: scopoletin and isofraxidin sulphate were concentrated in the cortical regions of *P. sidoides*, while umckalin dominated the vascular bundles of *P. reniforme*. This study demonstrates, for the first time, the utility of HPTLC-DESI-ToF-MS as a rapid, dual-platform approach for the simultaneous separation, identification and spatial resolution of compounds in Pelargonium plants. HPTLC-DESI-MS analysis is cost-effectiveness, adaptability to complex matrices, reducing ion suppression and allowing targeted interrogation of specific bands through visual localization. This technique maintains analytical rigor while addressing several limitations inherent to conventional UPLC-MS methodologies. The distinct spatial distribution of biomarkers provides a novel criterion for species authentication, overcoming limitations of traditional morphological and chromatographic methods. Our findings affirm DESI-MSI as a transformative tool for *in situ* phytochemical profiling, offering actionable insights for quality control of botanicals and mitigating risks of misidentification in commercial herbal products.

Key Words: Desorption electrospray ionization mass spectrometry imaging (DESI-MSI), *Pelargonium sidoides*, *Pelargonium reniforme*

1. Introduction

Pelargonium sidoides and *Pelargonium reniforme* are two indigenous South African medicinal plants widely used in traditional medicine for treating respiratory and gastrointestinal ailments [1]. These two species are close taxonomic allies and differentiation of these species remains challenging due to overlapping morphological traits. Several analytical techniques have demonstrated distinct chemical differences between the two species [2, 3], but these methods lack the spatial resolution to localize key markers *in situ*. Desorption electrospray ionisation-mass spectrometry imaging (DESI-MSI) allows the ionisation of different chemical compounds under ambient environment, and suitable for the non-destructive and rapid analysis. In this study, DESI imaging was used to map the distribution of coumarins in the roots of *Pelargonium* species for chemotaxonomic classification.

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2. Material and Methods

2.1. Sample collection

The roots of *P. reniforme* and *P. sidoides* were sourced from Parceval (Pty) Ltd., South Africa.

2.2. Sample preparation

The roots were washed and stored in -80°C freezer until use. The frozen tissues were stabilised for 1 hour at -20°C in a Dakewe 6250 cryostat microtome (Cell path services, South Africa). The root tissues were embedded on a sample holder (Specimen chuck) using deionised water. The specimen chucks were placed on the freezing stage and the tissues were sectioned to achieve a thickness of 80 µm slice at -20°C. The tissues were mounted on a glass slide (Lasec, South Africa) for DESI-MSI analysis.

2.3. DESI-MSI analysis

The imaging analysis was performed on a XevoTM G3 QTof mass spectrometer (Waters Corporation, Manchester, UK) equipped with a DESI-XS source, containing heated transfer line (HTL) or an ion inlet tube, high-performance DESI sprayer and a 2D moving stage imaging. Harvard Apparatus system (Holliston, USA) was used to deliver the solvent with a 1.00 mL syringe (Microsep, South Africa). The DESI-MSI parameters utilised for Pelargonium samples were as follows: capillary voltage, 0.70-0.80 kV; ion source temperature, 120 °C; nitrogen pressure, 0.1 MPa; capillary tip to the surface, 2 mm; capillary tip to MS inlet, 6 mm; MS inlet to surface, ~ 0.5 mm; spray solvent, methanol: water (98:2) with 0.01 % formic acid, containing leucine enkephalin of 50 pg/µL (internal standard for mass correction). The flow rate was set at 3 µL/min. A mass range of *m/z* 100-1200 was used.

2.4. Data processing

High-definition imaging (HDI) 1.8 (Waters Corporation, Manchester, UK) software was utilised for the processing and visualisation of the raw image data files. The relative ion intensity represented by the variation in colour intensity reflected the spatial distribution visualisation of the detected compounds. The blue colour indicated low relative concentration/intensity, while the yellow indicated relative high concentration/intensity for the spatial distribution of the *m/z* in the tissues, with intensity levels in the order of yellow > red > blue.

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3. Results and Discussion

Table: Putatively identified compounds in Pelargonium species and their spatial distribution in root transverse sections

Compound No.	Calculated mass $[M-H]^-$ *m/z*	Elemental Composition	Compound name	Distribution in *P. reniforme*	Distribution in *P. sidoides*
A	190.9898	$C_{10}H_8O_4$	Scopoletin	Ep	All regions
B	207.9845	$C_{10}H_8O_5$	Isofraxetin	Xy, Ph, Co, and Ep	pith, xylem, and phloem
C	221.0361	$C_{11}H_{10}O_5$	Umckalin/Isofraxidin	Ep	All regions
D	237.0308	$C_{11}H_{10}O_6$	Dihydroxy-dimethoxycoumarin	Xy, Ph, Co, and Ep	Pi, Xy, and Ph
E	272.9605	$C_9H_6O_8S$	Dihydroxycoumarin-sulphate	Ep	Pi, Xy, Ph, and Ep
F	300.9808	$C_{11}H_{10}O_8S$	Isofraxidin sulphite	Ep	Pi, Xy, Ph, and Ep
G	305.0590	$C_{15}H_{14}O_7$	Epigallocatechin	Pi, Xy, and Ph	Pi, Xy, and Ph
H	316.9862	$C_{11}H_{10}O_9S$	Hydroxy-dimethoxycoumarin-sulphate	Ep, Xy, and Ph	Low intensity in all regions
I	305.0951	-	Unidentified	Low intensity in all regions	Pi, Xy, Ph, and Ep
J	305.0953	-	Unidentified	Low intensity in all regions	High intensity in all regions
K	328.0337	$C_{10}H_{12}N_5O_6P$	cAMP	Low intensity in all regions	Pi, Xy, and Ph
L	344.0281	$C_{10}H_{12}N_5O_7P$	cGMP	Low intensity in all regions	High intensity in all regions
M	358.0438	$C_{11}H_{14}N_5O_7P$	Methyl-cGMP	Co, Ph, and Xy	Low intensity in all regions
N	382.9948	-	Unidentified	No detection	High intensity in all regions

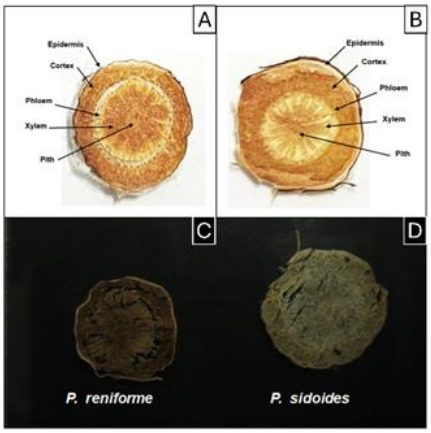

Figure 1: Transverse sections of root tissues from *P. reniforme* and *P. sidoides*, highlighting the anatomical regions (epidermis, cortex, phloem, xylem, and pith) found in root tissue.

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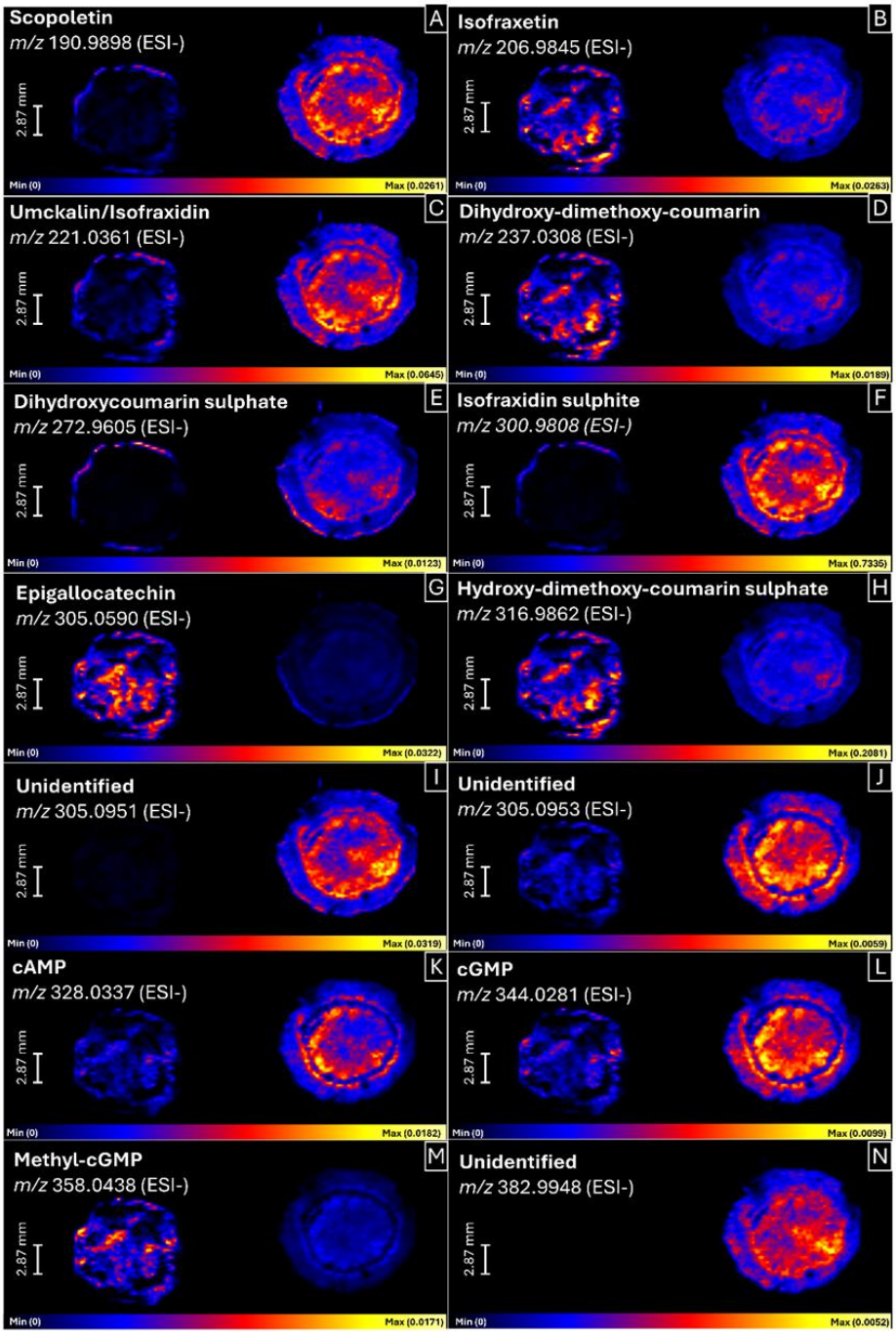

Figure 2. Comparative DESI-MSI images of transverse root tissues from *P. reniforme* (left) and *P. sidoides* (right) showing the spatial distribution of key metabolites across anatomical regions (epidermis, cortex, xylem, phloem, and pith).

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DESI-MSI analysis indicated that coumarins isofraxetin (m/z 206.9845), umckalin/isofraxidin (m/z 221.0361), and dihydroxy-dimethoxycoumarin (m/z 237.0399) were key chemomarkers for *P. sidoides* and *P. reniforme* differentiation. These findings reveal the precise tissue-specific accumulation patterns of these metabolites with previously unattainable spatial resolution.

4. Conclusion

This study demonstrates the capabilities of DESI-MSI as an analytical technique to distinguish the distribution of metabolites of *P. reniforme* and *P. sidoides*. Mapping the precise spatial distribution of metabolites enhances our understanding of phytochemistry, chemotaxonomy and in-cellular compound localization.

Acknowledgments

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Conflict of Interest

The authors declare no conflicts of interest.

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ANTIMICROBIAL ACTIVITY OF STARCH-BASED ORAL STRIPS CONTAINING PLANT ESSENTIAL OILS AND FRUIT EXTRACTS AGAINST *STREPTOCOCCUS MUTANS*

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Abstract: Dental caries is a biofilm-associated pathology in which the bacteria *Streptococcus mutans* has a significant role. This preliminary *in vitro* study evaluated starch-based edible films, designed as oral strips, incorporating cinnamon and clove essential oils, cranberry and pomegranate peel extracts against *S. mutans* using broth microdilution (MIC/MBC) and agar disk diffusion methods. Starch based films were formulated with individual active components: cinnamon essential oil (CIN, 1% w/w), clove essential oil (CLO, 1% w/w), cranberry extract (CRN, 1% w/w), and pomegranate peel extract (POM, 2% w/w), and a combination (COMB: 0.5% cinnamon, 0.25% clove, 1% cranberry, 1% pomegranate; 2.75% total). The starch–glycerol control dispersion showed no inhibition up to the highest tested level. MIC values (µg/mL of active) were: CLO 125, CIN 250, CRN 250, POM 1000, while COMB exhibited the lowest MIC of 62.5 µg/mL. MBC values followed the same pattern (CLO 250, CIN 500, CRN 500, POM >1000, COMB 125). MIC and MBC for COMB were significantly lower than those of all single-active dispersions ($p<0.05$), indicating enhanced antimicrobial potency at reduced individual component levels. CLO also showed a significantly lower MIC than CIN, CRN, and POM ($p<0.05$). In disk diffusion, the control discs showed only their intrinsic diameter (6 mm) being significantly lower than all ($p<0.05$). Among single-actives, CLO (20.5±1.3 mm) and CIN (18.2 ± 1.1 mm) generated significantly larger zones than CRN (13.4±0.9 mm) and POM (11.5±0.8 mm; $p<0.05$). COMB yielded the largest zones (23.0±1.4 mm), significantly exceeding all single-active formulations ($p<0.05$). The results indicate that starch-based oral strips incorporating these plant-derived actives, especially the combination formulation, demonstrate inhibitory and bactericidal activity against *S. mutans in vitro* and require further investigation in multi-species biofilm and in situ oral models.

Key Words: *Streptococcus mutans*, plant antimicrobials, edible films, essential oils, fruit extracts.

1. Introduction

Oral diseases remain a major global public-health challenge. The World Health Organization estimates that oral diseases affect nearly 3.7 billion people, and reports that untreated dental caries in permanent teeth is the most common health condition in the Global Burden of Disease 2021 assessment (World Health Organization [WHO], 2025). Dental caries is a biofilm-mediated disease driven by ecological shifts within dental plaque, where frequent exposure to fermentable carbohydrates promotes acidification, enamel demineralization, and selection of acidogenic/aciduric microorganisms. Among these, *Streptococcus mutans* is widely recognized as a key cariogenic species due to its capacity to synthesize extracellular polysaccharides (EPS) and to sustain biofilm growth under acidic conditions, thereby strengthening biofilm structure and persistence on tooth surfaces (Adil et al., 2014; Kim et al., 2015). A central virulence feature of *S. mutans* is EPS-matrix formation, largely produced via glucosyltransferase activity, which enhances adhesion, increases mechanical stability, and provides diffusion-limiting protection against environmental

stressors and antimicrobial agents (Adil et al., 2014; Kim et al., 2015). In mixed-species biofilms, disruption of the EPS-rich matrix can reduce cariogenicity by weakening the structural scaffold that supports microbial co-adhesion and localized acidification (Kim et al., 2015).

Preventing dental caries in the contemporary "biofilm-mediated dysbiosis" framework relies on interventions that (i) reduce cariogenic biofilm mass and virulence, (ii) increase resistance of enamel to acid challenge, and (iii) remove the dietary selective pressure that sustains low-pH ecology. Mechanical plaque control remains foundational: regular toothbrushing disrupts supragingival biofilm and, in controlled conditions, can reduce plaque scores by approximately 50%–60%, with powered brushes often achieving modestly greater disruption; however, real-world effectiveness is strongly technique- and adherence-dependent and is limited at interproximal stagnation sites, making interdental cleaning and behavioral reinforcement necessary adjuncts (Keller et al., 2023). Fluoride is consistently positioned as the cornerstone chemotherapeutic because it enhances remineralization, reduces enamel solubility, and suppresses bacterial acidogenesis via metabolic enzyme inhibition; clinical syntheses indicate meaningful reductions in caries incidence, particularly when professionally applied topical fluorides are added for high-risk patients (Luo et al., 2024). Antimicrobials such as chlorhexidine can transiently suppress cariogenic bacteria due to substantivity and broad activity, but biofilm-associated tolerance, rapid regrowth after cessation, and adverse effects (notably staining and taste disturbance) constrain chlorhexidine to short-term, risk-based use rather than continuous prevention (Poppolo Deus & Ouanounou, 2022). Dietary measures are therefore critical ecological levers: limiting the frequency of free-sugar exposure reduces repeated plaque pH drops and the selective advantage of aciduric taxa, while sugar substitutes (e.g., xylitol) can reduce cariogenic microbial levels with variable effect sizes depending on dose and background fluoride exposure (Luo et al., 2024). Emerging adjuncts emphasize microbiome and biofilm modulation rather than broad killing; probiotics/prebiotics and related approaches show growing evidence for reductions in *S. mutans* colonization and improvements in biofilm behavior, though optimal strains, delivery vehicles, and durations remain under investigation (Zhang et al., 2024). Finally, remineralization technologies and "smart" antibiofilm materials aim to strengthen enamel and disrupt EPS-rich matrix microenvironments, offering minimally invasive options to arrest or reverse early lesions, but require stronger long-term clinical validation before they can be considered equivalent to fluoride-based prevention in population settings (Taha et al., 2017).

Conventional chemical control strategies in dentistry frequently include chlorhexidine (CHX) mouthrinses due to their broad antibacterial and antiplaque effects. However, CHX use is limited by undesirable adverse effects—most notably tooth staining, taste alteration, mucosal irritation, and reduced compliance during prolonged use (Jindamporn et al., 2025; Adil et al., 2014). These limitations have strengthened interest in plant-derived antimicrobials that can be incorporated into oral-care matrices, potentially offering multi-target activity while aligning with consumer preference for "natural" ingredients. Plant essential oils and polyphenol-rich fruit extracts are among the most investigated natural antimicrobials. Clove essential oil, largely attributed to its major component eugenol, demonstrates broad antimicrobial activity and has been discussed as a bioactive compound capable of affecting microbial adhesion, biofilm formation, and virulence-related processes (Hu et al., 2018). In the context of oral biofilms, eugenol has been shown to suppress expression of biofilm- and quorum sensing–related genes in *S. mutans* at sub-inhibitory concentrations, suggesting potential to attenuate virulence without necessarily relying only on bactericidal effects (Adil et al., 2014).

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Cinnamon essential oil is also recognized for antimicrobial properties and, when combined with clove essential oil in biopolymer films, has been reported to improve antimicrobial performance in food-packaging film systems—supporting the broader feasibility of co-incorporating these oils into film matrices (Xu et al., 2019). Polyphenol-rich fruit extracts are additionally relevant for anticariogenic strategies. Cranberry flavonoids have been shown to modulate cariogenic properties of mixed-species biofilms through EPS-matrix disruption, providing mechanistic support for using cranberry-derived bioactives as antibiofilm agents in oral applications (Kim et al., 2015). In parallel, cranberry extracts have demonstrated antimicrobial and antibiofilm effects against *S. mutans in vitro*, reinforcing their potential relevance to dental biofilm control (Singhal et al., 2020). Pomegranate peel is another abundant source of phenolic compounds (e.g., hydrolyzable tannins) and has been investigated for antibacterial activity against *S. mutans*, including work evaluating pomegranate peel extracts against *S. mutans* isolated from orthodontically treated patients (Abd-El-Aziz & Sallam, 2020). Moreover, pomegranate-based mouthrinse formulations have been clinically explored with outcomes including reductions in salivary *S. mutans* counts and improved salivary parameters, indicating translational relevance of pomegranate bioactives for oral antimicrobial applications (Umar et al., 2016).

Beyond selecting active compounds, the delivery platform is critical for oral efficacy. Oral strips (orodispersible/oral dissolving films) provide a thin, flexible dosage form designed to disintegrate or hydrate in the oral cavity and can deliver active agents locally with improved convenience and potentially enhanced contact time at oral surfaces compared with conventional rinses (Bala et al., 2013). Recent film-based systems have incorporated essential oils into polymer matrices (including nanoemulsified approaches) to harness antibacterial activity in an oral film format (Aswathy et al., 2024). Although polymer selection and formulation strategies vary, these studies collectively support the feasibility of incorporating hydrophobic essential oils and hydrophilic plant extracts into edible film systems intended for oral use. Within this framework, the present preliminary *in vitro* study focuses on starch-based edible films designed as oral strips incorporating cinnamon essential oil (CIN), clove essential oil (CLO), cranberry extract (CRN), and pomegranate peel extract (POM and in combination (COMB) targeting *S. mutans* as main factor for oral health problems. The antimicrobial performance of these starch-based oral strips is evaluated using broth microdilution (MIC/MBC) and agar disk diffusion methods, aiming to generate comparative evidence on inhibition potential across single-active and combined formulations in alignment with caries-preventive, biofilm-relevant intervention concepts.

2. Material and Methods

All raw materials such as; cinnamon bark (*Cinnamomum verum*), dried clove flower buds (*Syzygium aromaticum* L.), dried cranberry (*Vaccinium macrocarpon*), and Pomegranate fruits (*Punica granatum* L.) were commercially obtained through traditional stores in Gaziantep, Türkiye.

2.1. Obtaining essential oils and fruit extracts

Cinnamon bark (*Cinnamomum verum*) and clove flower buds (*Syzygium aromaticum* (L.)) essential oils were produced by hydrodistillation using a Clevenger-type apparatus (InterLab, Adana, Türkiye) under the same laboratory setup and operating conditions reported by Barazi et al. (2023). Briefly, 150.0 ± 2 g of ground dried plant material was mixed with 1.5 liter of double-distilled water. The mixture was heated to

generate steam for distillation and hydrodistillation was continued for 5 h. The recovered oil phase was separated from the aqueous distillate, dried over anhydrous sodium sulfate, transferred into sterile amber (dark) bottles (10-20 mL approximately), and stored at 4°C until use. Pomegranate fruits were obtained locally. Peels were separated, washed, and dried at 40-45°C to constant weight, then milled to a fine powder. Peel powder was extracted with 70:30 (v/v) ethanol:water mixture at a 1:10 (w/v) solid-to-solvent ratio under agitation (150 rpm) for 48 h at room temperature. Then solutions were centrifuged at 6000 rpm for 10 min at 18°C (Eppendorf, Germany). The extract was filtered through (Whatman No. 1), concentrated by rotary vacuum evaporator at 40°C to remove ethanol, and dried to obtain a powder extract. The dried extract was stored in airtight containers at 4°C for further use. For microbiological assays, aqueous stock solutions were prepared in sterile distilled water with suitable concentrations. Cranberry extract was obtained from dried cranberries locally bought. The method was adapted from Singhal et al. (2020). Around 20 g of the dried fruit was chopped and kept at 40-45°C in oven to a constant weight. Then they were mixed with 200 mL of a hydroalcoholic solvent with 70:30 (v/v) ethanol:water in a conical flask. The extract was kept on shaker at 200rpm for 48 hours and the liquid part was filtered through Whatman no. 40 filter paper. The filtrate was transferred into a rotary evaporator and solvent was evaporated at 40°C under vacuum. The residual aqueous solution was stored at 4°C in an air tight container for further use.

2.2. Preparation of starch-based edible oral strip films

Starch-based oral strips were prepared by a film-forming dispersion and casting approach. Oral strip dispersions were prepared by casting a gelatinized starch–glycerol matrix into (PTFE/teflon) containers, with all formulations standardized to a final aqueous dispersion volume of 100 mL. The same core laboratory instruments and handling steps were applied and adapted to a starch matrix as described in previous study Barazi et al. (2023). Briefly, 3 g of starch was dispersed in 80 mL distilled water and heated to 85°C with continuous mixing on a magnetic stirrer/hotplate (Heidolph MR-Hei Standard, Germany) for 20 min to achieve gelatinization. After that, 0.9 g (30% of starch) of glycerol (ACS grade CAS 56-81-5, Millipore Merck, Darmstadt, Germany) was added as plasticizer and the dispersion was mixed for an additional 10 min and cooled to 45-50°C. Essential oils were premixed with Tween 80 and added dropwise to the cooled dispersion. For single-active treatments, the essential oils and fruit extracts was added at 1.0% of the total dispersion (i.e., 1.00 g / 100.0 g dispersion), with vigorous stirring to ensure uniform distribution. For the combination, 0.5% cinnamon, 0.25% clove, 1% cranberry, 1% pomegranate were added to get 2.75% total active substance concentration. A control film (CTRL) containing starch and glycerol without any active compounds was also prepared. The final film-forming dispersions were clarified by centrifugation at 6000 rpm for 6 min at 18°C (Eppendorf 5810R, Hamburg, Germany) to remove entrapped air and insoluble particles. A fixed volume (15 mL) of dispersion was cast onto leveled sterile PTFE containers and dried at 40°C for 24 h. Dried films were peeled, conditioned at 25°C (50% RH) for at least 24 h, and cut into standardized discs for antimicrobial assays. All film formulations and their compositions are given in Table 2.1.

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Table 2.1. Film formulations and active substances included

Film code	Film-forming matrix	Active compounds in dispersion	Active levels (% w/w)	Total active load (% w/w)
CTRL	Starch + glycerol (plasticizer); no actives	None	0	0
CIN	Starch + glycerol	Cinnamon essential oil *(Cinnamomum verum)*	1.00	1.00
CLO	Starch + glycerol	Clove essential oil *(Syzygium aromaticum)*	1.00	1.00
CRN	Starch + glycerol	Cranberry extract *(Vaccinium macrocarpon)*	1.00	1.00
POM	Starch + glycerol	Pomegranate peel extract *(Punica granatum)*	2.00	2.00
COMB	Starch + glycerol	Cinnamon EO + Clove EO + Cranberry extract + Pomegranate peel extract	Cinnamon 0.50; Clove 0.25; Cranberry 1.00; Pomegranate 1.00	2.75

2.3. Microorganism and culture conditions

Streptococcus mutans ATCC 25175 (American Type Culture Collection, Manassas, VA, USA) was used. Stock cultures were maintained on BHI agar at 4°C. Working cultures were prepared by two successive subcultures in BHI broth and incubation at 37°C for 24 h under 5% CO_2 (generating sachet, Oxoid, UK). The inoculum was standardized to 0.5 McFarland and diluted in broth to the working inoculum required for each assay.

2.4. Antimicrobial activity assays

2.4.1. Broth microdilution (MIC and MBC)

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth microdilution in 96-well plates according to standardized dilution susceptibility testing principles (Clinical and Laboratory Standards Institute [CLSI], 2024a). Two-fold serial dilutions of each film dispersions were prepared corresponded to final concentrations of 31.25-1000 µg/mL. Wells were inoculated to achieve a final bacterial density of approximately 5×10^5 CFU/mL and incubated at 37°C for 24 h under 5% CO_2 (Thermoscientific CO_2 gen sachet, OXOID, UK) in anaerobic jar. MIC was defined as the lowest concentration with no visible growth compared with the growth control. To determine MBC, samples (10 µl) from wells at the MIC level were plated onto BHI agar and incubated at 37°C for 24-48 h; MBC was defined as the lowest concentration yielding no colony growth.

2.4.2. Agar disk diffusion assay

Disk diffusion was performed following CLSI disk diffusion principles (CLSI, 2024b) using BHI agar. Plates were surface-inoculated with standardized with 0.5 McFarland *S. mutans* suspension. Sterile-cut film

discs (6 mm diameter) were placed on the agar surface and incubated at 37°C for 24 h under 5% CO_2. Inhibition zones were measured and reported as total zone diameter including the 6 mm disc.

2.4.3. Statistical analysis

All experiments were conducted with at least three independent replications. Disk diffusion zone diameters were reported as mean ± standard deviation (n = 6 per formulation, as indicated in Table 3.2). Group differences were evaluated by one-way analysis of variance (ANOVA) with $p < 0.05$ considered statistically significant, using SPSS 22.0 for Windows (IBM SPSS, Chicago, IL, USA). Differences in the sub groups were analyzed according to Tukey's post hoc tests aligned with ANOVA results.

3. Results and Discussion

3.1. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC)

The antimicrobial activity of the edible film dispersions including plant EOs and extracts against *S. mutans* was observed by broth microdilution. Film dispersions were prepared so that two-fold serial dilutions corresponded to final concentrations of 31.25–1000 µg/mL of the respective active compounds in the test wells (based on an "active per mL", not per mg of film matrix). The MIC and MBC values obtained for each formulation are summarized in Table 3.1.

