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Abstracts & Full Papers

11th mm International Mediterranean Symposium on Medicinal and Aromatic Plants

The 11th International Mediterranean Symposium on Medicinal and Aromatic Plants

6-8 May 2025 /Rimini -Italy

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The 11th International Mediterranean Symposium on Medicinal and Aromatic Plants

MESMAP – 11
PROCEEDINGS BOOK
ABSTRACTS & FULL PAPERS

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Dear Colleagues,

Having a respected scientific board and organizing committee members from all over the world, MESMAP Symposium series started in 2013. The first Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP-2013) was held on April 17–20, 2013 in Gazimagosa (Famagusta), Turkish Republic of Northern Cyprus (TRNC), which was organized by the Faculty of Pharmacy, Eastern Mediterranean University (EMU), in joint with AMAPMED (Association of Medicinal and Aromatic Plants of the Mediterranean).

MESMAP-2 Symposium was held on April 22–25, 2015, in Antalya – Türkiye, INDUSTRIAL CROPS AND PRODUCTS JOURNAL with a high impact factor from the Elsevier Group, published a special issue covering some of the full papers selected after scientific evaluation. MESMAP-3 Symposium, which was held on April 13–16, 2017 in Girne (Kryneia) in the Turkish Republic of Northern Cyprus (TRNC), was the third event of the MESMAP symposium series on Medicinal and Aromatic Plants. After scientific evaluation, selected full papers were published in the Indian Journal of Pharmaceutical Education and Research (IJPER), indexed by THOMSON REUTERS. MESMAP-4 Symposium, which was held on April 18–22, 2018 in Sherwood Breezes Resort Hotel Antalya, Türkiye, was the fourth event of the MESMAP Symposium Series on Medicinal and Aromatic Plants. Then, the fifth one was the MESMAP-5 symposium, which was organized as a joint meeting with ISPBS-5 at Cappadocia on April 24-28, 2019. After scientific evaluation, selected full papers from the MESMAP-5 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. Afterwards, the MESMAP-6 Symposium was organized on October 15–17, 2021, and this symposium was supported by the TÜBTAK 2223-B National Scientific Meetings Grant Program. After scientific evaluation, selected full papers from the MESMAP-6 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. Then, MESMAP-7 was organized during November 18–20, 2022, and hosted by Dokuz Eylul University and Torbalı (Izmir) Chamber of Commerce, Türkiye. MESMAP-8 was organized during October 20–22, 2022, in Izmir–Türkiye. After scientific evaluation, selected full papers from the MESMAP-8 Symposium were published in Molecules and the Brazilian Journal of Pharmacognosy, indexed with THOMSON REUTERS. Furthermore, MESMAP-8 was supported by the TÜBTAK 2223-B National Scientific Meetings Grant Program. MESMAP-9 was organized during May 03–05, 2023, in Ankara–Türkiye. After scientific evaluation, selected full papers from the MESMAP-9 Symposium were published in "Phytochemistry Letters" journal by Elsevier, indexed with THOMSON REUTERS. MESMAP-10, hosted by İstanbul University during 25-27 April, 2024 in İstanbul-Türkiye, was the tenth series of the meeting and had the "A Topical Collection by the "PLANT BIOSYSTEMS". MESMAP-11 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. We are delighted to announce that the 12th edition of the MESMAP series is scheduled to take place in THAILAND in January 2026. It would be our honor to welcome you once again to the MESMAP-12 Symposium.

Sincerely,
Symposium Chair
Prof. Dr. Nazım ŞEKEROĞLU
President of AMAPMED, General Coordinator of GOFMAP
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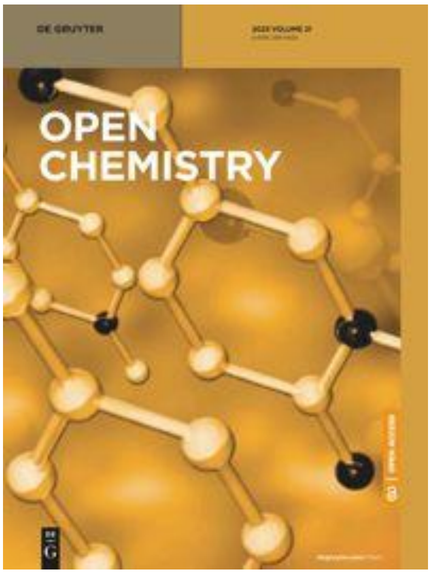