Table 3.1. MIC and MBC values of edible film dispersions against *Streptococcus mutans (values expressed as µg/mL of active compound in the test medium)*

Film code	Active compound in dispersion	MIC (µg/mL)	MBC (µg/mL)
CTRL	– (No actives present, starch–glycerol only)	>1000	>1000
CIN	Cinnamon essential oil	250	500
CLO	Clove essential oil	125	250
CRN	Cranberry extract	250	500
POM	Pomegranate peel extract	1000	>1000
COMB	Cinnamon + clove EO + cranberry + pomegranate extracts	62.5	125

No inhibitory effect was observed with the control film dispersion (CTRL) up to the highest tested concentration (equivalent to 1000 µg/mL), confirming that the starch–glycerol base itself did not affect *S. mutans* growth. Among the single-active dispersions, CLO (clove oil) exhibited the lowest MIC (125 µg/mL) and MBC (250 µg/mL), being the strongest bactericidal activity in this group ($p<0.05$). The CIN (cinnamon oil) and CRN (cranberry extract) dispersions showed intermediate MIC values (250 µg/mL) with MBCs of 500 µg/mL, suggesting a transition from bacteriostatic to bactericidal effects. On the other hand, the POM (pomegranate peel) dispersion only inhibited growth at 1000 µg/mL, and no clear bactericidal effect was observed within the tested range (MBC>1000 µg/mL). This gives a supporting view that pomegranate acts more as an anti-biofilm agent than as a potent bactericide at low concentrations. The COMB dispersion, containing all four actives at the ratios used in the combination film, demonstrated the lowest MIC (62.5 µg/mL total actives) and an MBC of 125 µg/mL. These values were significantly lower

(p<0.05) than the single-active formulations, despite the fact that the individual concentrations of each component in the COMB dispersion were below their respective single-agent MICs. This pattern strongly suggests additive or synergistic interactions between cinnamon and clove essential oils and the cranberry and pomegranate extracts. These levels are well below the MICs observed for the same components when tested individually, reinforcing the hypothesis of synergistic antimicrobial action within the combination system.

3.2. Agar disk diffusion assay

The qualitative antibacterial activity of the film formulations was further evaluated using an agar disk diffusion assay on BHI agar inoculated with *S. mutans*. Film-forming dispersions were prepared at a fixed total volume and cast under identical conditions; therefore, each 6 mm film disc contained a defined active loading corresponding to its formulation. Representative agar plates showed clear inhibition zones around all active formulations, whereas the CTRL discs produced no visible growth inhibition beyond their own diameter. Mean inhibition zone diameters (including the 6 mm disc) are presented in Table 3.2.

Table 3.2. Inhibition zone diameters of film dispersions against *Streptococcus mutans (disk diffusion assay on BHI agar, 24 h, 37 °C, 5% CO_2; mean ± SD, n = 6)*

Film code	Active compounds	Zone diameter (mm) (mean ± SD)
CTRL	–	6.0 ± 0.0
CIN	Cinnamon EO	18.2 ± 1.1
CLO	Clove EO	20.5 ± 1.3
CRN	Cranberry extract	13.4 ± 0.9
POM	Pomegranate peel extract	11.8 ± 0.8
COMB	CIN + CLO + CRN + POM	22.9 ± 1.4

The CTRL discs displayed only the baseline 6 mm diameter corresponding to the disc size, confirming the absence of intrinsic antibacterial activity in the film matrix. All active formulations produced significantly larger inhibition zones than CTRL (p<0.05). Among single-active preparations, CLO yielded the largest zone (20.5±1.3 mm), followed by CIN (18.2±1.1 mm). Both were significantly larger than those produced by CRN and POM (p<0.05), in line with the lower MIC values obtained in the broth microdilution assay. CRN and POM exhibited moderate inhibition (13.4±0.9 mm and 11.8±0.8 mm, respectively), reflecting their weaker direct bactericidal activity when tested alone. The COMB dispersion produced the largest inhibition zones overall (22.9±1.4 mm), significantly exceeding those of all single-active films (p<0.05 vs CLO; p<0.05 vs CIN, CRN, POM). The COMB halos were also visually more sharply defined, suggesting strong growth suppression around the discs even at the outer boundary of the zone. A moderate inverse correlation was observed between MIC values (Table 3.1) and inhibition zone diameters (Table 3.2) across formulations (i.e., lower MICs associated with larger zones), which is consistent with the expected relationship between diffusion-based and broth-based susceptibility tests. The COMB formulation, with the lowest MIC and MBC, also generated the widest inhibition zones, further supporting its superior anti-*S. mutans* activity. Inhibition zones for disc diffusion assay are indicated in Figure 3.1.

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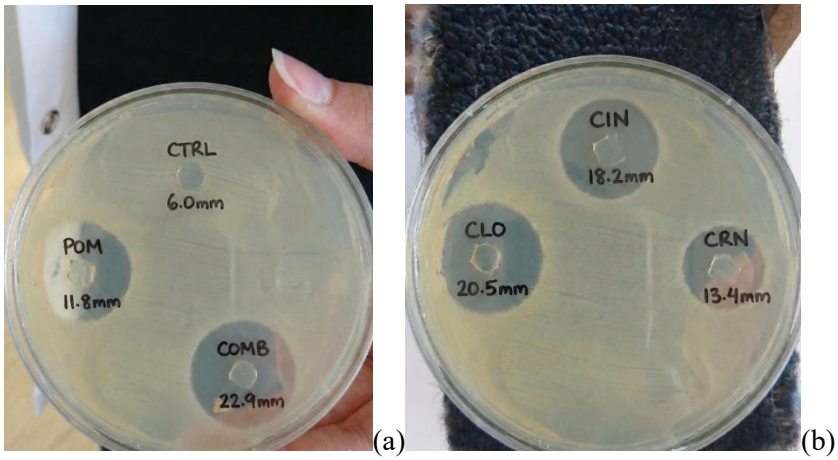

Figure 3.1. Disc diffusion assay results for film disc (a); CTRL, POM and COMB (b); CIN, CLO and CRN film samples. Inhibition zone dimaters include film diameters (6mm) and designated as average measurement from three replicate results.

4. Discussion and Conclusion

This preliminary *in vitro* study evaluated starch-based oral strips incorporating two essential oils (clove and cinnamon) and two fruit-derived extracts (cranberry and pomegranate peel) against *Streptococcus mutans* using broth microdilution (MIC/MBC) and agar disk diffusion. Overall, the antimicrobial agents displayed a clear potency gradient across assays: the essential oils were more active than the crude polyphenol-rich extracts, and the combined formulation (COMB) produced the strongest effect in both dilution and diffusion formats. This pattern is consistent with the broader literature on essential-oil-mediated membrane disruption and the more "anti-virulence/anti-biofilm–weighted" activity profiles often reported for berry and pomegranate polyphenols (Marchese et al., 2017).

Clove essential oil (CLO); CLO showed pronounced activity against *S. mutans* (MIC 125 µg/mL; MBC 250 µg/mL; inhibition zone ~20.5 mm). The close agreement between our MIC and the value reported by Rodríguez et al. (2014) for clove essential oil against *S. mutans* (MIC 125 µg/mL) provides strong external validity for the present results. The observed bactericidal threshold (MBC 250 µg/mL) is also consistent with the expected concentration gap between growth inhibition and killing for phenylpropanoid-rich oils. As mechanism of effect, clove oil activity is largely attributed to eugenol, which can disrupt Gram-positive membranes, increase permeability, and interfere with enzyme systems relevant to energy metabolism and biofilm development. In agar diffusion, a 20 mm inhibition zone aligns with "strong activity" classifications commonly reported for clove oil against *Streptococcus mutans* (Hu et al., 2018; Marchese et al., 2017).

Cinnamon essential oil (CIN); (MIC 250 µg/mL; MBC 500 µg/mL; ~18.2 mm zone) was also strongly active, though less potent than CLO in our system. Cinnamon oil activity is commonly attributed to cinnamaldehyde-dominant fractions that affect membrane integrity and key metabolic enzymes, including those linked with cariogenicity. Reported MICs vary substantially across assays, and studies confirm

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cinnamon oil can inhibit *S. mutans* and plaque biofilms *in vitro*, supporting our range as reasonable for a film-derived dispersion where emulsification and release may limit immediate bioavailability (Wiwattanarattanabut et al., 2017; Atarés & Chiralt, 2016). Accordingly, our CIN profile still represents strong antimicrobial performance for an oral strip matrix, and the ~18 mm diffusion zone supports appreciable mobility of active components from the strip/disc interface into agar.

Cranberry extract (CRN); showed intermediate antibacterial activity (MIC 250 µg/mL; MBC 500 µg/mL; zone ~13.4 mm). When compared to Singhal et al. (2020), who reported an MIC of 12.5 mg/dl (0.125 mg/mL) and MBC of 25 mg/dl (0.25 mg/mL) for an ethanolic cranberry extract against *S. mutans*, our MIC is within a 2-fold dilution of that MIC/MBC window and our MBC remains within the low range. Such shifts are expected because cranberry preparations vary substantially in phenolic composition and degree of standardization, and because matrix-associated delivery can alter bioavailability. The smaller inhibition zone in our diffusion assay does not necessarily imply weak anti-caries utility; rather, it may reflect that cranberries' strongest effects manifest in biofilm and adherence models. Philip et al. (2019) reported inhibitory effects of berry extracts on *S. mutans* biofilms, supporting the interpretation that berry-derived actives can be particularly relevant in biofilm-associated contexts such as dental caries.

Pomegranate peel extract (POM); demonstrated the lowest direct antibacterial potency among single actives (MIC 1000 µg/mL; MBC >1000 µg/mL; zone ~11.8 mm). This is, nevertheless, consistent with published microdilution findings indicating that pomegranate peel extracts frequently require concentrations in the ~1 mg/mL to several range to inhibit *S. mutans*, with bactericidal endpoints sometimes not reached within the same range. Alparslan et al. (2023) reported MIC values ≥1024 µg/mL for pomegranate peel extract against oral microorganisms, closely matching our MIC (1000 µg /mL). Additional formulations (e.g., pomegranate peel–based oral products) likewise support that inhibitory concentrations may fall in the low mg/mL range *in vitro,* depending on extract type and delivery system (Philip et al., 2019). From a mechanistic standpoint, pomegranate peel is rich in hydrolysable tannins and other polyphenols that can affect bacterial enzymes, metal availability, and adhesion-related functions, but the translation to rapid killing in broth can be limited by solubility, polymer binding, and assay conditions. Thus, POM may be most valuable as a complementary anti-virulence component rather than a stand-alone bactericide in a strip formulation.

Combination (COMB); formulation yielded the strongest antimicrobial response (MIC 62.5 µg/mL total actives; MBC 125 µg/mL; zone ~22.9 mm). Even though no identical combination study exists, the observed potency gain relative to single agents is consistent with the essential oil/extract synergy literature. Angane et al. (2024) demonstrated that pairing essential oils and extracts can generate synergistic interactions (assessed by checkerboard methodologies) and can reduce the effective concentrations needed for inhibition and killing. Likewise, Rezvani et al. (2017) reported enhanced anti-*S. mutans* activity for honey–cinnamon combinations compared with individual components, illustrating the broader principle that multi-component natural systems can improve antimicrobial outcomes via complementary mechanisms. This study should be interpreted as preliminary. First, essential oils and polyphenol extracts can show assay-dependent performance; disk diffusion is influenced by diffusion kinetics and hydrophobicity, while MIC/MBC depends on dispersion stability and adsorption to plastics or media components. Second, only planktonic endpoints were quantified here; because dental caries is fundamentally a biofilm-associated disease, follow-on experiments should include biofilm biomass, EPS

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quantification, and adhesion assays to capture the anti-virulence strengths of cranberry and pomegranate components. Finally, to support oral-strip applicability, future work should quantify release kinetics from the strip, evaluate stability of key actives, and assess cytocompatibility with relevant oral cell models and *in vivo* observation at realistic exposure times.

As a conclusion; the starch-based film matrix itself showed no antimicrobial effect, confirming that inhibition was driven by the incorporated plant actives. Among single agents, clove essential oil provided the strongest suppression of *S. mutans* and the most reliable bactericidal performance, while CIN and CRN produced meaningful inhibitory activity at moderate concentrations. Pomegranate peel extract required higher doses to achieve growth control and did not reach a clear bactericidal endpoint within the tested range, suggesting a secondary role focused on virulence modulation. Most importantly, the COMB delivered the lowest MIC/MBC values and the largest inhibition zones despite each component being below its stand-alone MIC, indicating additive or synergistic interactions between essential oils and polyphenols. These findings support using clove and cinnamon as the core antimicrobial drivers, retaining cranberry and pomegranate to strengthen biofilm/acidogenicity control, and prioritizing the combined formulation for validation in more representative oral biofilm models *in vivo*. Main highlights;

- Inert carrier: starch–glycerol film alone did not inhibit S. mutans.
- Potency ranking (single actives): CLO > CIN ≈ CRN > POM for planktonic susceptibility outcomes.
- Extract contribution: CRN and POM provide measurable antibacterial effects and are expected to support anti-biofilm/anti-virulence activity.
- Best formulation: COMB achieved superior efficacy at reduced per-component doses, consistent with synergy/additivity.
- Formulation decision: select CLO + CIN as primary actives; keep CRN + POM as supportive modifiers; advance COMB to complex biofilm/enamel-relevant testing.

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Conflict of Interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this original study.

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COMPARISON OF THE MORPHOLOGICAL AND KARYOLOGICAL CHARACTERISTICS OF *CENTAUREA ANTHEMIFOLIA* AND *C. SIPYLEA* (COMPOSITAE)

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Abstract: This study presents a comparative evaluation of the morphological and karyological characteristics of *Centaurea anthemifolia* Hub.-Mor. and *C. sipylea* Wagenitz, two closely related species belonging to the family Compositae. Although the morphological features of both taxa have been previously documented, their karyological properties are reported here for the first time. Detailed morphological examinations revealed clear diagnostic differences between the species, particularly in stem indumentum, leaf segmentation, involucre size and shape, appendage morphology, and pappus length. *C. anthemifolia* is characterized by its dwarf habit, dense white-tomentose stems, narrower involucres, more numerous cilia on the appendages, and shorter floral and pappus structures, whereas *C. sipylea* exhibits a taller stature, slightly arachnoid indumentum, larger ovoid involucres, fewer cilia, conspicuous hyaline auricles, and longer florets and pappus. Karyological analyses demonstrated that both species share the same diploid chromosome number (2n = 18, x = 9). However, notable differences were identified in chromosome morphology and karyotype asymmetry indices. *C. anthemifolia* exhibits a more heterogeneous and asymmetric karyotype (7m + 2sm; Stebbins 4B), while *C. sipylea* shows a more symmetrical chromosomal structure (8m + 1sm; Stebbins 4A). Additional asymmetry parameters (TF%, AsK%, A1–A2, CVcl, CVci) consistently indicate greater chromosomal variability in *C. anthemifolia*. Overall, the combined morphological and cytogenetic evidence clearly differentiates the two species. These findings contribute to a better understanding of species delimitation within *Centaurea* and provide valuable cytotaxonomic data for future evolutionary and systematic studies.

Key Words: Asteraceae, chromosome, endemic, knapweed

1. Introduction

The genus *Centaurea*, comprising approximately 650 species worldwide, is the largest genus within the subtribe Centaureinae [1]. Türkiye is one of the primary centers of diversity for *Centaurea* [2], and with the most recent publications, the number of taxa has reached 247 [3-9]. Of these taxa, 145 are endemic to Türkiye, yielding an endemism rate of 58.7%. Chromosomal evolution is a driving force underlying speciation in plants. Chromosome numbers, morphology, nuclear DNA amount, and composition exhibit great variation among plant species. In recent years, new tools have been developed that provide improved insights into chromosomal and genome evolution in plants, particularly in chromosome counting and the construction of karyotype structure. Among the most significant of these tools is next-generation sequencing (NGS) technology, which has revolutionized all biological disciplines in recent years [10]. Genome surveying and whole-genome sequencing have elevated classical cytogenetics to a new level within modern evolutionary cytogenomics [11]. Comparative plant (cyto)genomics has enabled the physical localization of DNA sequences on chromosomes and has established frameworks at both whole-genome and chromosomal scales. At the same time, it has provided new insights into nuclear repeats, chromosome structures, mechanisms of chromosomal rearrangements, and interphase chromosome organization [10]. Chromosomal evolution is a driving force underlying speciation in plants. Chromosome numbers, morphology, nuclear DNA amount, and composition exhibit great variation among plant species.

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The main objectives of this study can be summarized as follows: To perform a more detailed comparison of *C. anthemifolia* and *C. sipylea* using morphological and karyological data and to determine the chromosome numbers and morphologies of the species, thereby confirming the basic chromosome number with greater reliability and elucidating the significance of karyomorphological characters in distinguishing the species.

2. Material and Methods

The localities of the studied taxa are as follows: *C. anthemifolia* Türkiye: Konya; Halkapınar, Kayasaray village, around Düğünlük Stream, rocky slopes, 1691 m, 19.vii.2025, *E. Şirin* 1000. *C. siylea* Türkiye: Manisa; Mount Spil, southeastern slopes, rubble-strewn slopes, 1017 m, 15.vii.2025, *E. Şirin* 993 *Ö. Çeçen.* For the karyological investigation, fully developed achenes were chosen and germinated periodically. Somatic metaphase chromosomes were examined using the squash preparation method, with metaphase plates obtained from primary root meristem tissues. Prior to fixation, the root tips were treated with 0.002 M 8-hydroxyquinoline for 8 hours at 4 °C and subsequently fixed in Carnoy's solution for 24 hours at the same temperature. The material was then hydrolyzed in 5 N HCl for 30 minutes at room temperature and stained with 1% aceto-orcein. Permanent preparations were made according to the procedure described by [12]. For each species, at least ten well-defined metaphase spreads were evaluated, and high-quality images were taken at 100× magnification with an Olympus DP-72 digital camera attached to an Olympus BX53 microscope. The chromosomal terminology follows the system of [13], with metacentric and submetacentric chromosomes indicated as m and sm, respectively. Karyotype asymmetry was evaluated using several parameters, including the mean centromere index (CI), the ratio between the smallest and largest chromosomes, and the A1 and A2 asymmetrical indices. In addition, values for total form percentage (TF%; 14), the Arano karyotype asymmetry index (AsK%; 15), Romero-Zarco's interchromosomal and intrachromosomal asymmetry indices (A1–A2; 16), the coefficient of variation in chromosome length (CVcl; 17), as well as Stebbins' karyotype classification [18] were calculated. Idiograms for each taxon were prepared using the KaryoMeasure analysis software [19].

3. Results and discussion

The morphological characteristics of the studied species are as follows:
C. anthemifolia: Perennial with woody base, 10-20 cm. Stem erect-ascending, densely white tomentose, usually branched in upper part, 1.5-2 mm diameter in base. Leaves white tomentose; basal and lower 2-pinnatipartite with up to 8 pairs of linear segments, shortly petiolate, 16-55 x 8-18 mm, median stem leaves

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pinnatipartite with fewer segments, 12-18 x 6-12 mm. Involucre oblong, 8.5-12 x 3.5-6 mm. Bracts usually with 3-4 series. Appendages patent, straw coloured and often purple-tinged, with 5-8 pairs of cilia (1.4-2.2 mm), ending in a 2.5-4 mm spinule, outer appendages ovate-orbiculare, 3.5-4 x 1.5-2 mm, median appendages ovate-orbiculare, 7-7.5 x 1.5 mm, inner appendages linear-lanceolate, 8-8.5 x 1-1.5 mm. Flowers rose-purple, marginal 11-11.5 mm, lobes 5.5-6.5 mm, central flowers 9 mm, lobes 5-5.5 mm, anther tubes white and usually exceed the styles. Achene 3-3.5 x 1-1.5 mm, oblong, blackish brown, rounded at base, hilum lateral basal. Pappus scabrose, creamy white, outer pappus 2-2.5 mm, inner pappus 1-1.2 mm (Figure 1).

C. sipylea: Perennial, stem 26-50 cm. Stem erect-ascending, slightly arachnoid, usually branched in below and above, 2 mm diameter in base. Leaves arachnoid; basal and lower interruptedly 2-pinnatipartite with numerous oblong to lanceolate legments, 17-20 x 2-4 mm, median stem leaves pinnatipartite with linear segments, 10-40 x 5-10 mm. Involucre ovoid, 11-13 x 7-8 mm. Bracts usually with 3-4 series. Appendages straw coloured to light brown, decurrent with large hyaline auricles, with 3-5 pairs of cilia (1.4-2.1 mm), ending in a 1.5-2 mm spinule, outer appendages ovate-orbiculare, 5-6 x 1.5 mm, median appendages ovate-orbiculare, 7.5-8 x 1.5-2 mm, inner appendages linear-lanceolate, 10-11 x 1 mm. Flowers rose-purple, marginal 13.5-14 mm, lobes 5.5-6 mm, central flowers 11-12 mm, lobes 6.5-7 mm, anther tubes white and usually exceed the styles. Achene 3-3.5 x 1.5 mm, oblong, blackish brown, rounded at base, hilum lateral basal. Pappus scabrose, creamy white, outer pappus 2.5-3 mm, inner pappus 1.5-2 mm (Figure 2). Wagenitz (1975) noted that *C. sipylea* differs from *C. anthemifolia* in having stems 25–50 cm long (not 15–20 cm), an ovoid involucre measuring 12–13 × 7–8 mm (not rectangular, 8–9 × 3.5 mm), and appendages bearing 3-4 pairs of cilia on each side (not 5-7) [20]. *C. anthemifolia* is readily distinguished from *C. sipylea* by its markedly dwarf habit (10–20 cm tall) and densely white-tomentose stems, in contrast to the considerably taller stature (26–50 cm) and slightly arachnoid indumentum of *C. sipylea*. The leaves of *C. anthemifolia* are white-tomentose and possess fewer, strictly linear segments, whereas *C. sipylea* exhibits arachnoid leaves with numerous oblong to lanceolate segments, especially on the basal parts. The involucre of *C. anthemifolia* is smaller and oblong (8.5–12 × 3.5–6 mm), while that of *C. sipylea* is distinctly ovoid and larger (11–13 × 7–8 mm).

The appendages also provide clear diagnostic differences. In *C. anthemifolia*, the appendages bear 5–8 pairs of cilia and end in a longer spinule (2.5–4 mm), whereas *C. sipylea* has only 3–5 pairs of cilia and a shorter spinule (1.5–2 mm). Moreover, *C. sipylea* is characterized by appendages that are decurrent with conspicuous hyaline auricles—a feature absent in *C. anthemifolia*. The outer appendages of *C. sipylea* are notably larger (5–6 mm) than those of *C. anthemifolia* (3.5–4 mm). Floral characters also separate the two species: *C. sipylea* has significantly longer marginal florets (13.5–14 mm) and larger central florets (11–12 mm), while *C. anthemifolia* exhibits shorter marginal (11–11.5 mm) and central florets (9 mm). Pappus length further reinforces the distinction, being consistently longer in *C. sipylea* (outer pappus 2.5–3 mm; inner 1.5–2 mm) than in *C. anthemifolia* (outer 2–2.5 mm; inner 1–1.2 mm). In summary, *C. anthemifolia* is a smaller, densely tomentose species with narrower involucres, more numerous cilia on the appendages, and shorter florets and pappus; whereas *C. sipylea* is a taller species with larger involucres, fewer cilia on the appendages, prominent hyaline auricles, and longer florets and pappus structures (Table 1).

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Table 1. Comparative morphological characters of *C. anthemifolia* and *C. sipylea*

Character	*C. anthemifolia*	*C. sipylea*
Habit	10–20 cm, dwarf	26–50 cm, tall
Stem indumentum	Dense white tomentose	Slightly arachnoid
Lower leaves	Fewer linear segments	Numerous oblong-lanceolate segments
Involucre	8.5–12 × 3.5–6 mm, oblong	11–13 × 7–8 mm, ovoid
Appendage cilia	5–8 pairs	3–5 pairs
Appendage spinule	2.5–4 mm	1.5–2 mm
Hyaline auricles	Absent	Present and large
Outer appendages	Smaller (3.5–4 mm)	Larger (5–6 mm)
Marginal florets	11–11.5 mm	13.5–14 mm
Pappus	Shorter	Longer

This investigation is the first chromosome count and morphology report of the *C. anthemifolia*. Karyological data and asymmetry values are as follows: 2n=18, x=9, PL=2x, HCL=13.75, TF%=42.22, AsK%=57.77, S%=47.05, KF=7m+2sm, AI=3.48, A1=0.26, A2=0.22, X_{ca}=16.43, X_{ci}=0.41, CV_{cl}=22.95, CV_{ci}=15.17, Stebbins=4B (Figure 3). Similiarly this investigation is the first chromosome count and morphology report of the *C. sipylea*. Karyological data and asymmetry values are as follows: 2n=18, x=9, PL=2x, HCL=12.66, TF%=43.32, AsK%=56.67, S%=60.63, KF=8m+1sm, AI=1.28, A1=0.22, A2=0.14, X_{ca}=12.82, X_{ci}=0.43, CV_{cl}=14.96, CV_{ci}=8.58, Stebbins=4A (Figure 4). A comparative assessment of the karyological characteristics of *C. anthemifolia* and *C. sipylea* reveals both shared cytological patterns and notable differences in chromosomal morphology and asymmetry. For both taxa, this study provides the first chromosome counts and morphological descriptions, confirming the same diploid chromosome number (2n = 18, x = 9) and identical ploidy level (2x). Despite this shared basic cytogenetic structure, the two species exhibit distinct karyotype profiles. *C. anthemifolia* possesses a slightly higher haploid chromosome length (HCL = 13.75) compared with *C. sipylea* (HCL = 12.66), indicating marginally longer chromosomes overall. The karyotype formula also differs, with *C. anthemifolia* exhibiting 7m + 2sm chromosomes, whereas *C. sipylea* shows 8m + 1sm, suggesting a somewhat more symmetrical karyotype in the latter.

Karyotype asymmetry indices further support these differences. *C. anthemifolia* displays higher asymmetry, reflected in AsK% (57.77 vs. 56.67), S% (47.05 vs. 60.63), A1 (0.26 vs. 0.22), A2 (0.22 vs. 0.14), CV_{cl} (22.95 vs. 14.96), and CV_{ci} (15.17 vs. 8.58). The greater variability in chromosome length (CV_{cl}) and centromeric index (CV_{ci}) in *C. anthemifolia* indicates a more heterogeneous chromosome set. Conversely, *C. sipylea* exhibits lower asymmetry across multiple indices and a lower asymmetry index (AI = 1.28) than *C. anthemifolia* (AI = 3.48), underscoring its more balanced chromosomal architecture. Total form percentage (TF%) and Arano's asymmetry index (AsK%) differ only slightly between the taxa yet consistently point to a moderately more symmetrical karyotype in *C. sipylea*. Stebbins' classification further distinguishes the species, with *C. anthemifolia* falling into category 4B and *C. sipylea* into 4A, again indicating reduced asymmetry in the latter. Overall, although *C. anthemifolia* and *C. sipylea* share the same chromosome number and ploidy level, *C. sipylea* possesses a more symmetrical and less variable karyotype, whereas *C. anthemifolia* is characterized by higher chromosomal heterogeneity and asymmetry. These differences may reflect species-specific evolutionary trajectories within the genus *Centaurea*. Uysal et al. (2017) investigated the karyomorphology of 31 taxa belonging to the sections *Centaurea* and *Phalolepis* of

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the genus *Centaurea* and reported a basic chromosome number of x = 9 for all taxa except *C. hierapolitana*. The basic chromosome number was also determined as x = 9 for the species *C. mengenensis*, *C. ertugruliana*, *C. sakariyaensis*, and *C. pinetorum*, which are included in section *Centaurea* [21, 22, 6]. The basic chromosome number of *C. anthemifolia* and *C. sipylea* was determined as x = 9, consistent with related *Centaurea* species.

Figure 1. *C. anthemifolia* **a)** General habit, **b)** Flower

Figure 2. *C. sipylea* **a)** General habit, **b)** Flower

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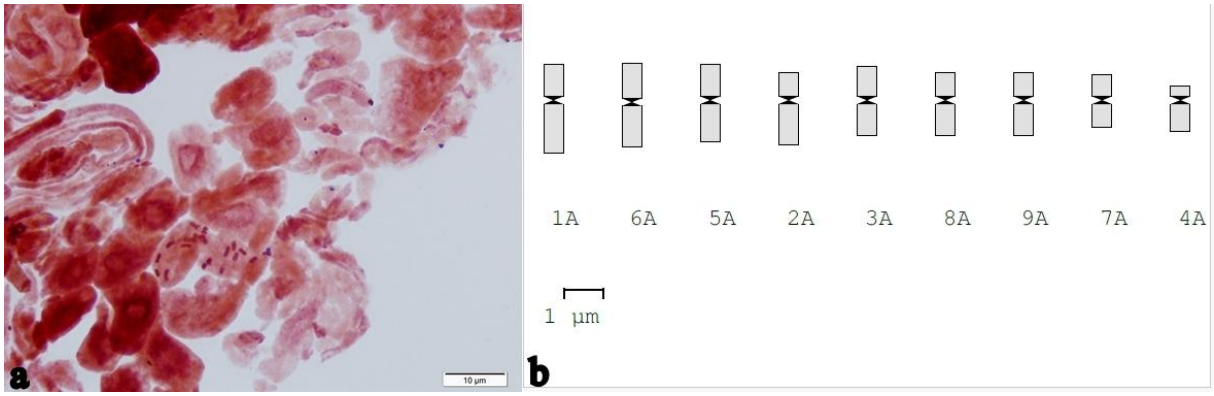

Figure 3. Metaphase (a) and idiogram (b) of *C. anthemifolia*

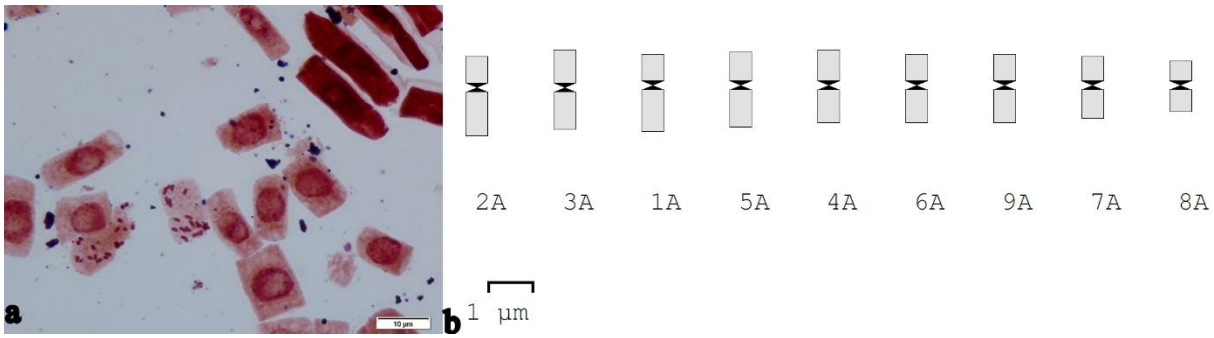

Figure 4. Metaphase (a) and idiogram (b) of *C. sipylea*

4. Conclusion

While the morphological characteristics of *C. anthemifolia* and *C. sipylea* have been examined in detail, their karyological features have been investigated here for the first time.