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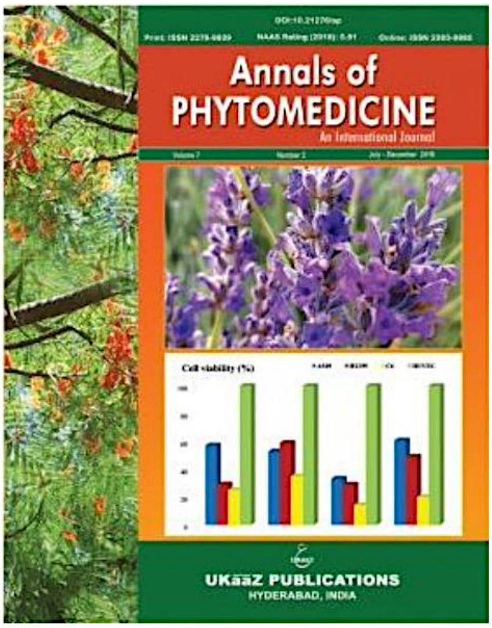

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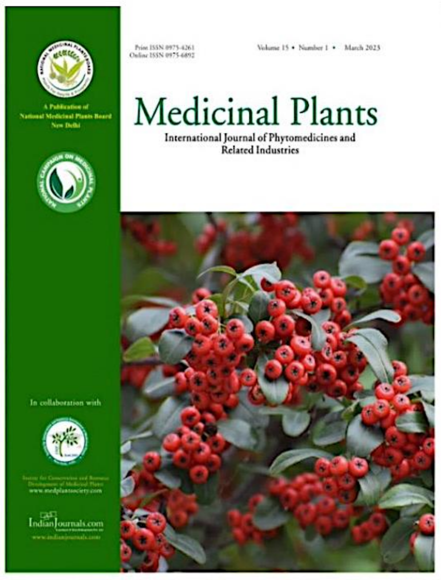

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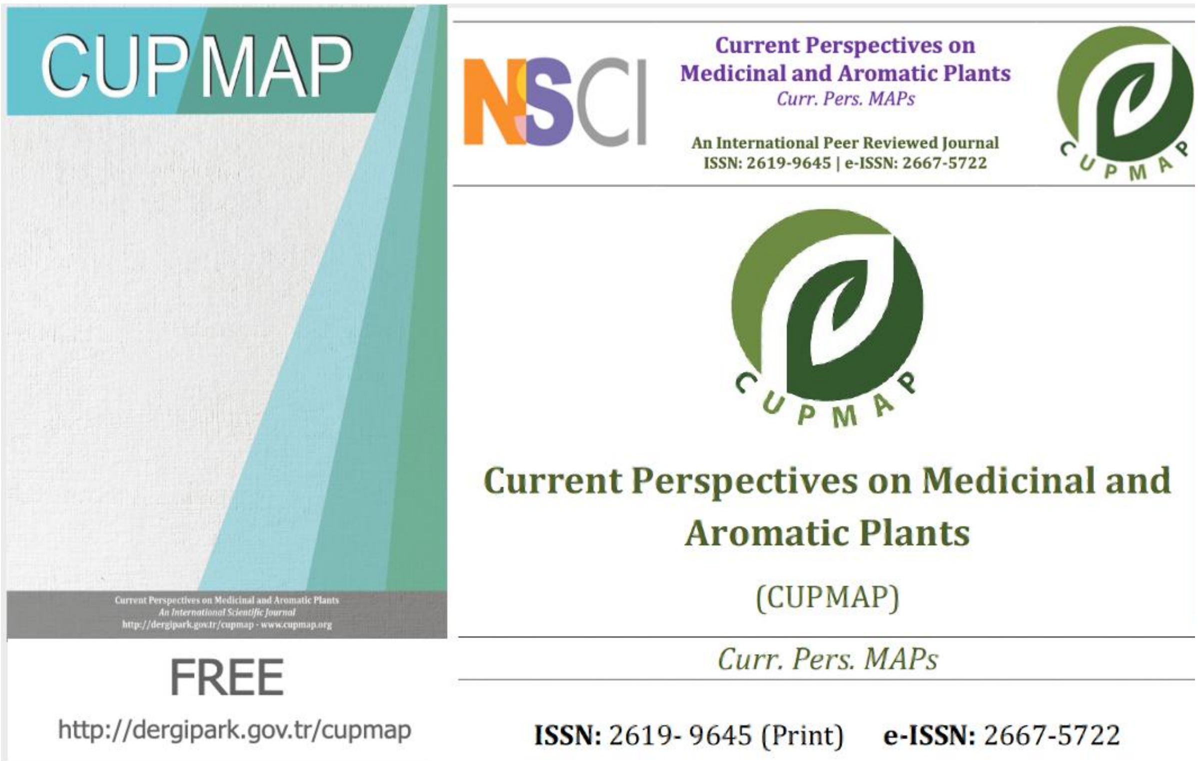