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Conflict of Interest

There is no conflict of interest.

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GENE-TARGET INTERACTION PROFILES OF BOSWELLIC ACID AND ITS DERIVATIVES: INSIGHTS INTO EURODEGENERATIVE DISEASES AND OXIDATIVE STRESS PATHWAYS

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Abstract: This study analyzes gene–chemical interaction data retrieved from the CTD database to explore the molecular targets of boswellic acid and its major derivatives—including 11-keto-boswellic acid and acetyl-11-keto-boswellic acid—in the context of neurodegenerative disorders. The results indicate that 11-keto-boswellic acid exhibits the highest density of interactions with apoptosis-related genes such as CASP3, CASP8, CASP9, and CYCS, which play central roles in pathways shared by Alzheimer's disease, Parkinson's disease, and oxidative damage responses. The acetylated derivative additionally targets genes involved in cell-cycle regulation (CDKN1A, CDKN1B), apoptosis modulation (BAX, BCL2), autophagy (ATG5, BECN1), and antioxidant defense (GSR, CYP1A1), suggesting a broader neuroprotective potential. In contrast, non-derivatized boswellic acid demonstrates minimal gene-level interactions. Collectively, the findings highlight that the more bioactive boswellic derivatives may serve as promising modulators of inflammation, oxidative stress, and neuro-apoptotic pathways in neurodegenerative diseases.

Key Words: Boswellic acids; Neurodegeneration; Oxidative stress

1. Introduction

Boswellic acids, a group of pentacyclic triterpenoids derived from the oleo-gum resin of Boswellia serrata, have attracted considerable attention due to their broad spectrum of pharmacological activities. Phytochemical investigations have identified several major bioactive constituents in B. serrata resin, including boswellic acid, α-boswellic acid, 11-keto-boswellic acid (KBA), and acetyl-11-keto-boswellic acid (AKBA), which are considered the primary contributors to its therapeutic effects (Siddiqui, 2011; Iram et al., 2017; Salati, 2024; Sonkar & Gupta, 2024). Among these, KBA and AKBA are recognized as the most biologically potent derivatives due to their enhanced anti-inflammatory and molecular regulatory properties. Boswellic acids have emerged as promising therapeutic agents in neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) owing to their multi-target activities. These compounds exhibit potent anti-inflammatory, antioxidant, and apoptosis-regulating properties, which are critical in modulating the complex and multifactorial pathogenic pathways involved in these diseases (Haghaei et al., 2020; Rajabian et al., 2020). Neurodegenerative disorders are characterized by progressive neuronal dysfunction driven by interactions among oxidative stress, neuroinflammation, and protein aggregation. The ability of boswellic acids to simultaneously target multiple pathological mechanisms positions them as attractive candidates for drug development.

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Specifically, boswellic acids can modulate inflammatory signaling pathways by inhibiting pro-inflammatory cytokines and enzymes, thereby attenuating neuroinflammation. In parallel, their antioxidant properties contribute to the reduction of oxidative stress, a major factor in neuronal damage and functional decline in AD and PD (Haghaei et al., 2020; Chen et al., 2021). Moreover, these compounds have been reported to influence key neurodegenerative processes, including tau and β-amyloid aggregation, stabilization of microtubule dynamics, reduction of amyloid plaque formation, and enhancement of cholinergic neurotransmission through acetylcholinesterase inhibition, collectively supporting cognitive function (Haghaei et al., 2020). Preclinical studies, both in vitro and in vivo, have provided substantial evidence supporting the neuroprotective effects of boswellic acids by modulating pathways relevant to AD and PD, reinforcing their potential as lead compounds for further drug development (Haghaei et al., 2020). Nevertheless, despite encouraging experimental findings, comprehensive clinical evaluations remain limited, and further clinical trials are required to confirm their safety and therapeutic efficacy in humans (Andrade et al., 2024). Given the complexity of neurodegenerative disease pathogenesis, a deeper mechanistic understanding and exploration of potential synergistic effects between boswellic acids and other natural compounds are essential to optimize their neuroprotective efficacy in clinical settings (Majhi & Singh, 2024; Andrade et al., 2024).

Among the identified boswellic acid derivatives, 11-keto-boswellic acid and its acetylated form, acetyl-11-keto-boswellic acid, are considered more pharmacologically active than the parent boswellic acid structure. AKBA, in particular, has demonstrated pronounced neuroprotective properties, including the promotion of nerve repair and protection against ischemic brain injury (Gong et al., 2022). At the molecular level, AKBA modulates gene expression by inhibiting NF-κB activation, a central regulator of inflammatory and apoptotic signaling pathways, providing a mechanistic basis for its therapeutic effects (Takada et al., 2006). Despite these promising attributes, the extent of gene-level interactions mediated by KBA and AKBA in neurodegenerative pathways remains insufficiently characterized. In this study, we utilized curated chemical–gene interaction data from the Comparative Toxicogenomics Database (CTD) to systematically map gene targets associated with boswellic acid and its derivatives, with a particular focus on genes implicated in Alzheimer's disease, Parkinson's disease, oxidative stress responses, and neurodegeneration with brain iron accumulation (NBIA). By identifying shared and compound-specific molecular targets, this analysis aims to provide novel insights into the multi-target neuroprotective potential of boswellic acids and to establish a molecular framework for future mechanistic and therapeutic investigations.

2. Materials and Methods

2.1. Identification of Active Phytochemical Constituents

The major chemical constituents of B. serrata resin were identified through a comprehensive search from the Comparative Toxicogenomics Database (CTD). The names of compounds associated with B. serrata were extracted from each database, and duplicate entries were removed. Based on this screening, the principal boswellic acid derivatives, including boswellic acid, 11-keto-boswellic acid, acetyl-11-keto-boswellic acid (AKBA), and α-boswellic acid, were selected for further analysis (Table 1).

2.2. Gene–Compound Interaction Analysis

To identify human genes interacting with the selected compounds, data were obtained from the Comparative Toxicogenomics Database (CTD). Each compound was searched individually in the database,

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and the corresponding gene–chemical interaction datasets were retrieved. Genes related to oxidative stress, apoptosis, and neurodegenerative pathways were selected for downstream analysis.

3. Result and discussion

3.1. Identification of Major Bioactive Compounds in Boswellia Resin

Phytochemical analysis of B. serrata resin, based on data retrieved from the Comparative Toxicogenomics Database (CTD), revealed that boswellic acids are the primary bioactive constituents of the resin. Among these, the most prominent and pharmacologically relevant compounds include 11-keto-boswellic acid (KBA), acetyl-11-keto-boswellic acid (AKBA), and α-boswellic acid. These pentacyclic triterpenoids are widely recognized for their anti-inflammatory, antioxidant, and therapeutic properties and are considered responsible for many of the medicinal effects attributed to B. serrata (Siddiqui, 2011; Iram et al., 2017; Sonkar & Gupta, 2024). 11-keto-boswellic acid exhibits significant anti-inflammatory activity through the inhibition of key inflammatory mediators, contributing to the overall therapeutic potential of Boswellia resin (Salati, 2024). Acetyl-11-keto-boswellic acid (AKBA), an acetylated derivative of KBA, is regarded as the most potent boswellic acid, particularly due to its strong inhibitory effect on 5-lipoxygenase (5-LOX), a central enzyme involved in leukotriene-mediated inflammation and oxidative stress (Siddiqui, 2011). AKBA has also been reported to exert protective effects in various pathological conditions, including neurodegenerative disorders and organ injury, highlighting its relevance in both traditional and modern therapeutic contexts (Table 1, Sharifi et al., 2023).

Although α-boswellic acid has been less extensively studied compared to KBA and AKBA, it contributes to the overall pharmacological profile of B. serrata and may act synergistically with other boswellic acids to enhance therapeutic efficacy (Sonkar & Gupta, 2024). Traditionally, B. serrata has been used in Ayurvedic and Unani medicine for the treatment of inflammatory diseases, respiratory disorders, and chronic conditions, a practice that is increasingly supported by contemporary pharmacological studies (Jana et al., 2020).

3.2. Gene–Compound Interaction Analysis

Analysis of chemical–gene interactions revealed that 11-keto-boswellic acid and acetyl-11-keto-boswellic acid (AKBA) interact with a broad network of genes involved in key cellular processes relevant to neurodegeneration. Notably, several apoptosis-related genes, including CASP3, CASP9, BAX, BCL2, and CYCS, were identified as major targets, indicating a potential role of these compounds in regulating mitochondrial-dependent apoptotic pathways. In addition, autophagy-associated genes such as ATG5 and BECN1 were found to be modulated, suggesting that boswellic acid derivatives may influence neuronal survival through the regulation of autophagic flux. Furthermore, interactions with genes implicated in oxidative stress response and neuroinflammation support the hypothesis that these compounds exert neuroprotective effects via multi-pathway modulation. Collectively, these gene–compound interactions provide a molecular framework explaining the multi-target therapeutic potential of 11-keto-boswellic acid and AKBA in neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Table 1).

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Table 1. Human genes targeted by boswellic acids in neurodegeneration and oxidative stress

No	Active Boswellic Compounds	Human Target Genes	Genes Involved in Alzheimer's Disease	Genes Involved in Parkinson's Disease	Genes Related to Oxidative Damage Response	Genes Associated with Neurodegeneration with Brain Iron Accumulation (NBIA)
1	boswellic acid	AKT1	-	-	-	-
2	boswellic acid	EGFR	-	-	-	-
3	boswellic acid	TOP1	-	-	-	-
4	boswellic acid	TOP2A	-	-	-	-
5	boswellic acid	VEGFA	-	-	-	-
6	11-keto-boswellic acid	CASP1	-	-	-	-
7	11-keto-boswellic acid	CASP10	-	-	-	-
8	11-keto-boswellic acid	CASP2	-	✔	-	-
9	11-keto-boswellic acid	CASP3	✔	✔	✔	
10	11-keto-boswellic acid	CASP6	-	✔	-	-
11	11-keto-boswellic acid	CASP8	✔			
12	11-keto-boswellic acid	CASP9	✔	✔	✔	
13	11-keto-boswellic acid	CYCS	✔	✔	✔	
14	11-keto-boswellic acid	TOP1	-	-	-	-
15	11-keto-boswellic acid	TOP2A	-	-	-	-
16	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CDKN1A	-	-	✔	-
17	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CDKN1B	-	-	✔	-
18	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	BAX	✔			
19	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CCNA2	-	-	-	-
20	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CDK2	-	-	-	-
21	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CDK4	-	-	-	-
22	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	MMP1	-	-	-	-
23	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	MMP2	-	-	-	-
24	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	MMP9	-	-	-	-
25	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	ATG5	-	-	-	✔
26	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	BCL2L1	-	-	-	
27	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	BECN1	-	-	-	✔
28	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CAT	-	-	-	-
29	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CCNE1	-	✔	-	-

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30	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CDK1	-	-	-	-
31	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	GSR	-	-	-	-
32	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	SQSTM1	-	-	-	-
33	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	BCL2	✔		✔	-
34	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CASP3	✔	-	-	-
35	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CCND2	-	-	-	-
36	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CEACAM20	-	-	-	-
37	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	CYP1A1	-	-	✔	-
38	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	IL1B	-	-	-	-
39	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	MAPK1	-	-	-	-
40	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	MAPK3	-	-	-	-
41	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	PARP1	-	-	-	-
42	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	TNF	-	-	-	-
43	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	TOP1	-	-	-	-
44	acetyl-11-ketoboswellic acid(Equivalent Term: 3-O-acetyl-11-keto-boswellic acid)	TOP2A	-	-	-	-
45	alpha-boswellic acid	GSR	-	-	✔	-
46	alpha-boswellic acid	IL1B	-	-	-	-
47	alpha-boswellic acid	TNF	✔	-	✔	-

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4. Conclusion

The present analysis confirms that boswellic acids constitute the major bioactive components of Boswellia serrata resin, with 11-keto-boswellic acid (KBA), acetyl-11-keto-boswellic acid (AKBA), and α-boswellic acid representing the most pharmacologically relevant compounds. Among these, KBA and AKBA exhibit superior biological activity, particularly due to their strong anti-inflammatory and antioxidant properties and their ability to modulate key molecular pathways associated with neurodegeneration. Gene–compound interaction analysis revealed that KBA and AKBA interact with a wide range of genes involved in apoptosis, autophagy, oxidative stress response, and neuroinflammation, including critical regulators such as CASP3, CASP9, BAX, BCL2, CYCS, ATG5, and BECN1. These interactions suggest that the neuroprotective effects of boswellic acid derivatives are mediated through multi-target regulation of pathways essential for neuronal survival, mitochondrial function, and cellular homeostasis. Collectively, these findings provide a molecular basis for the traditional and emerging therapeutic applications of Boswellia serrata and highlight 11-keto-boswellic acid and AKBA as promising lead compounds for further mechanistic studies and the development of multi-target therapeutic strategies against neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.

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STUDY ON PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF THAI MULBERRY LEAVES

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Abstract: Mulberry leaves (*Morus alba* L.) are used in many traditional remedies and health products because they contain natural phenolic compounds and have antioxidant activity. In Thailand, mulberry is grown in several regions, but there is not much simple information comparing the leaves from different areas. In this study, we examined the basic phenolic content and antioxidant activity of leaves collected from the northern, central, and northeastern parts of Thailand. We used a hydroalcoholic extraction and simple in-vitro tests. The early results showed that the leaves from different regions and cultivars were not the same, and some samples demonstrated stronger antioxidant properties. From the study, we found that the cultivar from Sakon Nakhon gave an interesting result in terms of antioxidant activity (0.43 mg/mL) in the 2nd round of extraction, while the Khon Kaen cultivar showed a significantly higher total phenolic content of 24.65 mg gallic acid equivalents (GAE) per gram of sample in the 2nd round of extraction. These findings suggest that Thai mulberry leaves may be considered as potential natural ingredients for herbal, health, and cosmetic products. Their in-vitro antioxidant activity indicates a possible relevance for applications related to oxidative stress and skin protection; however, these potential uses were not evaluated in this study and should be investigated in future research. More detailed studies can be conducted in the future.

Keywords: Antioxidant, Mulberry, Phenolic Compounds, Phytochemicals, Thailand

1. Introduction

Mulberry (*Morus alba* L.) leaves have long been used in traditional Asian remedies and are also used in modern health-related products. Previous studies have reported that mulberry leaves contain various phytochemical compounds, especially phenolic compounds, which are related to antioxidant activity and may help reduce oxidative stress (Kim et al., 1999). These compounds have been studied for their free-radical scavenging ability and possible use in herbal, nutraceutical, and cosmetic products. Mulberry leaves have also been reviewed as an important source of bioactive compounds, particularly phenolics and flavonoids, which contribute to their antioxidant and health-related properties (Gryn-Rynko et al., 2016). Phenolic compounds are commonly evaluated using standard antioxidant assays, such as the Folin-Ciocalteu method for total phenolic content and the DPPH radical scavenging assay, which are widely used in plant-based antioxidant studies. In Thailand, mulberry is grown in many regions and is mainly used for sericulture and herbal purposes. However, there is still limited information comparing the phenolic content and antioxidant activity of mulberry leaves collected from different regions of Thailand. Differences in growing conditions, such as climate, soil, and farming practices, may affect the phytochemical composition of mulberry leaves. Therefore, studying samples from different regions may help explain these variations (Zou et al., 2012 ,Kim et al., 2014; Jelen et al., 2025). Mulberry leaves have also been reviewed as an important source of bioactive compounds, particularly phenolics and flavonoids, which contribute to their antioxidant potential.

The aim of this study is to investigate the total phenolic content and *in vitro* antioxidant activity of mulberry leaves collected from the northern, central, and northeastern regions of Thailand. All samples

were extracted using the same hydroalcoholic method to allow comparison between cultivars. The results of this study provide basic information that may support future use of Thai mulberry leaves as natural ingredients for herbal, health, and cosmetic products.

2. Materials and Methods

2.1. Plant Materials

Mulberry (*Morus* sp.) leaf samples were collected from pesticide-free farms in different regions of Thailand. Four Thai mulberry cultivars were used in this study, including Chiang Mai 60, Kamphaeng Saen, Buriram, and Sakon Nakhon. Fresh leaves were transported to the laboratory and prepared for extraction.

2.2. Extraction of Mulberry Leaf Samples

Mulberry leaves were washed with tap water and air-dried. After that, the leaves were oven-dried until they were completely dry. The dried leaves were ground into powder and sieved to obtain a uniform particle size. The powdered leaves were extracted using methanol at room temperature for a fixed extraction time. After extraction, the mixture was filtered using Whatman No. 1 filter paper. The extraction was repeated to increase the amount of extractable compounds. Extracts from each cultivar were collected separately. The filtrates were concentrated using a rotary evaporator to obtain crude extracts. The extracts were dried to constant weight, and the extraction yield was calculated based on the dry weight of the extract compared to the dry weight of the leaf powder. All crude extracts were stored at -20°C until analysis.

2.3. Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of mulberry leaf extracts was determined using the Folin-Ciocalteu method, with slight modification from Syukriya et al. (2023). The Folin-Ciocalteu method is a widely accepted standard for determining total phenolic content in plant extracts (Folin, O., & Ciocalteu, V., 1927). The crude extracts were dissolved in distilled water and mixed with Folin-Ciocalteu reagent, followed by sodium carbonate (Na_2CO_3). The reaction mixtures were kept in the dark at room temperature for 60 min. After incubation, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. All measurements were performed in triplicate. Gallic acid was used as a standard, and the results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

2.4. Antioxidant Activity Assay (DPPH Radical Scavenging Assay)

The antioxidant activity of mulberry leaf extracts was evaluated using the DPPH radical scavenging assay, modified from Syukriya et al. (2023). The DPPH assay is a commonly used method for evaluating free radical scavenging activity of plant-derived antioxidants (Blois, M. S.1958).The crude extracts were dissolved in methanol and prepared at five different concentrations. Each concentration was mixed with a DPPH solution in equal volumes.

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The mixtures were incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Methanol was used as the control. All measurements were carried out in triplicate.

The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = [(A\_DPPH - A\_sample) / A\_DPPH] \times 100$$

where A_sample is the absorbance of the sample and A_DPPH is the absorbance of the control. A graph of scavenging activity versus extract concentration was used to calculate the IC_{50} value, which was expressed in mg/mL. Gallic acid was used as a reference standard**.**

2.5. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Differences among mulberry leaf samples were analyzed using one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used to compare mean values at $P \leq 0.05$. Statistical analysis was carried out using standard statistical software.

3. Results and Discussion

3.1. Total Phenolic Content

The total phenolic content (TPC) of mulberry leaf extracts showed differences among cultivars and between extraction rounds (Table 1). In the first extraction, the Sakon Nakhon cultivar showed the highest TPC value (19.48 ± 1.02 mg GAE/g sample). This value was not significantly different from that of the Buriram cultivar (17.90 ± 0.21 mg GAE/g sample). In contrast, lower TPC values were observed in the Chiang Mai 60 and Kamphaeng Saen cultivars, with values of 6.69 ± 1.22 and 4.86 ± 0.65 mg GAE/g sample, respectively. In the second extraction, higher TPC values were found in all cultivars compared to the first extraction. The Sakon Nakhon cultivar again showed the highest phenolic content (21.73 ± 0.98 mg GAE/g sample), followed by Buriram (19.84 ± 0.96 mg GAE/g sample). Chiang Mai 60 and Kamphaeng Saen showed similar TPC values in the second extraction, and no significant difference was observed between these two cultivars.

Table 1. Total phenolic content (TPC) of mulberry (Morus sp.) leaf extracts from different cultivars and extraction rounds

Mulberry cultivar	First extraction (mg GAE/g sample)	Mulberry cultivar	Second extraction (mg GAE/g sample)
Sakon Nakhon	19.48 ± 1.02^{b}	Sakon Nakhon	21.73 ± 0.98^{b}
Buriram	17.90 ± 0.21^{bc}	Buriram	19.84 ± 0.96^{c}
Chiang Mai 60	6.69 ± 1.22^{e}	Chiang Mai 60	16.70 ± 1.20^{d}
Kamphaeng Saen	4.86 ± 0.65^{e}	Kamphaeng Saen	16.63 ± 1.23^{d}

Note: Values are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences ($P \leq 0.05$) according to Duncan's Multiple Range Test (DMRT).

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3.2. Antioxidant Activity - DPPH Assay

The antioxidant activity of mulberry leaf extracts, expressed as IC_{50} values, differed among cultivars and extraction rounds (Table 2). In the first extraction, the Sakon Nakhon cultivar showed the lowest IC_{50} value (0.74 ± 0.01 mg/mL), indicating stronger DPPH radical scavenging activity compared to the other samples. The Chiang Mai 60 cultivar showed moderate antioxidant activity, while the Kamphaeng Saen and Buriram cultivars showed higher IC_{50} values. In the second extraction, clearer differences in antioxidant activity were observed among the cultivars. The Sakon Nakhon extract again showed the strongest activity, with the lowest IC_{50} value (0.43 ± 0.01 mg/mL). The Buriram cultivar also showed improved antioxidant activity, with an IC_{50} value of 0.52 ± 0.01 mg/mL. In contrast, the Kamphaeng Saen cultivar showed the highest IC_{50} value (2.23 ± 0.03 mg/mL), indicating weaker antioxidant activity among the tested samples.

Table 2. IC_{50} values of mulberry (*Morus* sp.) leaf extracts determined by the DPPH radical scavenging assay

Mulberry cultivar	First extraction IC_{50} (mg/mL)	Mulberry cultivar	Second extraction IC_{50} (mg/mL)
Sakon Nakhon	0.74 ± 0.01^{e}	Sakon Nakhon	0.43 ± 0.01^{e}
Buriram	1.86 ± 0.05^{b}	Buriram	0.52 ± 0.01^{e}
Chiang Mai 60	1.48 ± 0.02^{d}	Chiang Mai 60	1.30 ± 0.05^{d}
Kamphaeng Saen	1.84 ± 0.04^{b}	Kamphaeng Saen	2.23 ± 0.03^{a}

*Data are sorted according to IC_{50} values of the second extraction.
Note: Values are presented as mean ± SD (n = 3). Different superscript letters within the same column indicate significant differences ($P \leq 0.05$) based on Duncan's Multiple Range Test (DMRT). Lower IC_{50} values indicate stronger antioxidant activity.

The present study showed that the total phenolic content (TPC) and antioxidant activity of mulberry (*Morus* sp.) leaf extracts were different among cultivars and extraction rounds. Clear differences in phenolic content were found between Thai mulberry cultivars. The Sakon Nakhon and Buriram cultivars generally showed higher TPC values than Chiang Mai 60 and Kamphaeng Saen. A similar pattern was found for antioxidant activity using the DPPH assay. Extracts from the Sakon Nakhon cultivar had the lowest IC_{50} values, which means they showed the strongest antioxidant activity. These results indicate that mulberry leaves from different cultivars have different phytochemical properties, which affect their antioxidant potential. Total phenolic content and antioxidant activity of mulberry leaf extracts were related, but this relationship was different among cultivars. In general, samples with higher phenolic content showed stronger antioxidant activity. This was shown by lower IC_{50} values in the DPPH assay. A similar relationship between phenolic content and antioxidant activity was also reported in previous studies on mulberry leaves (Kim et al., 1999; Zou et al., 2012).

This pattern was found in the Sakon Nakhon cultivar, which showed high total phenolic content and low IC_{50} values in both extraction rounds. Phenolic compounds can act as antioxidants by reacting with free radicals. Therefore, extracts with higher phenolic content may show stronger antioxidant activity. In this study, the second extraction showed higher total phenolic content than the first extraction. Some cultivars, such as Sakon Nakhon and Buriram, also showed better antioxidant activity in the second extraction. This suggests that doing the extraction more than one time may help extract more phenolic

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compounds from mulberry leaves. However, not all cultivars showed the same result. Some samples had similar total phenolic content but different antioxidant activity. This indicates that antioxidant activity may not depend only on the total amount of phenolic compounds. Other factors, such as the type or mixture of phenolic compounds, may also affect antioxidant activity.

Overall, the results suggest that total phenolic content can be used as a basic indicator of antioxidant activity in mulberry leaf extracts. However, more studies are needed to understand which compounds are responsible for antioxidant activity in different mulberry cultivars.

4.Conclusion

This study showed that mulberry (*Morus* sp.) leaf extracts from different Thai cultivars differed in total phenolic content and antioxidant activity. Among the samples tested, the Sakon Nakhon cultivar consistently showed higher phenolic content and stronger radical scavenging activity compared to the other cultivars. Differences in antioxidant properties were observed among cultivars and between extraction rounds, indicating that both cultivar type and extraction conditions may affect antioxidant potential. In general, higher total phenolic content was associated with stronger antioxidant activity. However, this relationship was not the same for all cultivars, suggesting that antioxidant activity may not depend only on the total amount of phenolic compounds. Other factors, such as the composition of phenolic compounds, may also influence antioxidant performance. These results provide basic scientific information that may support the selection of suitable mulberry cultivars as natural sources of antioxidants. Further studies focusing on the identification of specific phenolic compounds and additional biological evaluation are needed to better understand the potential use of mulberry leaf extracts in herbal, health, and cosmetic products.

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The Importance of Rosehip Cultivation in the Yozgat Province of Türkiye

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Abstract

Türkiye is in a position to enable growth due to its geographical and ecological regions and has a large amount of genetic diversity and many fruits, vegetables, vineyards and ornamental plants. The genus *Rosa* covers more than 100 species in the Northern Hemisphere, including the temperate and subtropical zones, and Anatolia is home to 27 of these native species. Rosehip grows naturally in almost every region of Türkiye, particularly in the central and northern Anatolian regions, such as Kastamonu, Çorum, Amasya, Tokat, Sivas, Gümüşhane, Erzincan and Erzurum. In Yozgat, 254 species belonging to 47 families have been identified, 62 of which are endemic. In Yozgat, summers are generally hot and dry, winters are cold and rainy, and there are significant temperature differences between night and day, as well as between summer and winter. In Yozgat, 254 species belonging to 47 families have been identified, 62 of which are endemic. Rosehip is a species that may have economic importance in Yozgat. It has a high nutritional value and grows naturally in all of Yozgat's provinces. However, rosehip is not suitable for fresh consumption or as a table fruit; it is an industrial fruit that can only be consumed after processing. It is used to make products such as rosehip marmalade, fruit juice and rosehip tea, which are produced in 20 different facilities across the country. As well as being used for their nutritional value, rosehips are used for medicinal purposes, as rootstock for roses, as landscape plants and hedgerows, to prevent erosion, and the waste fruits and their seeds are used as animal feed. Increasing cultivation will be possible by cultivating cultivars detected through selection in naturally grown populations and by establishing gardens. This will lead to the cultivation of productive rosehip varieties with economic importance.

Key Words: Rose hips, Yozgat, Türkiye, nutritive value, usage patterns

Introduction

Yozgat is located on the Bozok Plateau in the Central Kızılırmak section of the Central Anatolia Region of Türkiye. While the semi-arid continental climate of Central Anatolia prevails in the province, the climate becomes milder in the Çekerek Valley, which enters the Yeşilırmak basin, and the effects of the Black Sea climate are observed. Generally, summers are hot and dry; winters are cold and rainy, with high temperature differences between summer and winter, and between day and night. 254 species belonging to 47 families have been identified in Yozgat, 62 of which are endemic. In addition to endemic plants, apples, pears, plums, sour cherries, peaches, apricots, almonds, walnuts, quinces, local grapes, and viburnum are cultivated, while wild fruits such as hazelnuts, cranberries, linden, hawthorn, rosehip, salep, wild pear, and apple are found (Tübives, 2018). Türkiye is quite rich in terms of plant species diversity, possessing 10,000 different plant species, 33% of which are endemic (Davis, 1965; 1988). It has a rich variety of fruit species that have not yet been cultivated, such as rosehip, hawthorn, blackberry, blackthorn, sea buckthorn, carob, hackberry, mastic, and buttum, and is also one of the oldest centers of cultivation for many cultivated fruit species (Özbek, 1977). It is virtually a collection garden in terms of cultivated and edible wild fruit species. Most of the edible wild fruit species available in our country are already used by our people for various purposes (Ercişli and Eşitken, 2004).

Rosehip (*Rosa* spp.) has a natural distribution in Central and Western Asia, Europe, and North Africa.

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Within these areas, including Anatolia, it grows densely with a rich variety of species and genotypes (Nilson, 1997; İlisulu, 1992, Güneş, 2013). Looking at its natural distribution areas in Türkiye, rosehip grows in almost every region, but is particularly dense in the Central, Northern, and Eastern Anatolia regions (Güneş, 2013). The *Rosa* genus, to which rosehip belongs, has more than 100 species in the temperate and subtropical regions of the Northern Hemisphere, while 27 species grow in Türkiye (Nilson, 1997; Tübives, 2018).

Plants have been widely used for centuries to meet a significant portion of people's basic needs such as nutrition, heating, and shelter. The rapid increase in the world's population, the indiscriminate use of plant resources to meet daily needs, land clearing, the replacement of native (traditional) varieties with improved varieties, the use of herbicides, consuming plants by uprooting them instead of producing them, natural disasters, road and dam construction, and uncontrolled urbanization and industrialization are causing a decrease and rapid loss of plant genetic resources. Both the development of new varieties to increase agricultural production and the preservation of natural (wild) plant species as raw materials for future generations without erosion can only be achieved through the objective identification, collection, and conservation of existing plant diversity (Şehirali and Özgen, 1987; Ercişli and Eşitken, 2004; Tan, 2010). The world's population is projected to exceed 8 billion in 2030 and 9.9 billion in 2050 (Ray et al., 2013). This population increase is causing a significant rise in food demand, making it necessary to develop more efficient and disease and pest-resistant plant varieties in agricultural production. To meet the nutritional needs of the growing population, approximately 70% more food needs to be produced than is currently the case. To prevent fluctuations in production caused by biotic and abiotic stress conditions due to the increase in the human population and climate change, the development of new, high-yielding, environmentally stress-resistant, and nutrient-rich plant varieties is one of the most critical needs in agriculture (Charles et al., 2014; Abberton et al., 2016; Raina et al., 2018).

Rosehip (*Rosa canina* L.), belonging to the genus *Rosa* of the Rosaceae family, is a perennial shrub that grows naturally in valleys, along roadsides, and in garden borders (Bilgener et al., 1996). Rosehip grows naturally in a wide geographical area including Central and Western Asia, the Caucasus, Europe, Northwest Africa, northern and western parts of Iraq and Iran, northern Afghanistan, Pakistan, Central Asia, and Russia. Within the genus Rosehip, 35 species, three of which are endemic, are naturally distributed in the flora of Türkiye (Güner et al., 2012; Riek et al., 2013). In particular, it grows in large populations in its natural environment in the provinces of Kastamonu, Çankırı, Çorum, Amasya, Tokat, Sivas, and Erzurum (Ağaoğlu and Gerçekcioğlu, 2013).

Türkiye is one of the genetic centers of rosehip, and in recent years, its use for both nutritional and medicinal purposes has been increasing, making it a fruit species with significant economic value (Doğan and Kazankaya, 2006). Rosehip is not only valued for its nutritional or medicinal uses, but also as a rootstock in landscape design and cut rose cultivation. Its colorful flowers (pink, light red, light yellow, and white) that bloom in summer and its bright red fruits that ripen in autumn are used aesthetically and functionally in landscape design, individually or in groups (Koçan, 2010). Today, fruit consumers worldwide and in our country consume fruit not only for its taste but also considering its nutritional content (Boyacı and Yılmaz, 2020). Therefore, rosehip, with its rich vitamin and mineral content, is among the most popular fruits of recent times. Rosehip (*Rosa* spp.), depending on the species, is a perennial shrub in the form of an upright or climbing plant with many stems and deep roots, reaching 0.5 - 4.0 m in height. It has both fibrous roots and taproots that can extend up to 4 meters. Its fleshy, red, soft roots are used in the dye industry, and the buds on them tend to produce root suckers. Its leaves are elliptical, toothed, and compound with 5 or 7 leaflets. The flowers are pink, yellow, cream, white, or

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light red, with 5 petals and 5 sepals, fragrant, and beautiful in appearance. However, in some species, the number of petals can be much more than 5, like in roses. Rosehip has hermaphrodite flowers and possesses numerous male and female organs. Its fruit is a false fruit. The fruit shape is round, egg-shaped, or elliptical. The fruit is fleshy and shiny, green before ripening, and changes from brick red to red when ripe. The outer part of the fruit is hairy or hairless depending on the species, while the inside is more or less hairy and contains numerous seeds. If not harvested, it can remain on the plant during the winter. The ripening period of the fruits varies between July and October depending on the species and the climatic conditions of the region (İlisulu, 1992; Türkben, 2003, Güneş, 2013).

Rosehip fruits, from which tea, juice and marmalade are produced, are reported to be very rich in vitamin C and are the richest among cultivated and naturally growing plants (Ağaoğlu et al., 1987). User (1967) reported that since the human body cannot synthesize and store vitamin C, it is necessary to take it daily from external sources; that vitamin C ensures the normal formation of collagen, an important component of skin and connective tissues, bone development, and healthy tooth development; that it neutralizes harmful substances known as free radicals with its antioxidant properties; that it ensures normal cholesterol levels in the blood; that it helps the body's defense system function normally by preventing symptoms that occur in colds and flu; and that it also enables the utilization of some vitamins and minerals such as vitamins E, A, B2, B5, folic acid, iron, and calcium (Güneş, 2013). The use of rosehips for thousands of years stems from their positive effects on human health. Rosehip, which has a very wide range of uses in terms of human health, especially in nutrition, unfortunately did not receive the necessary attention in our country until recent years. Rosehip, which is valued as a raw material in the pharmaceutical industry in many European countries, is also used in folk medicine; its roots and fruits are used in the treatment of hemorrhoids, as a painkiller, for colds and rheumatism, while its leaves and flowers are used in the treatment of bronchitis (Honda et al. 1996).

Table 1. Rosehip Calorie and Nutrition Table (per 100 grams)

Nutritional Value	Amount
Calories	51 kcal
Carbohydrates	12.1 g
Protein	0.9 g
Fat	0.3 g
Fiber	4.8 g
Vitamin C	426 mg
Vitamin A	1694 IU
Calcium	169 mg
Iron	0.5 mg
Potassium	288 mg
Magnesium	29 mg

Since rosehip is a wild-growing fruit species in our country, statistical data on the number of plants and production is unavailable. The processing of rosehip into many different products in the food industry highlights the need for cultivation (Şen and Güneş, 1996). As a result of the demand from existing industrial establishments and the public, local rosehip markets are forming. Rosehip is not suitable for fresh or table consumption; it is an industrial fruit species that can be consumed after being processed into various products. However, the fruits of some species have an appealing texture and consistency suitable for fresh consumption. As a result of processes such as cutting, chopping, drying, and heat treatment in the processing of rosehip, the amount of some organic substances

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decreases significantly (Güneş, 2013).

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Rosehip (*Rosa* spp.), depending on the species, is a perennial shrub with multiple stems and deep roots, growing to a height of 0.5-4.0 m, either erect or climbing. It possesses both fibrous roots and taproots that can reach up to 4 m. The fleshy, red, soft roots are used in the dye industry, and the buds on them tend to produce root suckers. The leaves are elliptical, toothed, and compound with 5 or 7 leaflets. The flowers are pink, yellow, cream, white, or light red, with 5 petals and 5 sepals, fragrant, and beautiful in appearance. However, in some species, the number of petals can be much more than 5, as in roses. Rosehip has hermaphrodite flowers with numerous male and female organs. The fruit is a false fruit. The shape of the fruit is round, egg-shaped, or elliptical. The fruit is fleshy and shiny, green before ripening, and changes from brick red to red when ripe. The outer part of the fruit is hairy or hairless depending on the species, while the inside is more or less hairy and contains numerous seeds. If not harvested, it can remain on the plant during the winter. The ripening period of the fruits is between July and October, depending on the species and the climatic conditions of the region (İlisulu, 1992; Türkben, 2003, Güneş, 2013).

Rosehip naturally grows in a very wide area, from sea level up to altitudes of 3000 m. It is a plant resistant to extreme climate and soil conditions, and because it blooms between May and July, it is not damaged by late spring frosts. Flowering time is delayed as altitude increases. Because its roots penetrate very deep, it is also resistant to drought conditions. Due to its high adaptability, rosehip is also successfully used in erosion control. While it thrives better in sandy soils, it shows the best growth in nutrient-rich, loose soils (Gökmen, 1973, İlisulu, 1992, Yamankaradeniz, 1982). It is not found on the Mediterranean coast because it cannot meet its winter chilling requirement. Rainfall during the growing season increases fruit size. Rosehip harvesting should be done at the "technological maturity" stage, when the fruit has reached its full ripe color and has the highest vitamin C content. Depending on altitude and other climatic factors, rosehips should generally be harvested in August-September, after they have reached their natural ripe color, before they soften on the plant. Significant losses in vitamin C content occur as the fruits begin to soften (Güneş, 2013). In Türkiye, rosehip harvesting is done by hand, with the branches bent appropriately.

Although our country has a very long history of fruit cultivation, we still have very few varieties of some fruit species, such as rosehip. These are YILDIZ (*Rosa canina*) in 2012, GERÇEKCİOĞLU (*Rosa montana* chaix subsp. *woronowii* (Lonacz.) Ö.Nillson) in 2015, and AY (*Rosa montana* chaix

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subsp.*woronowii* (Lonacz.) Ö.Nillson) in 2022 (TTSM, 2018). The fruit weight of the Yıldız variety was determined to be 2.90 g in Tokat city center and 2.15 g in Tokat Başçiftlik (Güneş, 2011), while the fruit weight of the Gerçekcioğlu variety was determined to be 3.17 g (Öz Atasever et al., 2016). Phenological observations and pomological analyses are ongoing on the candidate genotypes identified in the rosehip selection study conducted in Yozgat.

In rosehip orchards, planting distances can be 1x1.5 m; 1.5x2 m; 2x3 m; 2.5x3.5 m or 3x3 m due to the different growth, branching, and crown formation patterns of the species. While orchards can be established with a single species except *Rosa pendulina,* a mixed species is more beneficial for achieving high fruit set. Regular annual maintenance will increase fruit yield and quality. Soil tillage should be performed to kill weeds, improve soil aeration and fertilizer integration, reduce water loss, and eliminate overwintering pests. Irrigation should be done according to soil conditions during the first few years of seedlings. Fertilization promotes the formation of new shoots, significantly increasing plant height, fruit size and weight, and therefore yield. Pruning in rosehip orchards involves thinning branches in plants that grow in a clump and controlling basal shoots that extend beyond the clump (Güneş, 2013).

Although no studies have been conducted in Yozgat regarding rosehip pests and their natural enemies, the most common pest species in nature appears to be Diplolepis mayri Schld. In spring, unfertilized females lay their eggs in rose buds. Galls containing many larval chambers develop from these buds (Oğurlu et al., 1996).

Uses of Rosehip:

- Use as a Foodstuff

In Türkiye, the use of rosehip as a foodstuff has gained increasing importance in recent years. Especially in Central and Northern Anatolia, such as Tokat, Çorum, Erzincan, Gümüşhane, and Erzurum, rosehip is consumed not only as marmalade (pelver, pekmez) but also as fruit juice, jam, rosehip powder, compote, and tea. Rosehip fruit is generally consumed as fruit juice, rosehip marmalade, rosehip jam, vitamin C sandoz, and rosehip tea (İlisulu, 1992; Şen and Güneş, 1996; Güneş, 2013).

- Medicinal Use

Rosehip, evaluated in terms of human health, is used in folk medicine to alleviate the following ailments (Şen and Güneş, 1996; Doğan et al., 2006; Güneş, 2013):

- It strengthens the body's defense systems against infections and colds.
- It has nourishing and strengthening, mild laxative, and mild diuretic properties.
- It is beneficial for constipation and gallbladder, kidney, and bladder disorders.
- It has been scientifically proven to support the production of important hormones by affecting the adrenal glands.
- It has wound-healing and blood-cleansing properties.
- It is therapeutic for kidney and urinary tract stones and sand, and bloody urine.
- It is used to increase resistance against colds and flu.
- It is used in the treatment of stomach cramps, burns, and wounds.
- It has blood-building and blood pressure-regulating properties.

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- It is good for the eyes due to its rich vitamin content.
- It is protective against cancer due to its richness in antioxidants.
- It has a therapeutic effect on hemorrhoids.

- Use as a Rose Rootstock

In the cut flower industry, rosehip is used as a rootstock in the propagation of roses. Rosehip, which is not selective in terms of soil and climate requirements, is especially resistant to soil-borne nematodes and root cancer, so roses are grafted onto it (Kazaz et al., 2013; Güneş, 2013).

- Use in Landscape Design and as a Hedge Plant

Rosehip is a plant that forms a crown 1-3 m wide and high, captivating with its flowers in spring and its fruits in autumn. Its flowers are red, yellow, pink and various shades of these, and have a very beautiful appearance and fragrance. If the fruits are not harvested, they remain on the plant until the beginning of January or February. Rosehip is used in many European countries for aesthetic and functional purposes in roadside afforestation because it is resistant to environmental pollution. It has an ideal size and appearance as an ornamental plant (Şen and Güneş, 1996; Güneş, 2013; Korkmaz et al., 2013).

- Use as an Erosion Prevention Agent

The rosehip plant has excellent soil anchorage thanks to its taproots that can reach 4 m and its fibrous roots that can grow up to 2 m in diameter. It covers the surface with its abundant root suckers. Rain, hail, and snow cannot directly contact the soil due to its height and canopy (Yılmaz, 1996). In previous years, rosehip was planted in large quantities in barren, bare areas as part of erosion control campaigns. Planting rosehip on steep slopes, stream banks, and dam basins will prevent the loss of our fertile soils to erosion (Yılmaz, 1996; Güneş, 2013).

- Uses in Other Areas

In addition to its widespread uses, rosehip is used in the production of baby food, as a food coloring agent (carotene, beta-carotene), in the production of wine and liqueur, in the production of many cosmetic products such as sunscreen, shampoo, soap and moisturizer, in the production of rose water and rose oil, its fruits and seeds are used as feed for lambs, fish and birds, and due to the high vitamin C content of waste rosehip fruits, it is used as horse feed, especially in Arab countries, and as a tanning agent in the leather industry (Güneş, 2013).

Conclusion

Yozgat is a province rich in rosehip populations. Rosehips are collected by the local people and used to meet their own needs. Rosehip, which is of great importance for health, especially being rich in vitamin C and minerals, has many uses in addition to its nutritional value, and this will ensure that rosehip is given the importance it deserves. Since rosehip is an uncultivated plant, the stable production required for industry cannot be achieved, which causes the rosehip industry to operate irregularly. By establishing enclosed orchards with rosehip, which grows in every region of our country but does not yet have economic importance, agricultural activities in terms of fruit cultivation will be developed in the region, primarily meeting the needs of the region, contributing to family and national economies, preventing migration from rural areas, meeting the raw material needs of businesses related to processing rosehip into various products, creating new job opportunities and reducing unemployment. With its market opportunities and high sales price, rosehip has the potential to be an important source of income for our farmers.

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EFFECTS OF MULCHING PRACTICES ON ESSENTIAL OIL COMPOSITION OF ZAHTER (*Thymbra spicata* L. var. *spicata*)

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Abstract

Mulching is a commonly used agronomic practice in the cultivation of medicinal and aromatic plants to improve soil moisture conservation, suppress weed growth, and regulate soil temperature. However, its effects on the qualitative characteristics of essential oils have not been sufficiently clarified. This study aimed to evaluate the influence of mulching application on the essential oil composition of zahter (*Thymbra spicata* L. var. *spicata*), with particular emphasis on changes in major volatile constituents.

The experiment was conducted under field conditions using black polyethylene mulch as an inorganic mulching material, along with a non-mulched control treatment. Aerial parts of zahter plants were harvested at the full flowering stage, and essential oils were extracted by hydrodistillation. The chemical composition of the essential oils was determined using gas chromatography-mass spectrometry (GC-MS), and the relative abundance of individual compounds was compared among treatments.

The results revealed that mulching significantly affected the essential oil composition of zahter. Although total essential oil yield exhibited slight variations among treatments, substantial differences were observed in the proportions of major components such as *γ*-terpinene, *o*-cymene, caryophyllene, (+)-4-carene, *β*-myrcene. Mulched plots generally showed higher concentrations of phenolic monoterpenes, which are responsible for the characteristic aroma and strong biological activity of zahter essential oil. These variations are likely associated with improved soil microclimatic conditions and enhanced nutrient availability under mulching practices.

In conclusion, mulching application can be considered an effective cultural practice not only for improving soil conditions but also for enhancing essential oil quality in zahter cultivation. Selecting appropriate mulching materials may serve as a practical approach to optimize the chemical composition of zahter essential oil, thereby increasing its economic value and suitability for pharmaceutical, food, and aromatic applications.

Key Words: Zahter, *Thymbra spicata* L. var. *spicata,* mulching, essential oil composition, phenolic monoterpenes, GC-MS analysis

1. Introduction

Medicinal and aromatic plants have gained increasing importance in pharmaceutical, food, cosmetic, and aromatherapy industries due to their rich content of secondary metabolites. Among these compounds, essential oils are of particular interest because of their well-documented antimicrobial, antioxidant, and anti-inflammatory properties [1, 2]. The chemical composition of essential oils is influenced not only by genetic factors but also by environmental conditions, phenological stage, and applied agronomic practices.

Thymbra spicata L. var. *spicata,* commonly known as zahter, is an aromatic plant native to the Eastern Mediterranean region and widely distributed in southern Türkiye. The essential oil of zahter is characterized by a high content of phenolic monoterpenes, mainly carvacrol, thymol, *γ*-terpinene, and *p*-cymene, which are responsible for its distinctive aroma and biological activity [3, 4].

Recent studies emphasize that the qualitative composition of essential oils is often more critical than total oil yield in determining commercial and therapeutic value [5, 6]. Consequently, understanding how cultivation techniques influence essential oil composition has become a key research focus in medicinal

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plant agronomy. The qualitative and quantitative composition of essential oils is strongly influenced by genetic factors, phenological stage, ecological conditions, and agronomic practices [7, 8]. Recently, increasing attention has been given to the impact of cultural practices on secondary metabolite production in medicinal and aromatic plants.

Mulching is a widely adopted agricultural practice used to improve soil moisture retention, suppress weed growth, and regulate soil temperature [9]. In particular, black polyethylene mulch has been shown to modify soil microclimatic conditions and enhance plant growth in semi-arid environments. Recent evidence suggests that mulching practices can also affect secondary metabolite biosynthesis by altering plant stress responses and metabolic pathways [10, 11, 12].

Despite the growing body of research on mulching effects in medicinal plants, studies focusing on *T. spicata* remain limited. In particular, the influence of mulching on the relative abundance and stability of major essential oil constituents has not been sufficiently clarified.

Therefore, the present study aimed to evaluate the effects of black polyethylene mulching on the essential oil composition of *T. spicata* var. *spicata* under field conditions. The findings are expected to contribute to the development of quality-oriented cultivation strategies for zahter and similar aromatic plants.

2. Material and Methods

2.1. Experimental Site and Plant Material

The experiment was conducted at the experimental fields of the Faculty of Agriculture, Kilis 7 Aralık University, Türkiye. The study area is characterized by a typical Mediterranean climate with hot, dry summers and mild winters, which is suitable for the cultivation of aromatic and medicinal plants.

T. spicata var. *spicata* plants were grown under two different cultivation conditions: Unmulched cultivation and black polyethylene mulching. The experimental design consisted of six plots, including three mulched plots and three unmulched plots. All plots were managed using similar agronomic practices, except for the mulching treatment, in order to isolate the effect of mulching on essential oil composition.

Plant materials were harvested simultaneously at a similar phenological stage, corresponding to the flowering period, which is known to be the optimal stage for essential oil accumulation in *Thymbra* species [7, 8]. This approach minimized variations related to plant maturity and environmental conditions.

2.2. Essential Oil Extraction

Essential oils were extracted from the aerial parts of the plants by hydrodistillation using an alembic (imbik) apparatus. In this process, the plant material was placed in the alembic together with water and heated under controlled conditions until boiling. The generated water vapor carried the volatile constituents through the distillation system, where they were condensed and subsequently separated into oil and aqueous phases. The distillation was performed for a fixed period.

After distillation, the essential oils were dried over anhydrous sodium sulfate to remove residual moisture. The dried oils were then transferred into airtight amber glass vials and stored at +4 °C until GC–MS analysis. Low-temperature storage was applied to minimize oxidative degradation and prevent changes in chemical composition prior to analysis [1].

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2.3. GC–MS Analysis

The chemical composition of the essential oils was determined using an Agilent gas chromatography–mass spectrometry (GC–MS) system. Separation of the compounds was achieved by capillary gas chromatography, while identification was carried out using mass spectrometric detection.

Individual components were identified by comparing their mass spectra with those in commercial spectral libraries (such as NIST and Wiley) and by evaluating their retention behavior in accordance with published literature data [13]. Only compounds with reliable spectral matches and consistent retention characteristics were considered for identification.

GC–MS analysis led to the identification of 16 major compounds in the essential oil of *T. spicata* var. *spicata,* which together represented the predominant fraction of the total essential oil composition.

The relative abundance of each compound was expressed as percentage of total peak area (% area) obtained from the GC–MS chromatograms. The results were used to compare the essential oil composition between mulched and unmulched cultivation systems.

3. Results and Discussion

The effect of mulched and unmulched cultivation on the volatile oil profile of thyme presented in Table 1 and Figure 1. Among these, γ-terpinene, o-cymene, and caryophyllene were the dominant constituents across all treatments.

γ-Terpinene content ranged from 23.39–29.37% in Unmulched plots and from 25.55–27.79% in mulched plots. The highest value was recorded in Unmulched I (29.37%), whereas the lowest was observed in Unmulched II (23.39%). Mulched plots showed a narrower range, indicating more stable accumulation of this compound.

Table 3.1. The effect of mulched and unmulched cultivation on the essential oil profile of thyme

	Unmulched Pot I	Unmulched Pot II	Unmulched Pot III	Mulched Pot I	Mulched Pot II	Mulched Pot III
γ-Terpinene	**29.37**	**23.39**	**29.23**	**25.55**	**26.71**	**27.79**
o-Cymene	**20.11**	**14.83**	**17.44**	**19.50**	**20.60**	**18.48**
Caryophyllene	**6.07**	**4.79**	**5.97**	**6.05**	**6.02**	**6.14**
(+)-4-Carene	**4.69**	**3.64**	**4.41**	**4.30**	**4.38**	**4.40**
β-Myrcene	**2.71**	**2.11**	**2.55**	**2.39**	**2.43**	**2.42**
Terpinen-4-ol	1.02	0.97	1.25	0.99	1.04	1.06
Limonene	0.57	0.45	0.53	0.48	0.52	0.49
α-Phellandrene	0.46	0.36	0.43	0.39	0.40	0.41
β-Phellandrene	0.42	0.32	0.39	0.00	0.38	0.00
Humulene	0.26	0.21	0.27	0.27	0.27	0.27
Linalool	0.24	0.19	0.21	0.18	0.21	0.19
Camphene	0.19	0.14	0.18	0.16	0.18	0.16
Borneol	0.18	0.15	0.26	0.23	0.24	0.22
1-Octen-3-ol	0.46	0.34	0.47	0.34	0.42	0.38
α-Pinene	1.26	0.99	0.31	0.27	0.00	0.28
α-Terpineol	0.00	0.00	0.18	0.16	0.17	0.18

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o-Cymene percentages varied between 14.83–20.11% in unmulched and 18.48–20.60% in mulched plots. The highest o-cymene content was detected in Mulched Field II (20.60%). Greater variability was observed under unmulched conditions.

Caryophyllene content ranged from 4.79–6.07% in unmulched and 6.02–6.14% in mulched plots, showing only minor differences among treatments.

Other monoterpene hydrocarbons such as (+)-4-carene and β-myrcene showed relatively consistent values, while oxygenated monoterpenes (terpinen-4-ol, linalool, borneol, α-terpineol) tended to be slightly higher in mulched plots.

Overall, mulched plots exhibited a more homogeneous distribution of essential oil constituents compared to unmulched conditions.

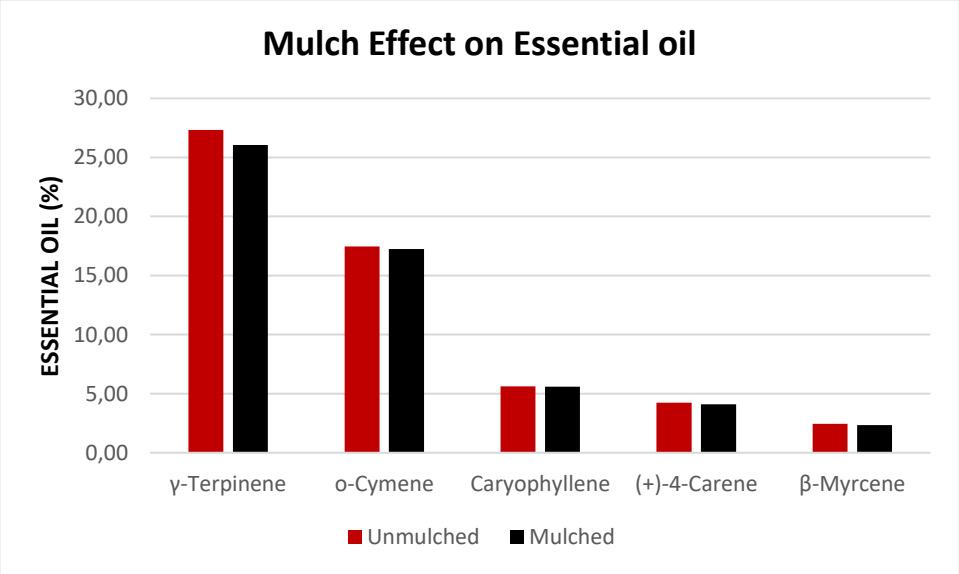

Figure 1. Effects of mulch on essential oil

GC-MS analysis of the essential oil obtained from *T. spicata* var. *spicata* revealed the presence of 16 major compounds, representing the dominant fraction of the total oil composition. Across all treatments, γ-terpinene, o-cymene, and caryophyllene were identified as the principal constituents, confirming the characteristic chemotype previously reported for this species [3, 8].

The proportion of γ-terpinene ranged from 23.39 to 29.37% in unmulched plots, whereas a narrower range of 25.55 to 27.79% was observed in mulched plots. The highest value was recorded in Unmulched I (29.37%), while the lowest occurred in Unmulched II (23.39%). The reduced variability observed under mulching conditions suggests that soil moisture conservation and moderated temperature fluctuations may contribute to a more stable biosynthesis of monoterpene hydrocarbons. Similar stabilizing effects of mulching on terpene accumulation have been reported in other aromatic plants.

The content of o-cymene, a key biosynthetic precursor of phenolic monoterpenes such as thymol and carvacrol, varied between 14.83 and 20.11% in unmulched and between 18.48 and 20.60% in mulched plots. Although mean values were comparable, unmulched conditions exhibited greater sample-to-sample variability. This fluctuation may be associated with increased sensitivity of o-cymene

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accumulation to environmental stress factors, including water deficit and temperature extremes [7]. Among sesquiterpene hydrocarbons, caryophyllene showed relatively consistent levels across treatments, ranging from 4.79 to 6.07% in Unmulched and 6.02 to 6.14% in mulched plots. The limited variation observed supports previous findings indicating that sesquiterpene biosynthesis is less responsive to agronomic and environmental changes compared to monoterpenes [1]. Other monoterpene hydrocarbons, including (+)-4-carene and β-myrcene, were detected at moderate levels. (+)-4-Carene ranged from 3.64 to 4.69% in Unmulched and 4.30 to 4.40% in mulched plots, while β-myrcene varied between 2.11 and 2.71% and 2.39 and 2.43%, respectively. These compounds showed relatively narrow concentration ranges, indicating a limited response to mulching treatment. Oxygenated monoterpenes, such as terpinen-4-ol, linalool, borneol, and α-terpineol, were present at lower concentrations but displayed a tendency toward higher values in mulched plots. For instance, borneol content increased from 0.15–0.18% in Unmulched to 0.22–0.24% under mulching. This trend may reflect reduced oxidative stress and enhanced enzymatic conversion toward oxygenated terpenes under improved soil microclimatic conditions [10].

Overall, the combined evaluation of quantitative values and compositional stability indicates that black polyethylene mulching promotes a more homogeneous essential oil profile in T. spicata. These findings demonstrate that agronomic practices influence not only yield but also the quantitative distribution and stability of bioactive essential oil constituents. The results of the present study clearly demonstrate that black polyethylene mulching influences the essential oil composition of *T. spicata* var. *spicata*, particularly with respect to the relative proportions and stability of major monoterpene constituents. Although total essential oil yield showed only minor variation between mulched and unmulched conditions, notable differences were observed in the distribution of key volatile compounds, indicating that mulching primarily affects essential oil quality rather than quantity. Across all treatments, γ-terpinene, o-cymene, and caryophyllene were identified as the dominant components, confirming the characteristic chemotype previously reported for T. spicata growing in the Eastern Mediterranean region [3, 4, 8]. However, the narrower concentration ranges and reduced variability observed in mulched plots suggest that soil microclimatic modification through mulching plays a stabilizing role in terpene biosynthesis.

γ-Terpinene, a key monoterpene hydrocarbon and biosynthetic precursor of phenolic compounds such as thymol and carvacrol, exhibited greater variability under Unmulched conditions compared to mulched plots. The stabilization of γ-terpinene levels under mulching may be attributed to improved soil moisture availability and moderated temperature fluctuations, which are known to influence enzymatic activity within the monoterpene biosynthetic pathway [12]. Similar findings have been reported in other aromatic species, where reduced environmental stress resulted in more consistent accumulation of monoterpene hydrocarbons [11]. The content of o-cymene, another important intermediate in the biosynthesis of phenolic monoterpenes, showed greater variability in unmulched plots. This compound is particularly sensitive to environmental stress, as it is rapidly converted to thymol and carvacrol under favorable metabolic conditions [7]. The relatively stable o-cymene proportions observed in mulched plots suggest that improved soil conditions may regulate the metabolic flux between precursor and end-product compounds, leading to a more balanced essential oil profile.

Sesquiterpene hydrocarbons, represented mainly by caryophyllene, displayed limited variation between treatments. This observation is consistent with previous studies indicating that sesquiterpene biosynthesis is less responsive to short-term environmental changes and agronomic practices compared to monoterpenes [1]. The relatively stable caryophyllene content across treatments supports the hypothesis that mulching primarily affects the plastidial monoterpene pathway rather than the cytosolic sesquiterpene pathway.

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Oxygenated monoterpenes such as terpinen-4-ol, linalool, borneol, and α-terpineol were detected at low but ecologically and pharmacologically significant levels. These compounds tended to be slightly higher in mulched plots, suggesting that improved soil moisture and reduced oxidative stress may favor enzymatic oxidation and hydroxylation reactions. Arslan et al. (2018) reported similar trends in medicinal plants grown under improved microclimatic conditions, where oxygenated terpenes increased in response to reduced abiotic stress [11]. From a qualitative perspective, the more homogeneous essential oil profile observed under mulching conditions is of particular importance for commercial zahter production. Consistency in chemical composition is a key requirement for pharmaceutical, food, and cosmetic applications, where variability in active compounds can affect product efficacy and sensory properties. Therefore, the application of black polyethylene mulch may contribute to standardization of essential oil quality in T. spicata cultivation.

Overall, the findings of this study highlight that mulching acts as a quality-modulating agronomic practice, influencing not only plant growth conditions but also the biochemical pathways responsible for essential oil biosynthesis. These results are in agreement with recent literature emphasizing the role of controlled cultivation techniques in optimizing secondary metabolite profiles in medicinal and aromatic plants [2]

4. Conclusion

The present study demonstrates that black polyethylene mulching significantly influences the essential oil composition of *T. spicata* var. *spicata* under field conditions. While total essential oil yield exhibited only minor variation between mulched and unmulched plots, mulching notably affected the relative abundance and stability of major volatile constituents, particularly monoterpene hydrocarbons. Mulched plots showed a more homogeneous essential oil profile, characterized by reduced variability in key compounds such as γ-terpinene and *o*-cymene, as well as slightly increased levels of oxygenated monoterpenes. These changes are likely associated with improved soil microclimatic conditions, including enhanced moisture retention and moderated temperature fluctuations, which contribute to more stable secondary metabolite biosynthesis. From an agronomic and industrial perspective, these findings indicate that mulching can be considered an effective cultural practice for improving essential oil quality in zahter cultivation. By promoting compositional stability and favorable chemical profiles, black polyethylene mulching may increase the economic value and marketability of *T. spicata* essential oil for pharmaceutical, food, and aromatic applications. Future studies should focus on evaluating different mulching materials, long-term cultivation effects, and their interactions with other agronomic factors to further optimize essential oil quality and yield in zahter and related aromatic plant species.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF THE BIOACTIVE POTENTIAL OF EXTRACTABLE FRACTIONS FROM *INONOTUS OBLIQUUS* AND *PHALLUS IMPUDICUS*

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Abstract

This research investigates the chemical composition, extraction efficiency, and biological properties of two historically valued macrofungi: Chaga mushroom (*Inonotus obliquus*) and the Common Stinkhorn (*Phallus impudicus*), aiming to unlock their potential for value-added industrial applications.

The objectives were to determine the proximate chemical composition, evaluate the efficiency of two advanced extraction methods, and assess the antioxidant and antibacterial activities of the resulting extracts derived exclusively from *I. obliquus* and *P. impudicus.*

Methods involved proximate analysis, which found that carbohydrates constituted the largest share in both fungi, while *P. impudicus* had the highest protein (20.44%) and fat (1.57%) content. Extracts were obtained using Supercritical Carbon Dioxide Extraction (SCE-CO_2) and Pressurized Liquid Extraction (PLE) with solvents of increasing polarity (*n*-hexane, acetone, ethanol). The biological activities were determined using DPPH, FRAP, and Folin-Ciocalteu assays for antioxidant properties, and the agar diffusion method against Escherichia coli and Bacillus subtilis for antibacterial activity. Efficient Liquid Chromatography (ELC) was used to quantify ergosterol.

Results showed that *P. impudicus* yielded the highest amount of non-polar extract in both SCE-CO_2 (0.80%) and PLE with *n*-hexane (0.93%). The highest yield of polar extract was achieved using PLE with ethanol. All extracts exhibited antioxidant properties, with the strongest activity observed in the acetone and ethanol extracts of Chaga mushroom (*I. obliquus*). Antibacterial assessment indicated strong selective activity; the SCE-CO_2 extract of *P. impudicus* showed the highest inhibition (14.0 mm) against *E. coli,* while the *n*-hexane extract of *P. impudicus* was most effective against B. subtilis (14.7 mm). Ergosterol was detected at the highest concentration in the acetone extract of P. impudicus, but was absent in the *n*-hexane extract of *I. obliquus.*

In conclusion, *I. obliquus* and *P. impudicus* are rich sources of bioactive compounds. The strong antioxidant capabilities of the polar extracts from Chaga and the significant antibacterial potential of the non-polar extracts from P. impudicus underscore their potential as valuable ingredients for the food, cosmetic, and pharmaceutical industries through targeted biorefining.

Key Words: Chaga mushroom, *Inonotus obliquus, Phallus impudicus*, Biorefining, Antioxidant activity, Pressurized Liquid Extraction, Ergosterol.

1.Introduction

Mushrooms have been valued since ancient times not only for their nutritional value, but also for their important medicinal properties. Scientists are actively searching for natural antioxidant and antibacterial compounds, and therefore interest in mushrooms is currently increasing due to their even wider applicability in medicine (Venturella et.al.,2021), pharmacy, food (Navarro-Simarro et al., 2024) and other fields (Taofiq et al., 2016).

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Mushrooms are rich in proteins, fats, phenolic compounds, flavonoids and vitamins, such as vitamins B1, B2, B3, C (Fogarasi et al., 2024) and ergosterol, which can be easily converted into vitamin D2 (Sun et al., 2022).
The scientific literature mentions the biological activities of bioactive compounds isolated from mushrooms: antioxidant, anti-inflammatory, cytotoxic properties (Souilem et al., 2017), antibacterial, antiviral, and immunomodulatory properties (Kumar & Baojun, 2025).
Black birch fungus also known as chaga (*Inonotus obliquus*) and common stinkhorn (*Phallus impudicus*) possess a variety of health benefits: immune modulatory activities, inhibits cancer cells, protects from oxidative stress (Cheung et al., 2023), (Khan et al., 2020). *P.impudicus*, belongs to *Phallus*, Phallaceae and Phallales species.
In order to discover natural biologically active compounds and apply them in the development of functional foods, supplements or drugs. Supercritical carbon dioxide extraction (SCE–CO_2) and solvent extraction under elevated pressure are effective methodologies to extract bioactive substances from natural raw materials (Joradon et al. 2024), which aim to achieve better and more selective product recovery, while contributing to sustainability and green chemistry principles (Akter et al. 2025).
The aim of this work is to determine the chemical composition of black birch (*I. obliquus*) and common stinkhorn (*P. impudicus*). To evaluate the antioxidant and antibacterial activity of extracts obtained by supercritical carbon dioxide extraction (SCE–CO_2) and stepwise extraction with increasing polarity solvents using pressurised liquid extraction (PLE) methods, and to investigate the ergoster content in extracts obtained by PLE.

2.Material and Methods

Sample Preparation

The chaga (*I. obliquus*) and the common phallus (*P. impudicus*) mushrooms were collected from different regions of Lithuania. Mushrooms were peeled, washed and freeze-dried. The dried samples were ground in a laboratory mill to a particle size of ≤ 0.5 mm. Mushrooms powder were stored in airtight containers in the dark until extraction.

Extraction Procedures

Bioactive compounds from mushrooms were extracted using high pressure extraction techniques as supercritical fluid extraction and pressurized liquid extraction (PLE) by sequentially changing solvents of increasing polarity - *n*-hexane, acetone and ethanol.
Supercritical fluid extraction was performed at a pressure of 350 bar and a temperature of 50 °C. The CO_2 flow was maintained constant (2 SL of CO_2 gases at RT/min) until complete extraction was achieved in 120 min. 20g of powdered mushroom samples were loaded into 50 ml extraction cell.
PLE was performed using three solvents of different polarity in order of increasing polarity - *n*-hexane, acetone and ethanol. Extraction with each solvent is performed in one extraction cycle at a pressure of 10 MPa, extraction time of 15 min, and 120% washout. The temperature was selected according to the solvent: *n*-hexane/acetone – 90 °C, ethanol – 100 °C. Sample amounts used: black birch mushroom – 8–9.5 g, common phallus – 11 g. The extracts were stored at 4°C for further analysis.

Phytochemical Activity Determination

The total amount of phenolic compounds was measured spectrophotometrically, according to the methodology of Singleton et al. (1999) with some modifications. The Folin-Ciocalteu reagent is used in the study, which forms blue complexes with phenolic compounds. The samples were mixed and kept in

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a dark place for 120 min. After the specified time, the absorbance of the samples measured using a spectrophotometer (λ=760 nm). The total content of phenolic compounds is expressed in gallic acid equivalents (µg GAE/g extract).
Antioxidant activity was measured spectrophotometrically, using the DPPH˙ radical-scavenging assay, according to the methodology of Brand-Williams et al. (1995) with slight modification. Light absorption was measured with spectrophotometer at a wavelength of 515 nm. Mushrooms extract samples were diluted with methanol to the final concentration of 1-3%. Extract antioxidant potential was calculated by the evaluating the percentage of DPPH˙ inhibition.
Antioxidant power (FRAP) assay was conducted according to the methodology of Huang et al. (2005) with slight modification. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine), and 20 mM $FeCl_3$ (ferric chloride) solution. To perform the measurement, 20 mL of the methanol extract was reacted with the FRAP reagent for 5 minutes. The reduction of the ferric-TPTZ complex to its blue-colored ferrous form was monitored by measuring the absorbance at 593 nm using a spectrophotometer.

Antibacterial Activity Assay

The antibacterial potential of mushrooms extracts was evaluated using the disc diffusion method against Gram-negative and Gram-positive model organisms. Two bacterial species, *Escherichia coli* and *Bacillus subtilis*, were utilized as test organisms. The cultures were maintained in Luria–Bertani (LB) medium. The antibacterial activity was determined according to the methodology of Alam et al. (2009) with slight modification. LB agar plates were uniformly swabbed with the standardized bacterial culture (10^6 CFU/mL). Sterile filter paper discs were impregnated with 25 µL of the mushrooms extract. The discs were placed onto the inoculated agar surface, and the plates were incubated at 37°C for 24 hours. Ciprofloxacin was employed as a positive control antibiotic to ensure assay sensitivity. Antibacterial activity was quantified by measuring the diameter (cm) of the clear zone of inhibition formed around each disc.

Determination of Ergosterol Concentration

Quantitative evaluation of extracts was performed using high-performance liquid chromatography (HPLC). The ergosterol analysis was performed using a Water Alliance 2695 Separation Module chromatography system. The sample was analysed using a Waters Symmetry (Milford, MA, USA) RP C18 column (75x4.6mm, 3.5mm) at 30°C. The analysis was performed by feeding a mobile phase mixture consisting of methanol - acetonitrile (30:70) at a rate of 1 ml/min. The sample injection volume was 20 µL. Ergosterol was analysed using a 2487 UV detector (Milford, MA, USA) at a wavelength of 280 nm (Chiocchio1 & Matković, 2011).
A certified ergosterol standard material was used to create the calibration curve, from which standard solutions (0.01–0.20 mg/ml) were prepared. The ergosterol concentration (mg/g) is calculated from the calibration curve.

3.Results and Discussion
Isolation of Bioactive Fractions

The supercritical carbon dioxide ($SCCO_2$) extraction of lipophilic fractions from the studied mushroom species revealed significant variations in yield. Under optimal conditions, *P. impudicus* provided the highest recovery at 0.82% (w/w). In contrast, *I. obliquus* yielded a lower lipophilic fraction of 0.35% (w/w). For both species, the extraction efficiency peaked at an optimal duration of 120 minutes; beyond

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this point, yield increments were negligible, indicating exhaustive recovery of the accessible lipid-soluble components. The observed differences in yield between the two species can be attributed to the distinct chemical compositions and structural characteristics of their respective fruiting bodies. The higher yield in *P. impudicus* suggests a richer profile of non-polar compounds, such as fatty acids, terpenes, and sterols, compared to *I. obliquus*. The observed extraction yield in the current study was lower than the 0.4–0.5% range reported in previous literature of Karina et al. (2022) for the same temperature and pressure settings. This discrepancy can be attributed to several overlapping factors, as environmental variability, technical and matrix factors or differences in the density of the fungal material.

The isolation of bioactive compounds from *P. impudicus* and *I. obliquus* was performed using sequential Pressurized Liquid Extraction (PLE) with solvents of increasing polarity: *n*-hexane, acetone, and ethanol (Table 1). The sequential extraction revealed distinct chemical profiles for both mushroom species. Using *n*-hexane, *P. impudicus* provided a significantly higher yield (0.93%) compared to *I. obliquus* (0.38%), suggesting a higher accumulation of non-polar, lipophilic substances. Notably, the PLE *n*-hexane yield for *I. obliquus* slightly exceeded results previously obtained via $SCCO_2$ extraction. In the second stage, acetone extraction favoured *I. obliquus*, yielding 2.44%, which was more than double the recovery from *P. impudicus* (1.19%). Finally, the ethanol fraction produced the highest overall yields for both species, with *P. impudicus* reaching a peak of 4.83% compared to 1.78% for *I. obliquus*.
The fact that *n*-hexane extraction performed via PLE was more efficient than $SCCO_2$ extraction for *I. obliquus* suggests that the elevated pressure and temperature used in PLE may better disrupt the fungal cell wall (chitin matrix), allowing for enhanced mass transfer of lipophilic components. Conversely, the high acetone yield in *I. obliquus* points toward a significant concentration of medium-polar compounds, likely including specialized triterpenoids or steroids common in Chaga mushrooms. These results demonstrate that sequential PLE is an effective strategy for the comprehensive fractionation of mushroom metabolites based on their lipophilic and hydrophilic characteristics.

Table 1. PLE yields by solvent polarity

Solvent	Polarity	*P. impudicus* yield (%)	*I. obliquus* yield (%)
n-Hexane	Non-polar	0.93	0.38
Acetone	Medium-polar	1.19	2.44
Ethanol	Polar	4.83	1.78

Determination of the Antioxidant Potential of Extracts

The antioxidant potential of *I. obliquus* and *P. impudicus* extracts was evaluated based on their ability to inhibit $DPPH^{\bullet}$ radicals. The results demonstrate a clear correlation between solvent polarity and antioxidant efficacy. The screening for radical scavenging activity revealed that polar extracts possess significantly higher antioxidant capacities compared to non-polar fractions. The acetone (7) and ethanolic (8) extracts of *P. impudicus* showed robust activity, reaching 66.31% and 53.74%, respectively. Similarly, the acetone (3) and ethanolic (4) extracts of *I. obliquus* yielded high inhibition values of 45.66% and 54.98% (Table 2).
Notably, when the extract concentrations were reduced by 50%, the inhibition values remained stable, indicating high potency even at lower dosages. Conversely, the non-polar extracts obtained via $SCCO_2$ and *n*-hexane extractions exhibited the lowest antioxidant activity, with values ranging narrowly between 12.26% and 13.65%. The significant disparity between the antioxidant activities of the extracts suggests that the bioactive compounds responsible for radical scavenging in these mushrooms are

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primarily polar to medium-polar in nature. The high activity of the acetone and ethanol fractions is likely related to the high concentration of phenolic compounds, flavonoids, and polysaccharides. These molecules possess hydroxyl groups that readily donate hydrogen atoms to neutralize free radicals. In contrast, the low activity of the *n*-hexane and $SCCO_2$ extracts indicates that lipophilic components (such as certain fatty acids or non-polar sterols) contribute minimally to the antioxidant activity.

The reducing capacity of the mushroom extracts, an essential indicator of their electron-donating antioxidant potential, was quantified using the FRAP assay. The results showed a significant dependence on solvent polarity, with polar and medium-polar extracts outperforming non-polar fractions.

The FRAP assay revealed that the highest reducing potential was localized in the acetone (3) and ethanol (4) extracts of *I. obliquus* (Table 2). These extracts exhibited Fe^{2+} ion concentrations in the range of 16.01–16.98 µmol, indicating a high density of reductants capable of converting the Fe^{3+}-TPTZ complex to its blue Fe^{2+} form. In contrast, the *n*-hexane (2) extract of *I. obliquus* demonstrated minimal antioxidant activity, with a Fe^{2+} concentration of only 1.25 µmol.

Table 2. The phytochemical activity of *I. obliquus* and *P. impudicus* extracts

Extract samples	DPPH• inhibition, %	FRAP value Fe^{2+}, (µmol/L)	TPC, mg (GAE/g extract)
1. *I. obliquus* ($SCCO_2$)	12.61	3.36	0.34
2. *I. obliquus* (PLE / *n*-hexane)	12.26	1.25	0.53
3. *I. obliquus* (PLE / acetone)	45.66	16.98	2.61
4. *I. obliquus* (PLE / ethanol)	54.98	16.01	2.68
5. *P. impudicus* ($SCCO_2$)	13.65	1.41	0.44
6. *P. impudicus* (PLE / *n*-hexane)	13.48	1.96	0.12
7. *P. impudicus* (PLE / acetone)	66.31	3.54	0.29
8. *P. impudicus* (PLE / ethanol)	53.74	5.38	0.40

The significant variation in FRAP values across solvents highlights the chemical nature of the antioxidants present in *I. obliquus*. The high reducing power of the ethanol and acetone fractions (16.01–16.98 µmol) suggests that the primary antioxidants in this species are polar secondary metabolites. The negligible activity found in the *n*-hexane extract (1.25 µmol) further confirms that non-polar, lipophilic components contribute very little to the ferric reducing capacity of the mushroom.

To further investigate the chemical basis of the observed antioxidant properties, the Total Phenolic Content (TPC) of the mushroom extracts was quantified. Phenolic compounds are well-known for their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. The TPC assay confirmed the presence of phenolic constituents across all analysed extracts, though concentrations varied significantly based on solvent polarity. The highest phenolic accumulation was identified in the ethanolic (3) and acetone (4) extracts of *I. obliquus*, with values ranging from 2.61 to 2.68 mg GAE/g extract (Table 2). In contrast, the non-polar *n*-hexane (6) extract of *P. impudicus* yielded the lowest phenolic concentration, recorded at only 0.12 mg GAE/g extract.

The results of the TPC analysis show a strong positive correlation with the data previously obtained from the DPPH• radical scavenging and FRAP assays. The extracts that exhibited the highest antioxidant activity (Samples 3 and 4) also possessed the highest concentration of phenolic compounds. This relationship suggests that phenolics are the primary bioactive constituents responsible for the antioxidant potential of *I. obliquus*. The disparity between the polar (ethanol/acetone) and non-polar (*n*-hexane) extracts is chemically consistent with the solubility of phenolic structures. Most fungal phenolics, such as phenolic acids and hispidin derivatives, contain multiple hydroxyl groups that increase their affinity

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for polar solvents. The low concentration found in the *n*-hexane extract (0.12 mg/g) indicates that lipophilic fractions are poor carriers of these specific bioactive metabolites. Comparing these results to literature, the TPC values for the birch mushroom extracts highlight its status as a potent source of natural antioxidants. The consistency across three different testing methods (DPPH•, FRAP, and TPC) provides a high degree of confidence that the antioxidant capacity of these species is intrinsically linked to their phenolic profile.

Antimicrobial Activity

The antibacterial efficacy of the mushroom extracts was evaluated using the disc diffusion method against *Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative). The results, measured as zones of inhibition (mm), provide insight into the spectrum of activity for each extraction method.
The experimental data indicates that samples 1-8 exhibited superior antibacterial activity against the Gram-positive bacterium *B. Subtilis* (**Table 3**). In contrast, *E. coli* (Gram-negative) showed greater resistance to most treatments. The most significant inhibitory effect against *E. coli* was observed in the non-polar extracts of *P. impudicus* (Samples 5 and 6), obtained via pressurized liquid extraction (PLE) with *n*-hexane and $SCCO_2$ extraction, respectively. These samples produced inhibition zones ranging from 13.7 to 14.0 mm. Mushrooms nonpolar extracts also exhibited the highest activity against *B. subtilis*, with inhibition zones of 14.7 mm and 14.3 mm, respectively. In contrast, *E. coli* showed complete resistance to the non-polar extracts of *I. obliquus* (1, 2) and the ethanolic extract of *I. obliquus* (8). While many samples demonstrated activity against the Gram-positive *B. subtilis*, none of the experimental extracts surpassed the inhibitory power of the control antibiotic, Ciprofloxacin, which produced zones of 30.0 mm (*E. coli*) and 25.0 mm (*B. subtilis*). The results indicate that *P. impudicus* contains non-polar bioactive metabolites with broad-spectrum antibacterial potential. The effectiveness of the *n*-hexane and $SCCO_2$ extracts—which were previously shown to have low phenolic content—suggests that the antibacterial mechanism in this species may be driven by lipophilic compounds (such as specific terpenoids or fatty acids) rather than polar phenolics.

Table3. Inhibition zones of mushroom extracts and control

ID. Extract samples	*E. coli* (mm)	*B. subtilis* (mm)
1. *I. obliquus* ($SCCO_2$)	0	13.2
2. *I. obliquus* (PLE / ***n*-hexane**)	0	13.3
3. *I. obliquus* (PLE / acetone)	11.3	13.0
4. *I. obliquus* (PLE / ethanol)	8.0	12.7
5. *P. impudicus* ($SCCO_2$)	14.0	14.3
6. *P. impudicus* (PLE / ***n*-hexane**)	13.7	14.7
7. *P. impudicus* (PLE / acetone)	11.0	14.0
8. *P. impudicus* (PLE / ethanol)	0	12.7
9. Control - Ciprofloxacin	30.0	25.0

A notable trend was the higher susceptibility of *B. subtilis* compared to *E. coli* across most samples. This is a common observation in natural product research, as the outer membrane of Gram-negative bacteria (*E. coli*) acts as a sophisticated permeability barrier, protecting the cell from many hydrophobic antimicrobial agents. The ability of the *P. impudicus n*-hexane extracts to inhibit *E. coli* at 14.0 mm is therefore significant, as it suggests these lipophilic compounds can effectively penetrate the Gram-negative outer membrane. These findings suggest with other authors that *I. obliquus* shows antibacterial activity, however this effect can be described as moderate to weak

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The lack of activity in *I. obliquus* non-polar extracts against *E. coli* suggests that its primary bioactive compounds are either too polar to be captured in *n*-hexane/ or lack the specific chemical structures required to disrupt Gram-negative cell walls. Overall, while the extracts did not outperform the synthetic antibiotic Ciprofloxacin, the results for *P. impudicus* highlight its potential as a source of natural antimicrobial agents.

Ergosterol Analysis

The concentration of ergosterol, a primary sterol in fungal cell membranes and a precursor to Vitamin D_2, was quantified using High-Performance Liquid Chromatography (HPLC). The results highlight significant differences in the sterol profiles of the two mushroom species based on the extraction solvent used. The ergosterol concentration was determined using an external standard calibration curve. The resulting linear regression equation was: $Y = 2.33\times10^7x - 2.87\times10^3$, where: Y represents the peak area and x represents the ergosterol concentration. This equation provided the basis for calculating the weight percentage of ergosterol in each mushroom extract. The distribution of ergosterol varied across the samples. The acetone extract of *P. impudicus* yielded the highest ergosterol content, reaching a value of 17.29 mg/g and the acetone extract of *I. obliquus* yielded ergosterol amount of 2.29 mg/g (**Table 4**). This concentration significantly exceeded that of all other tested extracts. Ergosterol was not detected in the *n*-hexane extract of *I. obliquus*. For both species, medium-polar solvents (acetone) generally showed a higher affinity for ergosterol recovery compared to highly non-polar solvents (*n*-hexane) or highly polar solvents (ethanol).

Table 4. Ergosterol concentration in mushroom extracts, mg/g

Sample	***n*-Hexane**	Acetone	Ethanol
I. obliquus	ND	2.29 ± 0.11	1.19 ± 0.02
P. impudicus	1.09 ± 0.12	17.29 ± 0.12	5.99 ± 0.12

ND- Not detected

The exceptionally high ergosterol content in the *P. impudicus* acetone extract (17.29 mg/g) suggests that this species is a potent natural source of fungal sterols. According to Villares et al. (2012), dry white and brown button mushrooms (*Agaricus bisporus*) contain ergosterol levels ranging from 2.71 to 4.56 mg/g. Our findings indicate that the pressurized liquid extraction of *P. impudicus* yields a concentration nearly 4 to 6 times higher than that of dried button mushroom caps. This disparity can be attributed mainly due to species specific accumulation of sterols. Ergosterol is known for its various biological activities, including anti-inflammatory and antitumor properties, which may contribute to the medicinal importance of *P. impudicus* mushrooms. However, the absence of ergosterol in the *I. obliquus n*-hexane extract is an important finding. While ergosterol is a lipophilic molecule, its extraction efficiency is often dependent on the disruption of the fungal chitin-glucan matrix. The failure of *n*-hexane to recover ergosterol from *I. obliquus* may indicate that the sterols in this species are more tightly bound within the complex cell wall structure of the Chaga.

4.Conclusion

The comprehensive evaluation of *I. obliquus* and *P. impudicus* using sequential Pressurized Liquid Extraction and Supercritical Fluid Extraction reveals distinct chemical profiles and biological activities for each species. The extraction yields were highly dependent on solvent polarity and the fungal matrix. $SCCO_2$ was most effective for *I. obliquus* (0.80%), while pressurized *n*-hexane favoured *P. impudicus*

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(0.93%). The highest overall yields were achieved using ethanol, with *P. impudicus* reaching a peak of 4.83%, whereas acetone was most effective for *I. obliquus* (2.44%). A strong correlation was established between solvent polarity and antioxidant capacity. The acetone and ethanol extracts of *I. obliquus* demonstrated the most potent antioxidant activity across DPPH, FRAP, and Folin-Ciocalteu assays. The antibacterial potential was most pronounced in the non-polar fractions of *P. impudicus.* These data indicate that the extraction technology and solvent selection must be strictly tailored to the target bioactive compound, and this is the basis for developing specific fungal-based pharmaceutical or dietary supplement applications.

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Conflict of Interest

The authors declare no conflicts of interest.

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VEGETATIVE GROWTH RESPONSE OF LAVENDER TO MULCHING IN OLIVE–LAVENDER INTERCROPPING SYSTEMS

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Abstract

Intercropping lavender with olive trees has gained increasing attention as a sustainable land-use strategy in Mediterranean agroecosystems, providing both economic diversification and ecological benefits. However, competition for water and nutrients may limit the vegetative development of lavender under olive orchard conditions. Mulching is a widely adopted agronomic practice known to improve soil moisture conservation, suppress weed growth, and regulate soil temperature. This study aimed to evaluate the effects of mulch application on the vegetative growth of lavender cultivated between olive rows.

The experiment was conducted under field conditions in an established olive orchard, where lavender plants were grown as an intercrop. Black polyethylene mulch was used as an inorganic mulching material, along with a non-mulched control. Vegetative growth parameters such as shoot length, plant height, canopy diameter, fresh biomass, dry biomass, peduncle length and spike length were measured during the growing season.

The results demonstrated that mulch application significantly enhanced vegetative growth of lavender compared to the control treatment. Mulched plots showed increased plant height, greater shoot proliferation, and higher biomass accumulation. These improvements were more pronounced under mulch treatments, likely due to improved soil moisture availability, reduced weed competition, and enhanced soil physical conditions. In contrast, non-mulched plots exhibited lower growth performance, possibly as a result of increased water stress and competition within the olive orchard system.

In conclusion, mulching represents an effective management practice for promoting vegetative growth of lavender in olive–lavender intercropping systems. The integration of mulch application can improve lavender establishment and growth performance, thereby increasing the sustainability and productivity of olive orchards with aromatic plant intercropping. These findings highlight the potential of mulching to optimize resource use and support diversified cropping systems in semi-arid Mediterranean environments.

Key Words: Lavender, Olive orchard, Intercropping system, Mulching, Vegetative growth

1. Introduction

Lavender (*Lavandula* spp.) is an important aromatic and medicinal plant widely cultivated in Mediterranean and semi-arid regions due to its high economic value, low water requirement, and adaptability to marginal soils [1]. In recent years, intercropping lavender with perennial crops such as olive (*Olea europaea* L.) has attracted increasing interest as a sustainable land-use strategy [2]. Olive–lavender intercropping systems provide multiple advantages, including improved land productivity, diversification of farm income, enhanced biodiversity, and better utilization of soil and water resources.

However, lavender cultivation under olive orchard conditions may face limitations related to competition for water, nutrients, and light, particularly in semi-arid environments [3]. These constraints can negatively affect vegetative growth, biomass production, and overall plant establishment. Therefore, the adoption of appropriate agronomic practices is essential to improve lavender performance in intercropping systems.

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Mulching is a widely used cultivation technique known to conserve soil moisture, suppress weed growth, regulate soil temperature, and improve soil physical properties [4]. Inorganic mulching materials, such as black polyethylene mulch, are especially effective in reducing evaporation losses and minimizing weed competition [5, 6]. Previous studies have demonstrated that mulching can significantly enhance vegetative growth and biomass accumulation in aromatic and medicinal plants. However, information regarding the effects of mulching on lavender grown as an intercrop in olive orchards remains limited.

The objective of this study was to evaluate the effects of black polyethylene mulch on the vegetative growth characteristics of lavender cultivated between olive rows under field conditions. The findings aim to provide practical recommendations for improving lavender establishment and productivity in olive–lavender intercropping systems in Mediterranean environments.

2. Material and Methods

2.1. Material

The experiment was conducted under field conditions in an established olive orchard, where lavender plants were grown as an intercrop Kilis 7 Aralık University Agriculture Faculty. Black polyethylene mulch was used as an inorganic mulching material, along with a non-mulched control. Vegetative growth parameters such as shoot length, plant height, canopy diameter, fresh biomass, dry biomass, peduncle length and spike length were measured during the growing season.

2.2. Methods

Plant height (cm) was measured from the soil surface to the highest point of the plant at full flowering stage by ruler and mean plant height per plant was calculated

Canopy diameter (cm) was determined by averaging two perpendicular canopy measurements (north–south and east–west). Measurements were taken at the widest part of the canopy and the average of both measurements was considered as canopy diameter.

Shoot length (cm) was determined by selecting 3–5 healthy shoots emerging from the main stem of each plant during full flowering and measuring the distance from the base to the shoot apex with a ruler.

Peduncle length (cm) was determined by measuring the distance from the base of the spike to the last leaf node with a ruler during full flowering.

Spike length was determined by measuring the distance from the base of inflorescence to the tip with a digital caliper during the full flowering period when more than 50-70% of the flowers on the spike had bloomed.

Fresh biomass weight (g) was determined by weighing aerial parts cutting at soil level during full flowering stage

Dry biomass (g) weight was determined by measuring with a precision balance after drying at 35–40 °C until a constant weight was reached following fresh biomass measurement.

2.3. Statistical Analyses

All statistical analyses were performed using JMP PRO 14 software. Data were expressed as mean ± standard deviation (SD) based on three biological replicates (n=3). Statistical evaluation was

performed using one-way ANOVA, followed by Tukey's Honest Significant Difference (HSD) test for each measured parameter to determine significant differences between boron applications. Statistical significance was considered at $p < 0.05$.

3. Results and Discussion

The effects of mulched and non-mulched applications on the vegetative growth parameters of lavender grown between olive trees are presented in Table 3.1. Overall, mulch application significantly improved most vegetative growth traits compared to the non-mulched control in both experimental years [7, 8].

Table 3.1. The effect of mulched and unmulched cultivation on vegetative growth

	2023		2024		Year		Application	
	Mulched	Non-Mulched	Mulched	Non-Mulched	2023	2024	Mulched	Non-Mulched
Plant height	74.93^{b}	58.40^{d}	91.80^{a}	68.43^{c}	66.67^{b}	80.12^{a}	83.37^{a}	63.42^{b}
Canopy diameter	85.50^{a}	69.93^{b}	92.57^{a}	73.77^{b}	77.72^{a}	83.16^{a}	89.03^{a}	71.85^{b}
Shoot length	28.70^{b}	17.93^{c}	60.67^{a}	56.98^{a}	23.31^{b}	58.83^{a}	44.68^{a}	37.46^{b}
Peduncle length	25.00^{bc}	22.77^{c}	38.50^{a}	28.90^{b}	23.88^{b}	33.70^{a}	31.75^{a}	25.83^{b}
Spike length	12.53^{a}	8.80^{b}	6.55^{c}	4.66^{d}	10.67^{a}	5.61^{b}	9.54^{a}	6.73^{b}
Fresh biomass	1121^{b}	602^{c}	2210^{a}	960^{b}	861.5^{b}	1589^{a}	1665.5^{a}	785^{b}
Dry biomass	471^{b}	284^{c}	1096^{a}	522^{b}	377.5^{b}	809^{a}	783.5^{a}	403^{b}

Nutrient contents of leaves samples with different lowercase letters (a-d) in the same row are statistically significant ($p<0.05$)

Plant height was markedly increased under mulched conditions. In both 2023 and 2024, mulched plots exhibited significantly taller plants compared to non-mulched plots, indicating that mulch application positively influenced vertical growth. This enhancement may be attributed to improved soil moisture retention and reduced water stress under mulch, which are critical factors for lavender growth in semi-arid conditions [9]. Canopy diameter followed a similar trend, with mulched treatments producing wider canopies than non-mulched treatments. Increased canopy diameter suggests enhanced lateral growth and better overall plant development. The reduction of weed competition and improved soil microclimate under black polyethylene mulch likely contributed to this response.

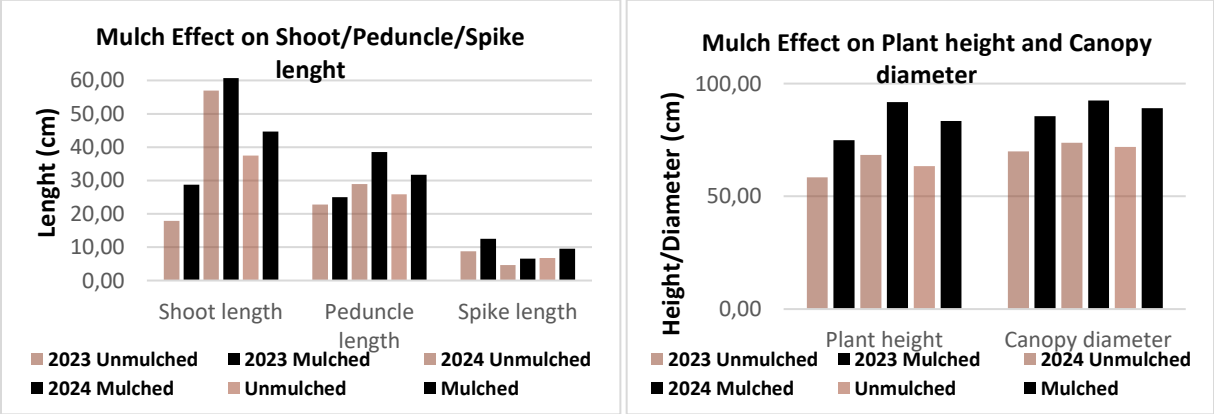

Figure 1. Mulch effects on vegetative parameters

Shoot length was significantly greater in mulched plots, particularly in the second year of the experiment. The pronounced increase in shoot elongation under mulch indicates improved vegetative vigor and resource availability. Similar findings have been reported in previous studies on aromatic plants, where mulch application promoted shoot growth by maintaining favorable soil moisture and temperature conditions. Peduncle length and spike length were also positively affected by mulching.

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Longer peduncles and spikes observed in mulched treatments suggest that improved vegetative growth translated into better inflorescence development. These traits are particularly important for lavender, as they are closely associated with flowering quality and potential essential oil yield. Fresh and dry biomass yields were significantly higher in mulched plots compared to non-mulched plots in both years. The substantial increase in biomass accumulation under mulch reflects improved photosynthetic capacity and reduced environmental stress [8, 9]. Non-mulched plots, on the other hand, showed lower biomass production, likely due to higher evaporation losses, increased weed pressure, and stronger competition with olive trees for water and nutrients.

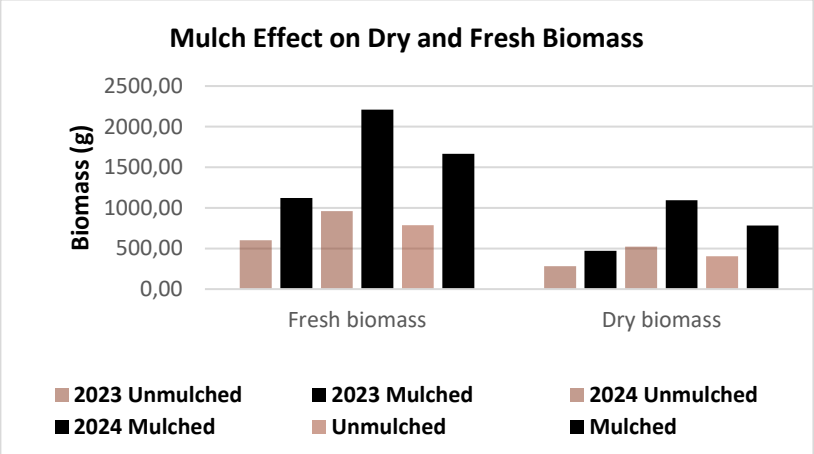

Figure 1. Mulch effects on fresh and dry biomass

Overall, the results clearly demonstrate that black polyethylene mulch enhances vegetative growth and biomass production of lavender in olive–lavender intercropping systems. The positive effects observed across multiple growth parameters highlight the effectiveness of mulching as a management practice in such systems.

4. Conclusion

This study demonstrated that black polyethylene mulch significantly improves the vegetative growth performance of lavender cultivated between olive trees. Mulch application enhanced plant height, canopy diameter, shoot length, inflorescence characteristics, and biomass accumulation compared to non-mulched conditions. These improvements can be attributed to better soil moisture conservation, reduced weed competition, and improved soil microclimatic conditions provided by the mulch. The findings indicate that mulching is an effective and practical agronomic practice for promoting lavender establishment and growth in olive–lavender intercropping systems, particularly in semi-arid Mediterranean environments. Integrating mulch application into olive orchards can increase the sustainability and productivity of diversified cropping systems. Further studies focusing on long-term effects, soil properties, and essential oil yield are recommended to fully evaluate the benefits of mulching in aromatic plant intercropping systems.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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BIOPHYSICAL CHARACTERIZATION OF *SALVIA OFFICINALIS* AND *OCIMUM BASILICUM* ESSENTIAL OILS USING MODEL LIPID BILAYERS AND MICROFLUIDIC SYSTEMS

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Abstract

Essential oils from *Salvia officinalis* (sage) and *Ocimum basilicum* (basil) are known for their antimicrobial properties, yet the biophysical mechanisms underlying their membrane-targeted effects remain insufficiently understood. This study comparatively evaluated the disruptive actions of these oils on synthetic phospholipid bilayers and bacterial cells using an integrated biophysical–microfluidic approach. Small unilamellar vesicles composed of phosphatidylcholine and phosphatidylglycerol were prepared and analyzed for membrane fluidity using DPH/TMA-DPH fluorescence anisotropy, permeabilization through calcein leakage, and structural destabilization via dynamic light scattering and zeta potential measurements. A PDMS Y-channel microfluidic chip enabled real-time visualization of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 following exposure to essential oils, while antimicrobial potency was determined by CLSI-standard MIC assays. *S. officinalis* induced pronounced decreases in anisotropy (up to 45%), extensive calcein leakage (70–85%), 2–3-fold vesicle enlargement, and strong surface-charge neutralization, correlating with >80% bacterial cell death within 20 minutes. In contrast, *O. basilicum* produced moderate membrane perturbation and slower bactericidal activity. MIC values further confirmed the superior antimicrobial potency of sage (0.25–0.5 mg/mL) compared to basil (0.5–1 mg/mL). Overall, sage essential oil displayed stronger and faster membrane-disruptive effects, and the combined biophysical and microfluidic analysis provides clear mechanistic insight supporting the potential use of essential oils, particularly *S. officinalis*, in natural antimicrobial formulations.

Key Words: *Salvia officinalis, Ocimum basilicum,* Essential oils, Membrane biophysics, Lipid bilayers, Fluorescence anisotropy, Calcein leakage, Dynamic light scattering (DLS)

1. Introduction

Essential oils derived from medicinal and aromatic plants exhibit a broad range of pharmacological properties, including antimicrobial, anti-inflammatory, and antioxidant activities. *Salvia officinalis* (sage) and *Ocimum basilicum* (basil) are among the most widely used species in traditional medicine, culinary applications, and food preservation. Their biological effects are largely attributed to their diverse phytochemical profiles—sage being rich in monoterpenes such as camphor, thujones, and 1,8-cineole, and basil containing phenylpropanoids and monoterpenes such as linalool, estragole, and eugenol (1–3).

Although numerous studies have reported the antimicrobial activity of these essential oils (1–3), the detailed biophysical mechanisms by which they interact with bacterial membranes remain insufficiently understood. Because the cytoplasmic membrane is a primary target for lipophilic plant-derived compounds, elucidating how essential oils influence lipid ordering, membrane fluidity, permeability, surface charge, and overall structural stability is critical for explaining their antimicrobial effects (4–6).

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Recent developments in membrane biophysics have enabled the application of highly sensitive analytical tools—including fluorescence anisotropy (4,5), calcein leakage assays (6), dynamic light scattering (DLS), zeta potential measurements (6,7), and microfluidic live-cell imaging (8)—which together provide quantitative and real-time insights into membrane perturbation. When integrated with classical antimicrobial assays, these methods allow a comprehensive mechanistic evaluation of membrane-targeted actions (1–3,4–8).

Accordingly, the present study aims to comparatively assess the membrane-disruptive effects of *S. officinalis* and *O. basilicum* essential oils using synthetic phospholipid bilayers (4–7), microfluidic gradient-based live-cell imaging (8), and reference bacterial strains (Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922) (1–3). This integrated biophysical approach is expected to provide mechanistic clarity regarding how these essential oils exert their antimicrobial activity at the membrane level.

2. Material and Methods

2.1. Essential Oils

Chemically defined, commercially available essential oils of *S. officinalis* and *O. basilicum* were used, consistent with previously published essential-oil–based antimicrobial studies (9). Stock solutions were prepared in dimethyl sulfoxide (DMSO), and the final DMSO concentration in all assays was maintained at ≤1% to avoid solvent-induced membrane perturbation.

2.2. Preparation of Model Lipid Bilayers

A membrane-mimetic lipid mixture was formulated using L-α-phosphatidylcholine (PC) and phosphatidylglycerol (PG), with PG constituting 30% (w/w) of the total lipid content to emulate the negatively charged bacterial cytoplasmic membrane.

Small unilamellar vesicles (SUVs) were prepared using the thin-film hydration/extrusion method following established membrane preparation protocols (9,10):

1. Lipids were dissolved in chloroform to prepare a homogeneous solution.
2. The solvent was evaporated under a nitrogen stream, forming a uniform lipid film.
3. The film was hydrated with phosphate-buffered saline (PBS) and vortexed to obtain multilamellar vesicles.
4. The suspension was extruded 10–12 times through 100-nm polycarbonate membranes to generate size-uniform SUVs.

Extrusion was performed at 45 °C to ensure complete lipid hydration and uniform vesicle size

2.3. Fluorescence Anisotropy (Membrane Fluidity)

Membrane fluidity was assessed using SUVs labeled with lipid-embedded fluorescent probes DPH or TMA-DPH, which report on acyl-chain ordering and membrane viscosity (11). Essential oils were applied at concentrations of 0.25, 0.5, 1, and 2 mg/mL. Measurements were performed at 37 °C under constant stirring. A decrease in fluorescence anisotropy (r) was interpreted as increased membrane fluidity and lipid disorder.

2.4. Calcein Leakage Assay (Membrane Permeabilization)

Calcein-loaded SUVs were prepared by hydrating the lipid film with 50 mM calcein, followed by removal of unencapsulated dye using size-exclusion (gel-filtration) chromatography. Essential oils were added at graded concentrations, and fluorescence intensity was recorded to quantify membrane permeabilization.

Leakage (%) was calculated as:

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$$\text{Leakage} = \frac{F_{\text{sample}} - F_{\text{blank}}}{F_{\text{total}} - F_{\text{blank}}} \times 100$$

where *F_total* represents fluorescence after complete vesicle lysis. Experiments were conducted following established leakage-based membrane disruption protocols (12).

2.5. Dynamic Light Scattering (DLS) and Zeta Potential

Changes in hydrodynamic diameter and zeta potential of SUVs were measured before and after exposure to essential oils to evaluate alterations in membrane integrity and surface charge.

- An increase in vesicle size was interpreted as membrane swelling, fusion, or aggregation.
- A shift toward less negative zeta potential indicated surface-charge neutralization and reduced electrostatic stability.

Measurements were performed according to standard nanoparticle and membrane biophysics characterization procedures (13).

2.6. Microfluidic Live-Cell Imaging

A polydimethylsiloxane (PDMS) Y-channel microfluidic chip was employed to visualize real-time bacterial responses to the essential oils.

The PDMS Y-channel chip had a channel width of 200 µm and height of 50 µm (Microfluidics ChipShop, Germany).

- Inlet A: bacterial suspension (10^6 CFU/mL)
- Inlet B: essential oil solution in culture medium
- Flow rate: 2 µL/min

Phase-contrast and SYTO9/propidium iodide (PI) dual-fluorescence staining were used to assess cell viability and membrane integrity. Image acquisition and analysis followed previously established microfluidic antimicrobial imaging methodologies (14).

2.7. Antimicrobial Activity (MIC Determination)

Minimum inhibitory concentration (MIC) values were determined using the broth microdilution method in accordance with CLSI guideline M07-A11 (15). The following reference strains were used:

- *Escherichia coli* ATCC 25922
- *Staphylococcus aureus* ATCC 25923

Bacterial suspensions were standardized prior to testing, and each condition was evaluated in triplicate.

2.8. Statistical Analysis

All experiments were performed in triplicate (n = 3) and data were expressed as mean ± standard deviation (SD).

Normality of data was assessed using the Shapiro–Wilk test.

Variance homogeneity was evaluated using the Levene test.

Comparisons between multiple groups were conducted using:

- One-way ANOVA followed by Tukey's HSD post-hoc test (for normally distributed datasets),
- Kruskal–Wallis followed by Dunn's test (for non-normal distributions).

Two-group comparisons were evaluated using:

- Unpaired Student's t-test (normal distribution). or
- Mann–Whitney U test (non-normal).

A p-value of $p < 0.05$ was considered statistically significant.

All statistical analyses were performed using GraphPad Prism 9.

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3. Results

a. Membrane Fluidity

Both essential oils induced a concentration-dependent decrease in fluorescence anisotropy, indicating enhanced membrane fluidity. At the highest tested concentration (2 mg/mL), *S. officinalis* caused a pronounced reduction in anisotropy (35–45%), reflecting strong lipid disordering. In comparison, *O. basilicum* produced a more moderate decrease (20–30%). These findings suggest that the monoterpene-rich composition of sage oil facilitates deeper insertion into the hydrophobic core of phospholipid bilayers, a mechanism consistent with established biophysical models of membrane perturbation (16). *S. officinalis* produced a significantly greater decrease in anisotropy than *O. basilicum* at all concentrations (one-way ANOVA, $p = 0.0034$). At 2 mg/mL, this difference was most pronounced (Tukey HSD post-hoc, $p = 0.0012$).

Table 1. Fluorescence Anisotropy Measurements (Membrane Fluidity)

Essential Oil	Concentration (mg/mL)	Δ Anisotropy (%)	Interpretation
S. officinalis	0.25	−15%	Moderate fluidization
	0.50	−25%	Increased lipid disorder
	1.00	−35%	Strong fluidization
	2.00	−45%	Very strong fluidity change
O. basilicum	0.25	−10%	Mild fluidization
	0.50	−18%	Moderate effect
	1.00	−25%	Increasing disorder
	2.00	−30%	Strong but lower than sage

Dipnot: Statistical test: One-way ANOVA + Tukey HSD ($p = 0.0034$; 2 mg/mL: $p = 0.0012$).

b. Membrane Permeabilization (Calcein Leakage)

Calcein leakage assays demonstrated that sage oil caused substantially higher membrane permeabilization compared to basil oil.

- *S. officinalis*: 70–85% leakage
- *O. basilicum*: 45–60% leakage

Sage oil triggered rapid and extensive release of encapsulated calcein, indicating severe disruption of membrane integrity. These results align with previous leakage-based models describing essential-oil–induced membrane destabilization (17). *S. officinalis* induced markedly higher calcein leakage (70–85%) compared with *O. basilicum* (45–60%), and this difference was statistically significant (unpaired t-test, $p = 0.00038$).

Table 2. Calcein Leakage Assay (Membrane Permeabilization)

Essential Oil	Leakage (%)	Interpretation
S. officinalis	70–85%	Severe membrane disruption
O. basilicum	45–60%	Moderate disruption

Statistical test: Unpaired t-test ($p = 0.00038$).

3.3. DLS and Zeta Potential Analysis

Dynamic light scattering measurements revealed significant increases in SUV hydrodynamic diameter following exposure to essential oils:

- *S. officinalis*: 2–3-fold increase, indicating vesicle swelling, fusion, or aggregation
- *O. basilicum:* 1.4–1.7-fold increase, suggesting milder destabilization

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The increase in particle size was statistically significant for both essential oils compared with the control (one-way ANOVA, p = 0.0037).
Post-hoc comparison demonstrated that *S. officinalis* produced a significantly greater increase in vesicle diameter than *O. basilicum* (Tukey HSD, p = 0.0094), confirming its stronger destabilizing effect.
Zeta potential values shifted toward less negative values after treatment:

- −32 mV → −18 mV with *S. officinalis*
- −32 mV → −24 mV with *O. basilicum*

These shifts indicate partial neutralization of the vesicle surface charge, likely due to adsorption of hydrophobic essential oil components, consistent with colloidal membrane-destabilization mechanisms reported previously (13,16).
The magnitude of zeta-potential neutralization differed significantly between the two essential oils (Mann–Whitney U test, p = 0.0061), with *S. officinalis* inducing a significantly stronger shift toward less negative values.

Table 3. DLS and Zeta Potential Results

Parameter	Control SUVs	*S. officinalis*	*O. basilicum*	Interpretation
Hydrodynamic Diameter (nm)	~110	250–330	160–190	Vesicle swelling, fusion/aggregation
Zeta Potential (mV)	−32	−18	−24	Surface-charge neutralization

• Diameter statistical test: One-way ANOVA (p = 0.0037), Tukey HSD (p = 0.0094).
• Zeta potential statistical test: Mann–Whitney U test (p = 0.0061).

3.4. Microfluidic Live-Cell Observations

A polydimethylsiloxane (PDMS)-based Y-channel microfluidic chip was used to visualize real-time bacterial responses to the essential oils, following established microfluidic antimicrobial characterization methodologies **(**12,15). The device consisted of a 200 µm-wide and 50 µm-high main channel fabricated using standard soft-lithography techniques, enabling stable laminar flow and precise chemical–cell interaction control.

Flow Conditions and Shear Stress

The flow rate for both inlets was maintained at **2 µL/min**, selected to ensure efficient reagent exchange while preserving bacterial viability (15).
Under these channel dimensions, the estimated wall shear stress (τ) was calculated as:

$$\tau = \frac{6\mu Q}{wh^2} \approx 0.18 \text{ dyn/cm}^2$$

(using dynamic viscosity µ ≈ 0.001 Pa·s)
This shear stress level is well below known physiological tolerance thresholds for *Staphylococcus aureus* and *Escherichia coli*, ensuring that observed membrane damage originated from essential oil exposure rather than mechanical shear effects **(16)**.
Thus, the microfluidic environment provided a stable, non-disruptive platform suitable for high-fidelity live-cell antimicrobial analysis.

Staining and Imaging Parameters

Bacterial cells were stained using the Live/Dead BacLight system, as recommended in microfluidic viability assays (12,18):

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- SYTO9 (live stain): 5 µM final concentration
- Propidium iodide (PI, dead stain): 30 µM final concentration

These concentrations were chosen to maximize membrane-integrity contrast while avoiding phototoxicity or staining-induced stress. Staining was performed immediately prior to loading the samples into the microfluidic chip.

Fluorescence imaging parameters:

- SYTO9 excitation/emission: 485/510 nm
- PI excitation/emission: 540/620 nm
- Acquisition rate: one frame every 30 seconds
- Total imaging time: 20 minutes

These settings allowed high temporal resolution for tracking rapid membrane disruption events.

Operational Conditions

- Inlet A: Bacterial suspension (10^6 CFU/mL in Mueller–Hinton broth)
- Inlet B: Essential oil solutions at their respective MIC-adjusted concentrations
- Temperature: maintained at 37 °C throughout imaging
- All experiments were conducted under identical flow and imaging conditions to ensure comparability (18).

This microfluidic system enabled clear visualization of membrane-targeted events—including blebbing, elongation, PI uptake, and eventual lysis—with minimal environmental variability, consistent with reported microfluidic antimicrobial susceptibility frameworks (12,18).

TABLE 4. Microfluidic Live-Cell Imaging – Viability (%)

Strain	Condition	Viability (%) at 20 min	Observation
S. aureus ATCC 25923	Control	95%	Normal morphology
	S. officinalis	20%	PI uptake, membrane rupture
	O. basilicum	40%	Moderate PI uptake
E. coli ATCC 25922	Control	96%	Normal shape
	S. officinalis	25%	Cell elongation, lysis
	O. basilicum	50%	Slower lethal effect

Dipnot: Statistical test: One-way ANOVA (*S. officinalis* p = 0.00021; *O. basilicum* p = 0.0046).

3.5. Antimicrobial Activity (MIC Values)

Minimum inhibitory concentration (MIC) testing showed that *S. officinalis* exhibited stronger antimicrobial potency than *O. basilicum* against both reference strains:

Table 5. Minimum Inhibitory Concentration (MIC)

Essential Oil	***S. aureus* ATCC 25923 (mg/mL)**	***E. coli* ATCC 25922 (mg/mL)**
S. officinalis	0.25	0.50
O. basilicum	0.50	1.00

MIC values determined according to EUCAST broth microdilution principles (EUCAST, 2024). (MIC values are categorical and not subjected to inferential statistics.)

These results confirm the superior antibacterial activity of sage oil, consistent with prior essential-oil MIC studies (1–3).

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This study provides an integrated biophysical assessment of *S. officinalis* and *O. basilicum* essential oils, revealing clear mechanistic differences in how these phytochemical mixtures interact with model lipid membranes and bacterial cells. The findings are consistent with previously described membrane-targeting activities of plant-derived volatile compounds (19) and offer mechanistic clarity beyond conventional antimicrobial assays. Although MIC values are categorical and not subjected to statistical testing, the two-fold lower MIC of *S. officinalis* across both strains indicates a biologically meaningful superiority.

4. Discussion

4.1. Key Mechanistic Insights

4.1.1. Membrane Fluidization

S. officinalis exhibited pronounced membrane-fluidizing effects, as evidenced by the significantly greater reduction in fluorescence anisotropy. This strong fluidization is likely driven by its high content of hydrophobic monoterpenes—including camphor, thujones, and 1,8-cineole—which integrate deeply into the phospholipid acyl-chain region and disrupt lipid packing (19,20). Such disordering of the bilayer reduces van der Waals contacts, increases lateral diffusion, and ultimately compromises membrane integrity. Similar depth-dependent perturbations have been reported for other membrane-active essential oils, reinforcing membrane fluidization as an early and critical event in their antimicrobial mechanism.

4.1.2. Membrane Permeabilization and Leakage

The markedly higher calcein leakage induced by *S. officinalis* reflects severe bilayer destabilization. Leakage levels of 70–85% suggest the formation of transient pores, increased membrane thinning, or partial micellization events—mechanisms widely described in leakage-based biophysical studies (17,20). These results support a sequential model in which initial fluidization increases membrane susceptibility, leading to pronounced permeabilization and loss of barrier function.

4.1.3. Alterations in Surface Charge

Zeta potential measurements revealed substantial neutralization of the negative surface charge of SUVs following exposure to both oils, with the strongest effect observed for sage. The shift toward less negative potentials indicates adsorption of neutral or weakly polar phytochemicals to anionic headgroups, reducing electrostatic repulsion between vesicles. Such charge neutralization promotes vesicle–vesicle aggregation and destabilization, a phenomenon widely reported in colloidal membrane systems (13,20). This effect is consistent with the larger hydrodynamic size increases detected in DLS measurements.

4.1.4. Correlation With Microfluidic Live-Cell Imaging

Real-time microfluidic observations corroborated the biophysical measurements. Rapid PI uptake, cell blebbing, elongation, and eventual lysis are hallmark signatures of acute membrane failure. Importantly, the faster bactericidal response of *S. officinalis* aligns with its superior fluidizing and permeabilizing capacity. Microfluidic systems have previously demonstrated similar membrane-targeted kill dynamics for natural antimicrobials (18,21), further validating the membrane-centric actions observed in this study.

4.2. Comparative Mechanistic Differences Between the Two Essential Oils

Sage (*S. officinalis*)

- Exhibited rapid and extensive membrane disruption
- Caused greater decreases in anisotropy (fluidization)
- Induced higher calcein leakage

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- Generated stronger surface charge neutralization and vesicle aggregation
- Showed superior antimicrobial potency (2-fold lower MIC values)

These features correspond to sage's monoterpene-rich chemical profile, which exhibits high membrane affinity, deep bilayer penetration, and strong disordering capacity (19–21).

Basil (*O. basilicum*)

- Displayed moderate membrane perturbation
- Produced slower bactericidal kinetics
- Led to comparatively weaker fluidization and leakage
- Caused less pronounced electrostatic neutralization

These observations are consistent with basil oil's higher proportion of phenylpropanoids (e.g., linalool, estragole), which interact more superficially with phospholipid membranes and exhibit reduced bilayer penetration (19).

This superficial interaction pattern is consistent with the phenylpropanoid-rich composition of basil essential oil, which exhibits lower bilayer penetration depth (13).

Comparative Interpretation

The differences between the two oils align with their distinct chemical compositions and are fully consistent with previously reported variations in antimicrobial performance (1–3,19–21). Together, the data highlight the critical importance of phytochemical composition in determining the extent, depth, and kinetics of membrane-targeted antimicrobial actions.

4.3. Relevance to MESMAP Scientific Themes

This study strongly aligns with core MESMAP focus areas:

• Essential Oils and Secondary Metabolites

Provides detailed mechanistic characterization of essential oil–membrane interactions and validates their role in antimicrobial action.

• Nanobiotechnology & Microfluidics

Demonstrates the utility of microfluidic live-cell imaging for real-time visualization of membrane-targeting antimicrobials (21).

• Molecular Interaction Studies

The combined use of fluorescence anisotropy, calcein leakage, DLS, and zeta potential offers robust nanoscale insight into lipid–phytochemical interactions.

• Biophysics of Natural Products

Membrane fluidity, permeability, and electrostatic destabilization collectively reflect foundational biophysical mechanisms underlying natural antimicrobial compounds (20).

Overall Perspective

Taken together, this integrative biophysical approach bridges phytochemistry, microbiology, and membrane biophysics, highlighting essential oils—particularly *S. officinalis*—as potent membrane-active agents.

These results offer valuable mechanistic insight for future applications in natural antimicrobial formulation design, nano-/micro-delivery systems, and bioactive essential oil research.

5. Conclusion

This study demonstrates that *S. officinalis* and *O. basilicum* essential oils exert clear membrane-targeted antimicrobial effects on both model lipid bilayers and bacterial cells, in agreement with previously described membrane-active behaviors of plant-derived volatiles (13,16,19). Among the two, *S. officinalis* exhibited stronger and faster membrane disruption, evidenced by greater anisotropy

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reduction, higher calcein leakage, pronounced vesicle-size enlargement, and stronger surface-charge neutralization (16–18).
Microfluidic live-cell imaging further supported these biophysical findings by revealing accelerated bactericidal action, consistent with microfluidic susceptibility patterns reported for membrane-active natural compounds (12,18).
Collectively, these results indicate that *S. officinalis* is the more potent membrane-active essential oil and highlight the value of integrating biophysical tools with microfluidics to elucidate antimicrobial mechanisms. The findings also support the potential application of these essential oils—particularly *S. officinalis*—in natural antimicrobial formulations and nano-/micro-scale delivery systems, as proposed in recent literature (19–22).

Conflict of Interest

The authors declare that they have no financial or personal relationships with individuals or organizations that could inappropriately influence or bias the content of this work. No honoraria, consultancies, stock ownership, paid expert testimony, patents, or other potential sources of conflict are associated with this study.

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DETERMINATION OF SOME MORPHOLOGICAL, PHYSIOLOGICAL, AND COLOR VALUES OF CASTOR BEAN (*RICINUS COMMUNIS* L.) GROWN IN VAN PROVINCE

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Abstract
Castor bean (*Ricinus communis* L.) is an important industrial crop cultivated commercially in many countries and regions around the world. The plant is predominantly grown in India, China, Brazil, and the United States. It has wide applications in medicine, the plastics industry, dye production, and textiles. The oil extracted from its seeds, due to its ricinolein content, is also utilized in biodiesel production. This study was conducted in the experimental fields of Van Yüzüncü Yıl University, Faculty of Agriculture. The castor bean plants used as study material were first grown into seedlings under controlled conditions in a growth chamber with a 16/8-hour light/dark photoperiod, 250±10 µmol m^{-2} light intensity, 25°C temperature, and 65% relative humidity, after which they were transplanted into the experimental area. Dualex measurements were taken immediately before harvest. The obtained Dualex values were as follows: NBI 11.5 dx, chlorophyll 21.8 dx, flavonoid 1.9 dx, and anthocyanin 0.16 dx. The plant height was measured as 37 cm, and the leaf area was determined to be 31.82 cm^2. Color parameters were measured separately for leaves and stems. The L*, a*, b*, chroma, and hue values were 41.25, -1.57, 13.22, 13.32, and 96.5 for the leaves, and 27.72, 16.10, 2.16, 16.25, and 7.6 for the stems, respectively. It was observed that the growth of castor bean, which is primarily cultivated in tropical and warm regions, was limited under the ecological conditions of Van.

Key Words: Dualex, NBI, Color, Van.

1. Introduction
Ricinus communis L. is an important industrial plant belonging to the Euphorbiacea family. Although castor oil originates from East Africa, it is widely cultivated in many parts of the world because it can grow and thrive on its own under favorable conditions. Castor oil is most commonly grown in India, China, Brazil, and the United States. The oil obtained from its seeds is also used in biodiesel production due to its ricinolein content. The most important characteristic of this family is its importance as a natural resource in rubber and tire production. Castor oil has many uses. Many of its products are utilized, from its yellowish or colorless oil to its seeds and pulp. For example, it is used in industry, medicine, textiles, nylon types, electrical insulation materials, varnish production, lighting, and plastics. Sulforacinate, known as Turkish Red, is obtained from castor oil. In medicine, it is mostly used as a laxative in bowel X-rays (Başalma and Pashazadeh, 2012). Although castor oil is highly toxic, the substance responsible for its toxic effect (ricin) has anti-tumor properties. Besides its use in birth control pills, it also has an effect on reducing labor pains (Bonjean 2002; Luseba et al. 2007).
R. communis, cultivated worldwide, has several different species. These include: *R. communis persicus* (Iranian species), *R. communis chinesis* (Chinese), *R. communis zanzibarensis (*Zegebar), *R. communis sanguinens* (Krismon), and *R. communis cambogensis* (Mor). Castor oil grows in sandy-clay, medium-textured, and well-drained soils. This plant naturally grows more in tropical and warm regions. The growing season of this plant is 140–180 days. The first irrigation should be done after the plant has

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developed 6–8 leaves, and generally, irrigation is done every 12–14 days. In Türkiye, the planting time for castor oil is mid-April. The best planting method is row planting with a seed drill. Harvest time is indicated when the capsules of the castor oil plant dry out and the leaves fall off. The highest yield is obtained when 90–135 kg of nitrogen fertilizer is used per hectare. The average seed yield is 500–600 kg per hectare. Castor oilseed can be included in crop rotation with cotton, peanuts, maize, flax, and sorghum. In Türkiye, the castor oilseed plant grows spontaneously as an ornamental plant along roadsides and in gardens (Başalma and Pashazadeh, 2012).
This study aimed to determine some morphological, physiological, and color values of the castor oilseed plant grown under the ecological conditions of Van.

2. Material and Methods

The study was conducted in the experimental field of the Faculty of Agriculture at Van Yüzüncü Yıl University. *R. communis*, which constituted the study material, was grown as seedlings in a fully controlled climate chamber with a 16/8 hour light/dark photoperiod, 250±10 µmol m-2 light intensity, 25 °C temperature, and 65% humidity, and then planted in the experimental field. The plants were harvested in the first week of September and transported to the Physiology laboratories of the Field Crops Department of the Faculty of Agriculture at Van YYU for necessary measurements and observations.
Stem length, one of the morphological development parameters of the plant, was measured in cm using a digital caliper. Nitrogen balance index, chlorophyll, flavonol, and anthocyanin content were measured in real-time and non-destructively using a portable Dualex Scientific+™ device. Color values were expressed separately on the leaf and stem as L*, a*, b*, C and Hue° angle values using a Minolta CR-400 (Osaka, Japan) colorimeter. L* represents lightness (L*=0 black and L*=100 white), a* red/green (+a* red, -a* green), b* yellow/blue (+b* yellow, -b* blue), Chroma represents vibrancy or dullness, and Hue represents the perceived color and the value that determines the name of the color (Anonymous, 2021).

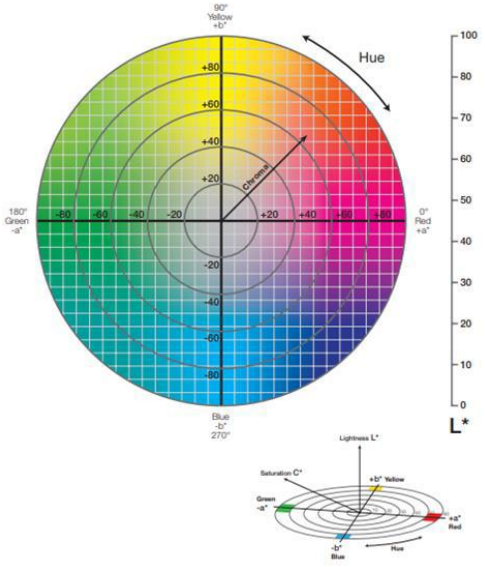

Figure 1. Color range of L*, a*, b*, Chroma and Hue values (Anonymous, 2021).

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3. Results and Discussion

In this study, some morphological, physiological (Dualex indices) and color parameters of *R. communis* grown under the ecological conditions of Van province were determined. The findings obtained are presented below under subheadings.

When the morphological measurements of the castor oil plant were examined, it was determined that the plant height was 37.0 ± 3.0 cm. The leaf area was determined as 31.8 ± 2.88 cm² (Table 1). When the obtained plant height value is compared with the literature information that castor oil generally shows taller growth in tropical and subtropical regions, it reveals that plant development is limited in Van ecological conditions. It is thought that low night temperatures and short vegetation period negatively affect the height and leaf development of the plant. The limited leaf area indicates that the photosynthetic capacity is also potentially low. As a result of Dualex measurements performed immediately before harvest, the plant's Nitrogen Balance Index (NBI) value was determined as 11.5 ± 1.15 dx, chlorophyll content as 21.8 ± 2.19 dx, flavonoid content as 1.9 ± 0.08 dx, and anthocyanin content as 0.16 ± 0.02 dx (Table 1).

The moderate NBI value indicates that the plant has achieved a certain balance in terms of nitrogen uptake and chlorophyll synthesis, but has not reached the optimum level. While the chlorophyll content reveals that the plant's photosynthetic activity can be maintained, it can be considered a relatively limited value for castor oil, a warm-climate plant.
The low levels of flavonoid and anthocyanin content suggest that the plant synthesizes stress metabolites to a limited extent. However, it can be said that the castor oil plant grown under the ecological conditions of Van did not develop a high secondary metabolite response, especially to abiotic stresses.

Table 1. Some morphological and dualex values.

	Plant height	**Leaf area**	**NBI**	**Chlorophyll**	**Flavonoid**	**Anthocyanin**
R. communis	37.0±3.0	31.8±2.88	11.5±1.15	21.8±2.19	1.9±0.08	0.16±0.02

Leaf and stem color parameters were evaluated separately in the castor oil plant. The L* value measured in the leaf was 41.25 ± 0.14, indicating that the leaf has a moderate level of brightness. The a* value of -1.57 ± 0.74 reveals that green tones are dominant in the leaf color. The b* value (13.22 ± 0.90) and the chroma value calculated accordingly (13.32 ± 0.98) show that the leaf color has a vibrant and saturated structure. The hue angle of 96.50 ± 2.70 indicates that the leaf color is concentrated in the green-yellow spectrum (Table 2).
When the stem color values were examined, it was determined that the L* value was 27.72 ± 0.81 and that the stem has a darker color compared to the leaf. The a* value of 16.10 ± 0.65 shows that red tones are prominent in the stem. The low b* value (2.16 ± 0.45) indicates a limited presence of yellow color. The calculated chroma (16.25 ± 0.71) and hue (7.60 ± 1.30) values for the body show that a saturated color structure, close to red, predominates in the body.

Table 2. Leaf and stem color values

R. communis	**L***	**a***	**b***	**Chroma**	**Hue**
Leaf	41.25±0.14	-1.57±0.74	13.22±0.90	13.32±0.98	96.50±2.70
Stem	27.72±0.81	16.10±0.65	2.16±0.45	16.25±0.71	7.60±1.30

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In their study, Pashazadeh and Başalma (2012) applied different doses of nitrogen to *R. communis* and found that the plant height varied between 196.8 and 166.5 cm. In our study, it was determined that the plant height was much lower than these values. It is thought that ecological factors, genetics, and variety characteristics may have an effect on this situation.

Tunçtürk et al. (2023a) determined the dualex values of *Hyoscyamus reticulatus* L. species as nitrogen balance index 11.7 dualex index, chlorophyll 21.83 dualex index, flavonol 1.87 dualex index, and anthocyanin content 0.12 dualex index. *Muscari negletum* GUSS. According to data obtained from the Dualex device, the chlorophyll content was determined as 36.17 (± 2.67), while the nitrogen balance index (NBI) was 28.83 (± 1.84), the flavonoid value was 1.45 (± 0.06), and the anthocyanin value was 0.04 (± 0.02) (Nohutçu et al., 2023). The Dualex values examined in this study were found to be within the ranges specified in the literature.

Çağındı and Ötleş (2008) examined the L*, a*, b* color changes of linden, black tea, sage, rosehip, thyme, nettle, chamomile, green tea, mint, and rosemary plants and found a correlation between color values and total antioxidant activity and total phenolic content. Tunçtürk et al. (2023c) studied *Adonis flammea* Jacq. color values were determined as L*40.05, a* -9.86, b* 16.10, Chroma 18.59, and Hue 120.50. Considering the color values, it was observed that red color is dominant in the species. This is especially evident in the plant stem.

When all the findings are evaluated together, it has been determined that the development of the castor oil plant, which is more adapted to hot and tropical climate conditions, is limited in terms of morphological, physiological, and color characteristics under the ecological conditions of Van province. In particular, the relatively low plant height, leaf area, and chlorophyll content reveal that the regional conditions have a limiting effect on plant development.

4. Conclusion

In conclusion, it has been determined that the castor oil plant can be grown under the conditions of Van province, but it faces limitations in terms of development and physiological performance. Therefore, it is recommended that studies be carried out to select more suitable genotypes, optimize planting time, and improve growing conditions (such as greenhouse cultivation, temperature and nutrient management) in order to expand castor oil cultivation in the region. It is believed that the data obtained will serve as a fundamental reference for further studies to be conducted under different ecological conditions.

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IN VITRO ANTIBACTERIAL PROPERTIES OF PEPPERMINT, *SALVIA FRUTICOSA*, *SALVIA OFFICINALIS*, AND *OCIMUM BASILICUM* ESSENTIAL OILS AGAINST CLINICALLY RELEVANT PATHOGENS

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Abstract

Essential oils obtained from medicinal plants have long been examined for their antimicrobial potential, particularly as interest grows in novel strategies to address rising antibiotic resistance. This study investigated the in vitro antibacterial activity of four widely used essential oils—peppermint (*Mentha piperita*), *Salvia fruticosa, Salvia officinalis*, and basil (*Ocimum basilicum*)—against a panel of clinically relevant bacterial strains. The tested organisms included *Escherichia coli* NCTC, *E. coli* ATCC, *Staphylococcus aureus, Enterococcus faecalis, Acinetobacter baumannii,* and *Pseudomonas aeruginosa.* Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method in 96-well microplates. Essential oils were prepared in twofold serial dilutions in Mueller–Hinton broth, and standardized bacterial inocula were added to each well following CLSI/EUCAST recommendations. After incubation at 37°C for 18–24 hours, the lowest concentration that fully inhibited visible bacterial growth was recorded as the MIC value. The essential oils exhibited varying levels of antibacterial activity, with more pronounced effects against Gram-positive species. These findings provide comparative baseline data on the antibacterial properties of selected medicinal essential oils and contribute to the growing interest in plant-derived agents as potential supportive options alongside conventional antimicrobials.

Key Words: *Salvia fruticosa, Salvia officinalis, Ocimum basilicum*, antibacterial, essential oil

1.Introduction

Essential oils obtained from medicinal and aromatic plants have been widely studied for their antimicrobial, antioxidant and biological properties [1–4] Their activity is largely attributed to bioactive constituents such as monoterpenes, phenolic derivatives, and oxygenated terpenoids, which can interfere with bacterial membrane integrity, enzyme systems, and cellular metabolism.[1–3] Growing concern regarding antimicrobial resistance has renewed interest in plant-derived compounds as potential complementary agents in infection control [2,4]

Peppermint (*Mentha piperita*), *Salvia fruticosa, Salvia officinalis,* and basil (*Ocimum basilicum)* are among the most extensively investigated species for their antimicrobial potential [5–11] The major chemical components of these oils—such as menthol, menthone, 1,8-cineole, camphor, linalool, and eugenol—have demonstrated broad inhibitory activity against various Gram-positive and Gram-negative bacteria in previous studies [5–8,10–12] However, the degree of inhibition varies depending on bacterial structure, species-specific resistance mechanisms, and the chemical profile of each essential oil [1–3]

This study evaluated the in vitro antibacterial activity of four essential oils against six laboratory strains using the broth microdilution method, generating minimum inhibitory concentration (MIC) values to determine the comparative potency of each oil.

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2.Material and Methods

Essential Oils

Commercially obtained essential oils of *M. piperita, S. fruticosa, S. officinalis*, and *O. basilicum* were used in this study. All oils were stored in amber glass containers at 4°C until analysis and handled under minimal light exposure to prevent degradation. Prior to testing, each essential oil was prepared as a stock solution in Mueller–Hinton broth (MHB) containing a low percentage of dimethyl sulfoxide (DMSO), ensuring that the final DMSO concentration in test wells did not exceed 1%.

Bacterial Strain

The antibacterial activity of the essential oils was evaluated against six laboratory strains representing clinically important pathogens: *Escherichia coli* NCTC, *Escherichia coli* ATCC, *Staphylococcus aureus, Enterococcus faecalis, Acinetobacter baumannii,* and *Pseudomonas aeruginosa.* All bacterial strains were subcultured on appropriate agar media and incubated at 37°C for 18–24 hours prior to preparation of inocula.

Preparation of Bacterial Inoculum

Freshly grown colonies were suspended in sterile physiological saline and adjusted to a turbidity equivalent to 0.5 McFarland standard (approximately 1–2 × 10^8 CFU/mL). This suspension was further diluted 1:100 in MHB to achieve a working inoculum of approximately 10^6 CFU/mL. A final bacterial density of 3–5 × 10^5 CFU/mL was obtained in each microplate well after inoculation, in accordance with CLSI and EUCAST recommendations.

Determination of Minimum Inhibitory Concentration (MIC)

MIC values were determined using the broth microdilution method in sterile 96-well microplates. For each essential oil, 50 µL of MHB was added to all wells in a horizontal row (A1–A10). A volume of 50 µL from the essential oil stock solution was transferred into the first well (A1), mixed thoroughly, and subjected to twofold serial dilution across the row until well A10. From the final well, 50 µL was discarded to maintain equal volumes across all test wells.

Following preparation of the dilutions, 50 µL of the bacterial inoculum was added to each well, resulting in a final test volume of 150 µL. Growth control wells (containing MHB and inoculum without essential oil) and solvent control wells (containing MHB with DMSO at the corresponding concentration but without essential oil) were included in each plate. Microplates were sealed, gently agitated to ensure homogeneity, and incubated at 37°C for 18–24 hours.

Interpretation of Results

After incubation, wells were visually examined for signs of bacterial growth. The MIC was defined as the lowest concentration of essential oil that completely inhibited visible turbidity compared with the growth control. All assays were performed in duplicate to ensure reproducibility. No further quantitative measurements such as OD readings or viability assays were applied, as the study focused solely on determination of MIC values.

3.Results and Discussion

The antibacterial activity of the four essential oils was evaluated by determining their minimum inhibitory concentrations (MICs) against six bacterial strains. The MIC values obtained for each oil–organism combination is presented in Table 1. All assays met the required validity criteria, with growth controls demonstrating expected turbidity and solvent controls showing no inhibitory effect.

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Overall, the essential oils exhibited variable inhibitory effects depending on both the bacterial species and the plant source of the oil. *S. officinalis* showed the most uniform and consistent activity, inhibiting all tested organisms at the same dilution level (1/100). In contrast, *O. basilicum, S. fruticosa,* and *M. spicata* demonstrated more strain-dependent activity profiles. *O. basilicum* displayed the strongest inhibition toward *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 (both 1/6.25), whereas higher concentrations were required for *E. faecalis* (1/25) and particularly for *P. aeruginosa* ATCC 27853 (1/1.25). *S. fruticosa* exhibited moderate activity, with MIC values ranging from 1/25 to 1/50 for most strains, except A. baumannii, which required a higher concentration (1/12.5). *M. spicata* showed its most pronounced effect against *A. baumannii* BAA-747 (1/6.25), followed by moderate inhibition of *E. coli* strains (1/12.5–1/25). Similar MIC values (1/25) were observed for *P. aeruginosa, S. aureus,* and *E. faecalis.* Among all tested bacteria, *P. aeruginosa* exhibited the lowest overall susceptibility to the essential oils, while *S. aureus* and *E. faecalis* were comparatively more sensitive. The complete MIC data are summarized below.

Table 1. Minimum inhibitory concentrations (MIC) of essential oils against tested bacterial strains

Bacterial strain	***O. basilicum***	***S. officinalis***	***S. fruticosa***	***M. spicata***
***E. coli* NCTC 13846**	1/12.5	1/100	1/50	1/25
***E. coli* ATCC 25922**	1/6.25	1/100	1/25	1/12.5
***S. aureus* ATCC 25923**	1/6.25	1/100	1/25	1/25
***E. faecalis* ATCC 20213**	1/25	1/100	1/50	1/25
***A. baumannii* BAA-747**	1/12.5	1/100	1/12.5	1/6.25
***P. aeruginosa* ATCC 27853**	1/1.25	1/100	1/25	1/25

The MIC results obtained in this study confirm that essential oils exhibit species-dependent antibacterial activity, which is consistent with the established literature describing the heterogeneous sensitivity of bacteria to plant-derived compounds.[1–4] Essential oils generally act by disrupting cytoplasmic membranes, altering permeability, and interfering with cellular functions, mechanisms that help explain their broad but variable activity profiles.[1–3]. In this study, *S. officinalis* demonstrated the most consistent inhibitory effect, with all strains inhibited at the same dilution (1/100). This uniform potency aligns with previous reports showing strong antimicrobial activity of *S. officinalis* essential oil, attributed to its dominant constituents such as 1,8-cineole, camphor, and thujone.[8] The ability of this oil to inhibit both Gram-positive and Gram-negative organisms supports earlier findings that certain *Salvia* chemotypes possess broad-spectrum effects.[7,8]

The activity of *O. basilicum* was more variable, showing stronger inhibition against E. *coli* ATCC and *S. aureus,* while far higher concentrations were required to inhibit *P. aeruginosa.* This pattern reflects the recognized resistance of non-fermenting Gram-negative bacteria, particularly due to their outer membrane impermeability and efflux systems. [3,11] Studies evaluating *O. basilicum* essential oil similarly report reduced activity against *P. aeruginosa* compared with other species.[10,11]. Salvia fruticosa showed moderate inhibition across most strains, which is consistent with prior analyses of this species' essential oils that describe intermediate antimicrobial potency influenced by chemotypic variation and environmental factors.[7] Gram-positive organisms such as *S. aureus* and *E. faecalis* showed greater sensitivity to S. fruticosa, reflecting the general trend that essential oils penetrate Gram-positive cell walls more efficiently than Gram-negative barriers.[1–2]

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An interesting observation was the relatively strong inhibitory effect of *M. spicata* on *A. baumannii* (1/6.25), despite the well-documented resilience of this pathogen. The antimicrobial properties of Mentha species are often attributed to compounds such as carvone, menthone, and limonene, which have been shown to disrupt membrane integrity and inhibit enzyme activity.[5,6] This may explain the enhanced susceptibility of *A. baumannii* observed in this study.

As expected, *P. aeruginosa* exhibited the lowest overall susceptibility to all essential oils. Its resistance profile corresponds with previous reports describing its robust lipopolysaccharide barrier, adaptive virulence factors, and strong biofilm-forming ability.[11,12] The higher MIC values observed in our results reinforce the challenge posed by this species in natural product-based antimicrobial research.

Collectively, the results support the growing body of evidence that essential oils may serve as complementary antibacterial agents rather than replacements for conventional antibiotics. Their activity, while measurable, is influenced by chemical composition, bacterial physiology, and strain-specific resistance mechanisms.[1–4,9–12] Further studies involving synergistic combinations, chemical profiling, and extended testing on resistant clinical isolates could provide deeper insight into their practical value.

4.Conclusion

This study demonstrated that essential oils derived from *S. officinalis, O. basilicum, S. fruticosa,* and *M. spicata* exhibit measurable antibacterial activity, with the degree of inhibition varying across bacterial species. *S. officinalis* showed uniform and consistent inhibitory effects against all tested strains, while the other oils demonstrated more strain-dependent activity profiles. Gram-positive organisms were generally more susceptible, whereas Pseudomonas aeruginosa displayed the greatest resistance.
Although essential oils cannot substitute for conventional antimicrobial agents, the observed inhibitory effects highlight their potential as complementary candidates in antimicrobial research. Further studies incorporating chemical characterization, synergistic assays, and evaluations using resistant clinical isolates will help clarify their potential applications and mechanisms of action.

Conflict of Interest

The authors declare that they have no financial or personal relationships with individuals or organizations that could inappropriately influence or bias the content of this work. No honoraria, consultancies, stock ownership, paid expert testimony, patents, or other potential sources of conflict are associated with this study.

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HEAVY METAL ACCUMULATION AND ESSENTIAL OIL COMPOSITION OF LAVENDER (*LAVANDULA ANGUSTIFOLIA MILL.*) CULTIVATED ON METAL-CONTAMINATED SOILS

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Abstract
This study evaluates heavy metal accumulation and essential oil composition in lavender (*Lavandula angustifolia Mill*., syn. L. vera DC) cultivated on soils contaminated by industrial emissions in the vicinity of the Non-Ferrous Metal Works (NFMW), Plovdiv, Bulgaria. Experimental plots were established along a contamination gradient at distances of 0.5, 1.0, 2.0, and 5 km from the pollution source. Lavender plants were harvested at the full flowering stage. Heavy metal concentrations in inflorescences were determined by inductively coupled plasma optical emission spectrometry (ICP-OES), while essential oils were obtained by steam distillation and analyzed for metal residues and chemical composition using GC–FID and GC–MS.
The results demonstrate that lavender exhibits high tolerance to heavy metal contamination, with no significant adverse effects on essential oil yield or quality. Although Pb, Zn, and Cd accumulated substantially in flowering tips at sites closest to the emission source, their transfer into essential oils was negligible. Heavy metal concentrations in the oil fraction remained well below internationally accepted safety limits. Chemical profiling revealed a composition consistent with ISO 3515:2002 standards for Bulgarian and French lavender oils, with linalool (22.95–27.18%) and linalyl acetate (23.60–25.54%) as dominant constituents and low camphor content (0.56–0.65%).
These findings confirm that lavender can be safely cultivated on metal-contaminated soils while producing high-quality essential oil and highlight its potential role as a phytostabilization crop combining environmental remediation with economic valorization.

Key Words: heavy metal accumulation, essential oils, phytoremediation, chemical composition, soil contamination, lavender oil quality.

1.Introduction

The genus *Lavandula* (Lamiaceae) includes approximately 39 species of perennial aromatic plants, widely appreciated for their fragrant flowers and the essential oils obtained from them. From an economic perspective, the most important representatives are *Lavandula angustifolia* Mill. (syn. *L. officinalis* L., *L. vera* DC), lavandin (*Lavandula* × *intermedia Emeric ex Loisel*.), and spike lavender (*Lavandula latifolia Medik*.), which are extensively cultivated for industrial purposes (Kara and Baydar, 2013).
L. angustifolia originates from the central and western Mediterranean Basin, where it naturally occurs on sunny slopes characterized by shallow, calcareous, and nutrient-poor soils. Its high adaptability to marginal edaphic conditions, combined with relatively low agronomic requirements, has enabled the widespread cultivation of lavender far beyond its native range. At present, Bulgaria and France are among the leading global producers, followed by Morocco, Italy, Spain, Hungary, Romania, Ukraine, Turkey, and Russia (Sellar, 2001; Lawless, 2002; Fakhari et al., 2005; Zheljazkov et al., 2012).
Lavender essential oil is a valuable raw material for several industrial sectors, including perfumery, cosmetics, pharmaceuticals, and food production. It is commonly used as a fragrance ingredient in high-quality perfumes, soaps, creams, and other personal care products, as well as a flavoring agent in

confectionery, beverages, honey, and chewing gum (Fakhari et al., 2005). In addition to its commercial importance, lavender oil exhibits a wide range of biological activities. Numerous studies have reported antibacterial, antifungal, sedative, anti-inflammatory, and anxiolytic effects, which underpin its traditional use in the treatment of stress, anxiety, insomnia, headaches, gastrointestinal disorders, and rheumatic conditions (Hajhashemi et al., 2003; Sabara and Kunicka-Styczyńska, 2009).
From a chemical standpoint, lavender essential oil is a complex mixture of volatile secondary metabolites, dominated by monoterpenes and their oxygenated derivatives. The quality of the oil is largely determined by the relative proportions of linalool and linalyl acetate, accompanied by low levels of camphor. These parameters are critical for both the sensory properties and the market value of the oil and are explicitly regulated by international quality standards (Kreis and Mosandl, 1992; Adam, 2006). Variations in the chemical profile may also affect the biological activity of lavender oil, including its antimicrobial and antifungal potential (Glinka and Glinka, 2008; Janeczko et al., 2008).

In recent decades, increasing attention has been directed toward the behavior of medicinal and aromatic plants grown on soils contaminated with heavy metals (Bağdat and Eid, 2007; Ali et al., 2013). The uptake, accumulation, and distribution of metals within plant tissues, as well as their possible transfer into plant-derived products, depend on a complex interplay of soil geochemical characteristics, environmental conditions, and species-specific physiological mechanisms (Abu-Darwish et al., 2013; Blagojević et al., 2009; Chan, 2003). For aromatic plants, a key issue is whether soil contamination influences essential oil yield, composition, and ultimately product safety. Available evidence suggests that lavender essential oil content and composition are relatively stable across a range of contamination levels, even though high metal loads may negatively affect plant growth and biomass production, leading to reduced total oil yield per unit area (Zheljazkov et al., 2013). Importantly, heavy metals are typically absent from the essential oil fraction, indicating a low risk of direct contamination of the final product (Zheljazkov et al., 2013; Angelova et al., 2015a). On this basis, lavender and other aromatic species have been proposed as suitable non-food crops for cultivation on contaminated or marginal lands, including their potential use in phytoremediation-oriented land management systems (Angelova, 2012; Zheljazkov et al., 2013; Angelova et al., 2015a).

Despite these encouraging findings, field-based studies that simultaneously examine soil heavy metal concentrations, metal accumulation in lavender flowering tops, and essential oil yield and quality along well-defined contamination gradients remain limited. In particular, there is still insufficient information on how severe industrial pollution influences the balance between metal uptake, plant performance, and the stability of the essential oil chemotype under real agricultural conditions. Therefore, the present study aims to evaluate (i) the accumulation of Pb, Zn, and Cd in lavender flowering tips, (ii) essential oil yield and chemical composition, and (iii) the suitability of *L. angustifolia* as a phytoremediation-oriented crop under field conditions along a clearly defined gradient of soil contamination.

2.Material and Methods

Study area and experimental design

The study was conducted on lavender (*L. angustifolia*) plantations located at different distances from the Non-Ferrous Metal Works (NFMW) in Plovdiv, Bulgaria, a major source of industrial heavy metal emissions. Experimental sites were established at distances of 0.5 km, 1.0 km, 2.0 km and 5.0 km from the pollution source.
Lavender is one of the most important essential oil crops cultivated under Bulgarian agroecological conditions. The plantations closest to the NFMW were established in 2001–2002 and occupy areas of 165 decares at 0.5 km and 1.0 km and 51 decares at 2.0 km and 5.0 km from the emission source. The more distant lavender field (5 km) are located in the Dolni Voden municipality. Lavender was cultivated

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using conventional agricultural practices. At each site, five representative plants were randomly selected for analysis. Plants were harvested at the full flowering stage, and inflorescences were used for the determination of Pb, Zn, and Cd concentrations.

Soil and plant analysis

Pseudo-total concentrations of heavy metals in soil samples were determined in accordance with ISO 11466 (1995). The bioavailable (mobile) fractions of Pb, Zn, and Cd were extracted using a DTPA solution (1 M NH_4HCO_3 and 0.005 M DTPA, pH 7.8), following standard procedures (ISO 14780, 2001). The concentrations of Pb, Zn, and Cd in plant material (lavender inflorescences) and in essential oils were determined after microwave mineralization. Quantitative analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-OES; Jobin Yvon Emission, JY 38 S, France). The accuracy and analytical performance of the ICP method were validated using a certified reference material (apple leaves, SRM 1515; National Institute of Standards and Technology, NIST).

Essential oil extraction and chemical analysis

Lavender essential oil was obtained by steam distillation under industrial conditions. The extracted oils were subsequently analyzed for heavy metal content and chemical composition.

For chromatographic analysis, essential oil samples were diluted in hexane (1:1000, v/v) and analyzed using an Agilent 7890A gas chromatography system equipped with a flame ionization detector (FID) and coupled to an Agilent 5975C mass spectrometer (GC–MS). Identification of individual compounds was based on mass spectral libraries and retention indices, while quantification was performed using FID peak area normalization.

Statistical analysis

All analyses were performed in triplicate. Statistical analyses were performed using SPSS software (IBM SPSS Statistics; IBM Corp., Armonk, NY, USA). Differences among means were assessed by one-way ANOVA at $p < 0.05$.

3.Results and Discussion

Soil contamination gradient and physicochemical characteristics

The physicochemical characteristics of soils from the NFMW area are presented in Table 1. Soil pH values ranged from neutral to alkaline (7.6–8.3), conditions known to reduce the mobility and bioavailability of cationic heavy metals (Kabata-Pendias, 2011). The slightly lower pH observed at S1 and S2 compared to S3 and S4 may have contributed to enhanced metal retention and accumulation closer to the pollution source.

Organic carbon content varied between 1.5 and 2.9%, with higher values recorded at S1 and S3. The elevated organic matter content at S1 likely enhanced soil sorption capacity, promoting the retention of Pb, Zn, and Cd through complexation processes (Kabata-Pendias, 2011).

Table 1. Physicochemical properties and total concentrations of Pb, Zn, and Cd in soils from the NFMW industrial area

Parameter	pH	Organic C,%	N Kjeldal,%	P, mg/kg	K, mg/kg	Pb, mg/kg	Zn, mg/kg	Cd, mg/kg
0.5 km (S1)	7.7	2.9	0.22	961	5807	2561	2673	51.8
1.0 km (S2)	7.7	2.8	0.22	1262	5168	2113	2513	43.6
2.0 km (S3)	8.3	1.5	0.13	474	4490	515.7	1078	7.4
5.0 km (S4)	8.3	2.1	0.16	722	8470	105.3	227.8	2.7

MPC (pH >7.4) – Pb – 120 mg/kg, Cd – 3.0 mg/kg, Zn - 400 mg/kg

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Total nitrogen (Kjeldahl N) and available phosphorus showed higher values at S1 and S2, suggesting differences in soil fertility that may influence plant growth and stress responses. Potassium content increased with distance from the contamination source, reaching its maximum at S3 (8470 mg kg^{-1}), which may contribute to improved plant nutritional status at less contaminated sites.

The studied soils exhibit a clear gradient of heavy metal contamination related to their distance from the NFMW industrial complex near Plovdiv, a phenomenon widely documented in the vicinity of non-ferrous metal smelters (Angelova et al., 2010; Kabata-Pendias, 2011). The highest concentrations of Pb, Zn, and Cd were recorded at the sites located 0.5 km and 1.0 km from the emission source (S1 and S2), whereas metal concentrations decreased markedly at 2 km (S3) and reached their lowest values at 5 km (S4). This spatial pattern confirms the dominant role of industrial emissions in shaping soil contamination in the study area.

At site S1and S2 (0.5 and 1.0 km), lead concentrations ranged between 2112 and 2561 mg kg^{-1}, zinc between 2513 and 2673 mg kg^{-1}, and cadmium between 43.6 and 51.8 mg kg^{-1}, indicating severe soil contamination well above typical background levels. In contrast, soils at 2 km (S3) showed substantially lower metal contents (Pb – 515.7 mg kg^{-1}, Zn – 1078.0 mg kg^{-1}, Cd - 7.4 mg kg^{-1}), corresponding to moderate contamination. The lowest concentrations were observed at 5 km (S4), where Pb (105 mg kg^{-1}), Zn (228 mg kg^{-1}), and Cd (2.7 mg kg^{-1}) approached or only slightly exceeded natural background values.

Table 2 presents the results for the mobile (bioavailable) forms of Pb, Zn, and Cd in the studied soils. DTPA-extractable metal concentrations indicate that, in contaminated soils (S1 and S2), the mobile fraction of Cd represents the largest proportion of its total content, ranging from 64.6 to 70.6%. This is followed by Pb, with mobile fractions between 38.7 and 56.5%, and Zn, with values ranging from 13.5 to 27.3%. Comparable trends were observed in the unpolluted soils (S3, S4, and S5), although at considerably lower absolute concentration levels.

Table 2. Bioavailable (DTPA-extractable) fractions of Pb, Zn, and Cd in soils from the NFMW area

Soils	Pb		Zn		Cd	
	mg kg^{-1}	%*	mg kg^{-1}	%	mg kg^{-1}	%
S1	1078.7	42.1	387.7	14.5	51.8	43.3
S2	816.9	38.7	340.0	13.5	30.8	70.6
S3	289.6	56.2	291.4	27.3	7.4	64.9
S4	35.1	33.3	16.5	7.2	1.03	38.1

*DTPA -extractable / total content

Despite the neutral to alkaline soil reaction, the results indicate a very high proportion of DTPA-extractable (potentially mobile) forms of Pb and especially Cd in the contaminated sites (S1 and S2). At first glance, this appears to contradict the generally accepted assumption that alkaline pH limits the mobility of cationic heavy metals; however, this can be explained by several key factors.

First, the extremely high total concentrations of Pb and Cd in close proximity to the pollution source likely lead to a substantial saturation of the soil sorption capacity. Under such conditions, a significant fraction of the metals remains bound in weakly stable forms that are easily chelatable and accessible to DTPA extraction, regardless of the alkaline soil reaction.

Cadmium exhibits higher mobility compared to Pb and Zn because it forms weaker associations with soil carbonates, clay minerals, and Fe/Mn oxides. As a result, Cd often maintains a high proportion of mobile fractions even under alkaline conditions, which explains the observed values exceeding 70–80% DTPA-extractable content in the most heavily contaminated soils.

An additional influencing factor is the technogenic origin of the metals in the NFMW area. Atmospherically deposited Pb and Cd enter the soil predominantly in the form of fine particles, sulfides, oxides, and salts, which are poorly integrated into the soil matrix. This results in a higher proportion of

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potentially mobile forms compared to geogenic metals. The role of organic matter also contributes to this behavior. Organic matter can form both stable and soluble complexes with Pb and Cd, the latter being readily extractable by chelating agents such as DTPA. Consequently, the presence of organic matter may increase the amount of mobile metal forms. It is important to emphasize that DTPA extraction determines potentially bioavailable metals rather than the actual concentration of metals in the soil solution. Therefore, high percentages of DTPA-extractable forms reflect an increased ecological risk and potential uptake by plants, rather than necessarily high metal migration under the existing pH conditions. The lower relative proportion of mobile Zn forms compared to Pb and Cd is consistent with its stronger fixation in alkaline soils and its tendency to form more stable mineral and sorption complexes.

Content of heavy metals in lavender flowering tips and essential oil

Soil conditions and plant species significantly influence the uptake and accumulation of heavy metals, as metal entry into plant tissues largely depends on their concentration and mobility in soils. Plants cultivated on contaminated soils tend to accumulate heavy metals primarily in their vegetative organs. In most cases, the highest concentrations are detected in the roots due to their direct contact with contaminated soil; however, in some plant species, substantial proportions of heavy metals may also accumulate in aboveground biomass (Peng et al., 2006). Table 3 presents the concentrations of Pb, Zn, and Cd in the flowering tips and essential oils of lavender. In plants grown at a distance of 0.3 km from the NFMW, the concentrations of Pb, Zn, and Cd in flowering tips reached up to 1147.3 mg kg^{-1}, 349.3 mg kg^{-1}, and 15.6 mg kg^{-1}, respectively. With increasing distance from the pollution source, a clear decreasing trend in heavy metal concentrations was observed in lavender inflorescences. At 1 km from the NFMW, Pb concentrations declined markedly to 31.4 mg kg^{-1}, Zn to 57.1 mg kg^{-1}, and Cd to 0.62 mg kg^{-1}.

Comparable values were recorded for lavender cultivated on unpolluted soils, where Pb concentrations ranged from 28.6 to 35.6 mg kg^{-1}, Cd from 0.58 to 0.64 mg kg^{-1}, and Zn from 26.9 to 38.5 mg kg^{-1}. A portion of the heavy metals detected in lavender flowering stems is likely attributable to atmospheric deposition. Lavender inflorescences are densely arranged at the uppermost parts of the plant canopy, covering a large proportion of leaves and stems, which makes them particularly susceptible to aerosol-derived pollutants. Heavy metal concentrations were also determined in lavender essential oils. The results demonstrate that the majority of heavy metals accumulated in the flowering tips do not transfer into the essential oil during steam distillation, resulting in substantially lower concentrations in the oil fraction. In essential oils obtained from lavender grown on highly contaminated soils (S1), Pb concentrations reached up to 0.28 mg kg^{-1} and Zn up to 0.84 mg kg^{-1}, while Cd remained below the quantitative detection limits of the analytical method. In oils from contaminated soils at 1 km distance (S2), Pb concentrations reached up to 0.25 mg kg^{-1} and Zn up to 0.74 mg kg^{-1}. Similar values were observed in essential oils obtained from lavender grown on unpolluted soils, with Pb concentrations ranging from 0.24 to 0.26 mg kg^{-1} and Zn from 0.65 to 0.70 mg kg^{-1}. No significant differences were detected in heavy metal concentrations between oils derived from contaminated and uncontaminated soils. Overall, despite substantial soil contamination and pronounced accumulation of heavy metals in lavender flowering tips, metal concentrations in the essential oils remained very low and well below accepted maximum permissible limits, confirming compliance with the requirements for environmentally friendly products. These findings are consistent with the results reported by Zheljazkov et al. (2013), who demonstrated that heavy metal concentrations in lavender essential oil are very low and largely independent of soil contamination levels.

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Table 3. Content of Pb, Zn, and Cd (mg/kg) in lavender flowering tips and essential oils grown at different distances from the NFMW

Element		Distance from NFMW			
		0.5 km (S1)	1.0 km (S2)	2.0 km (S3)	5 km (S4)
Pb	Flowering tips	1840.2	1147.3	31.4	34.0
	Oil	0.28	0.28	0.25	0.25
Cd	Flowering tips	15.9	15.6	0.62	0.64
	Oil	nd	nd	nd	nd
Zn	Flowering tips	724.5	349.3	57.1	26.9
	Oil	0.84	0.84	0.74	0.65

nd- non detected

Essential oil composition of lavender grown on contaminated soils

The essential oil composition of *L. angustifolia* cultivated on heavy metal–contaminated soils in the vicinity of the NFMW industrial area (Plovdiv, Bulgaria) is presented in Table 4. Despite adverse soil conditions, the oils exhibited a typical lavender chemotype dominated by oxygenated monoterpenes, with linalool and linalyl acetate as the major constituents.

Table 4. Composition (%) of lavender essential oil obtained from fresh flowering tips grown in the vicinity of the NFMW

№	Name	RI	S1	S2	S3	S4
1	α-Pinene	939	0.47	0.41	0,45	0,41
2	Camphene	954	0.36	0.31	0,34	0,31
3	β-Pinene	979	1.17	1.03	1,11	1,01
4	β-Myrcene	991	0.56	0.49	0,53	0,48
5	3-Octanol	999	0.45	0.40	0,43	0,39
6	p-Cymene	1019	0.65	0.57	0,62	0,56
7	Limonene	1025	1.31	0.94	1,01	0,93
8	Eucalyptol	1031	1.64	1.44	1,56	1,42
9	(Z)-β-Ocimene	1039	2.10	2.72	1,93	2,68
10	(E)-β-Ocimene	1049	1.78	1.56	1,69	1,54
11	γ-Terpinene	1060	0.53	0.46	0,50	0,46
12	cis-Linalool oxide	1073	0.18	0.16	0,17	0,16
13	trans-Linalool oxide	1078	0.36	0.31	0,34	0,31
14	Linalool	1097	22.95	26.51	25,60	27,18
15	1-Octen-3-yl-acetate	1113	0.35	0.31	0,34	0,31
16	Camphor	1146	0.65	0.57	0,61	0,56
17	Borneol	1169	0.37	0.33	0,35	0,32
18	Lavandulol	1173	4.53	3.97	4,29	3,92
19	Terpinene-4-ol	1177	6.48	5.68	6,13	5,61
20	Cryptone	1183	0.23	0.20	0,21	0,20
21	α-Terpineol	1189	5.90	5.18	5,59	5,11
22	Geraniol	1253	1.19	1.05	1,13	1,03
23	Linalyl acetate	1275	23.60	24.64	24,59	25,34

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24	Phellandral	1279	0.41	0.36	0,39	0,35
25	Bornyl acetate	1285	0.49	0.43	0,46	0,42
26	(±)-Lavandulyl acetate	1290	2.91	2.55	2,75	2,52
27	Neryl acetate	1366	2.16	1.89	2,04	1,87
28	Geranyl acetate	1382	3.18	2.79	3,01	2,75
29	β-Bourbonene	1388	0.36	0.32	0,34	0,31
30	β-Caryophyllene	1419	6.76	5.93	6,39	5,85
31	(E)-β-farnesene	1458	1.33	1.17	1,26	1,15
32	Germacrene D	1464	1.18	1.03	1,11	1,02
33	Caryophyllene oxide	1580	1.75	1.54	1,66	1,52
Monoterpene hydrocarbons (MH)			8.92	8.49	8.16	8.39
Oxygenated monoterpenes (OM)			77.22	78.05	79.21	79.09
Sesquiterpene hydrocarbons (SH)			9.63	8.45	9.11	8.34
Oxygenated sesquiterpenes (OS)			1.75	1.54	1.66	1.52
Non-terpene derivatives (NT)			0.81	0.71	0.76	0.70
Total			98.32	97.23	98.90	98.03

The chromatographic profile reveals a complex mixture of components in the composition of lavender essential oil. As shown in Figure 1, oxygenated monoterpenes are the dominant group (77.22–79.21%), followed by sesquiterpene hydrocarbons (8.34–9.63%), monoterpene hydrocarbons (8.16–8.92%), oxygenated sesquiterpenes (1.52–1.75%), and non-terpene derivatives (0.70–0.85%). This distribution further confirms the preservation of the typical chemical profile of *L. angustifolia* even under conditions of heavy metal soil contamination.

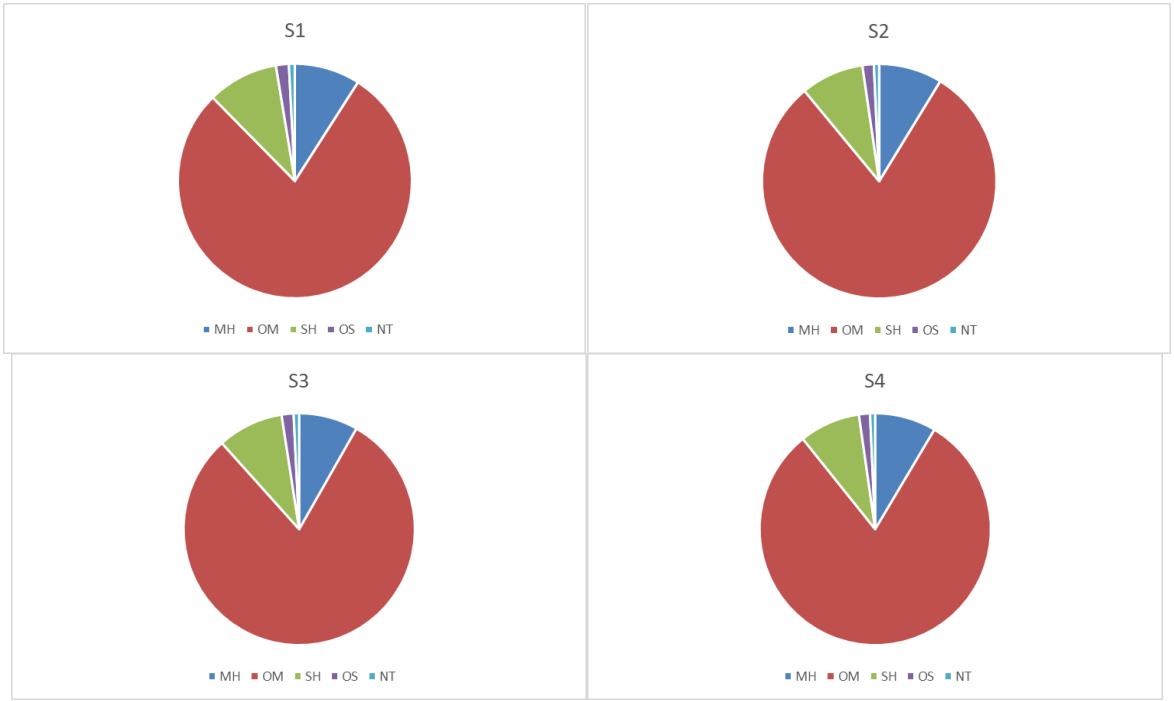

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Figure 1. Functional group classification of identified lavender essential oil constituents from plants grown near the NFMW (OM - oxygen-containing monoterpenes; MH - monoterpenic hydrocarbons; SH - sesquiterpene hydrocarbons; NT - and non-terpene derivates) .Linalool and linalyl acetate are the principal components determining the aromatic profile, commercial value, and pharmacological properties of *L. angustifolia* essential oil (Cavanagh, and Wilkinson, 2002). High concentrations of these oxygenated monoterpenes, combined with low levels of undesirable constituents such as camphor and 1,8-cineole, constitute a key quality criterion according to the international standard ISO 3515 (2002). Consequently, any deviation in the ratio between these components is often interpreted as an indicator of physiological stress or disruption of secondary plant metabolism (Lis-Balchin, 2002; Adam, 2006).
The results of the present study demonstrate that, despite the extremely high total and DTPA-extractable concentrations of Pb and Cd in soils located in close proximity to NFMW, the composition of lavender essential oil remains stable and characteristic of the species. The contents of linalool and linalyl acetate show no significant quantitative differences between plants grown on heavily contaminated soils and those grown on control soils. This clearly indicates that heavy-metal soil contamination does not lead to a substantial disruption of the biosynthetic pathways responsible for the formation of the main aromatic profile of lavender oil. Similar stability in chemical composition has also been reported by Zheljazkov et al. (2013) and Angelova et al. (2015b), who emphasize that the dominant lavender chemotype is largely genetically determined and only weakly influenced by abiotic stress factors.

Particularly noteworthy is the fact that the high mobility of Cd and Pb in soils, as established in the present study by DTPA extraction, is not reflected in increased levels of undesirable components such as camphor in the essential oil. This is of major importance, as elevated camphor concentrations lead to a reduction in essential oil quality. High camphor contents have been observed in plants subjected to severe physiological stress (Kreis and Mosandl, 1992; Hassiotis et al., 2014). The absence of such an effect in the present study suggests that metal stress, although significant in the soil environment, does not reach a threshold capable of disrupting terpene metabolism in the glandular trichomes—the structures responsible for essential oil biosynthesis and accumulation. The stability of the chemical composition can be explained by the action of several interrelated mechanisms associated with both soil characteristics and species-specific physiological traits. The neutral to alkaline soil reaction, combined with relatively high organic matter content, promotes the binding of heavy metals in the solid soil phase through sorption and complexation processes. Despite the high relative proportion of mobile fractions, the effective bioavailability of metals to plants remains limited. Furthermore, lavender shows accumulator-like behavior, with higher concentrations of heavy metals in the aboveground biomass than in the roots. However, these metals are not transferred to the essential oil, suggesting an effective physiological barrier that prevents their incorporation into the glandular structures responsible for essential oil synthesis and storage (Zheljazkov et al., 2013; Angelova et al., 2015b). The stability of the linalool- and linalyl acetate-dominated chemotype along the entire contamination gradient confirms that heavy-metal loading exerts a stronger influence on growth parameters and metal accumulation in plant tissues than on the quality of secondary metabolites. A similar “metabolic prioritization” has been described in other aromatic plants, where secondary metabolism is preserved or even stabilized under moderate abiotic stress conditions (Gershenzon, 1984; Selmar and Kleinwächter, 2013).

Quantitative analysis of the essential oil further supports these observations. Linalool content ranged from 22.95 to 26.51% in plants grown on contaminated soils, compared to 27.18% in the control sample, while linalyl acetate accounted for 23.60–24.64% in contaminated variants and 25.34% in the control. These values clearly indicate that soil contamination does not exert a significant effect on the biosynthesis of the main aroma-determining components. High linalyl acetate content is associated with superior oil quality, as it is responsible for the characteristic sweet, floral aroma and enhances the oil’s

calming properties. The slight variations observed in certain monoterpene and oxygenated terpene compounds (α-pinene, β-pinene, limonene, eucalyptol) may be interpreted as adaptive physiological responses to stress conditions without altering the overall chemotype. The stable or slightly increased levels of terpinen-4-ol, α-terpineol, and borneol in contaminated variants suggest that secondary metabolism remains functionally active even under unfavorable soil conditions. Sesquiterpene hydrocarbons and oxygenated sesquiterpenes also exhibit comparable levels across all treatments, indicating that the later stages of terpene biosynthesis are not substantially affected by heavy-metal stress. Despite the presence of moderate quantitative variations, no pronounced qualitative changes in essential oil composition were observed. This reflects high physiological plasticity and preserved integrity of the biosynthetic pathways. The absence of extreme deviations from control profiles confirms that heavy-metal contamination primarily affects vegetative growth and metal accumulation in plant tissues, rather than secondary metabolism, which is fully consistent with the non-accumulating behavior of *L. angustifolia.* According to ISO 3515, high-quality lavender oil is characterized by linalool contents ranging from 20–45%, linalyl acetate from 25–46%, low camphor levels, and balanced terpene fractions. The analyzed oils fall entirely within or very close to these ranges, without accumulation of undesirable components, confirming that lavender oil obtained from plants grown on contaminated soils retains its quality and commercial value.

4.Conclusion

Lavender cultivated on heavy metal–contaminated soils in close proximity to the industrial zone of NFMW retains a characteristic and relatively stable essential oil composition dominated by linalool and linalyl acetate. The obtained results indicate that soil contamination does not induce significant qualitative or quantitative changes in the essential oil profile, and in most cases the analyzed oils comply with the ISO standards for *L. angustifolia.*

Across the entire contamination gradient, the linalool- and linalyl acetate–dominated chemotype is preserved, with only minor quantitative fluctuations observed in some secondary components. The statistical analysis did not reveal the presence of heavy metal–induced chemotypic shifts, indicating a high resilience of lavender secondary metabolism under conditions of metal stress.

The preservation of essential oil quality even on heavily contaminated soils has important practical implications. It supports the use of lavender as a non-food crop for contaminated and marginal lands, combining economic benefits with reduced environmental risk. In this context, the results contribute to a better understanding of soil–plant interactions in polluted environments and provide a scientific basis for incorporating aromatic plants into sustainable land management and phytostabilization strategies.

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Conflict of Interest

The author declares no conflict of interest.

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