CUPMAP is an open access, peer-reviewed and refereed international journal published by MESMAP scientific group. The main objective of the CUPMAP is to provide an intellectual outlook on the scientific researches on Medicinal and Aromatic Plants. CUPMAP have distinguished goals to promote interdisciplinary 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 7 Issue 1, which is scheduled to be published on December, 2024. **Last date of submission: May 30, 2025.**

MESMAP-11 Sempozyumunda toplam 125 bildiri sunulmuştur, bunlardan 80'sı sözlü, 45 tanesi ise poster sunum şeklinde olup; sunulan **sözlü bildirilerin %58,75'lik kısmı yabancı katılımcılar** tarafından sunulmuştur. Sempozyuma yaklaşık **24 farklı ülkeden** bilim insanı katılım sağlamıştır. **Katılım Sağlayan Ülkeler:** Germany, Thailand, Portugal, Slovenia, United Kingdom, Saudi Arabia, Israel, Serbia, Italy, Croatia, India, Poland, Romania, Poland, Slovakia, Algeria, Albania, Tunisia, Iran, Australia, Turkish Republic of Northern Cyprus, Bulgaria, Austria and Türkiye.

Sempozyuma katılım sağlayan katılımcılar ve sunum başlıkları 'MESMAP-11 Abstracts & Proceedings Book' kitabının içindekiler kısmında sunulmuştur. MESMAP-11 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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INNOVATIVE EXTRACTION METHODS FOR MEDICINAL PLANTS GAS EXTRACTION (FE), ASE, ISOELECTRIC FOCUSED ADSORPTIVE BUBBLE CHROMATOGRAPHY, SPME, HEADSPACE GC

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Medicinal plants, with their biologically active components, have a wide range of uses in the pharmaceutical, cosmetic and food industries. Efficient extraction of this circuit is challenging due to the limitations of conventional extraction methods. In particular, problems such as high solvent consumption, long processing times and low yields have necessitated the development of more environmentally friendly and efficient technologies. In this context, advanced extraction methods play an important role in the isolation of actives from medicinal plants. In this study, the theoretical foundations, application areas and combined form of techniques such as Gas Extraction (FE), Accelerated Solvent Extraction (ASE), Isoelectric Focused Adsorptive Bubble Chromatography, Solid Phase Micro Extraction (SPME) and Headspace Gas Chromatography (GC) are discussed. As a result, advanced extraction methods optimize the isolation of bioactive structures from therapeutic plants by offering environmentally friendly, highly efficient and specific solutions. This dose enables biological activity analysis of pharmacological, cosmetic and food applications and promotes multidisciplinary collaborations. In the future, groups dedicated to the routines of these methods are expected to make important contributions to the development of more efficient extraction and sustainable technologies in the bioactive era.

Key Words: Medicinal plants, extraction, biological, solvent consumption, isolation, food applications

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MEDITERRANEAN DIET AND THE GUT-BRAIN AXIS

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Recent studies have indicated that our gut health and diet affect a range of health conditions, including those linked to the brain. The Mediterranean diet, which is rich whole grains, fruits, and vegetables, can slow down memory loss and lower the risk of diseases like Alzheimer's and Parkinson's. In Parkinson's, gut bacteria may influence the disease's progression, and probiotics or dietary changes could help manage symptoms. Mental health too can be linked to gut bacteria; poor gut health may contribute to anxiety and depression, and eating the right foods or taking probiotics could help improve mood.

Research has shown that specific diets—like the Mediterranean diet—may help protect brain function as we age. This has triggered a wide interest into how diet, gut bacteria, and brain health are all connected. A complex web of connections has come into view; our gut – or by extension, even our whole body – can be considered as an ecosystem. Our gut is the physical environment in which our dietary intake interacts with microbial and human cells. It will take a concerted effort and multidisciplinary collaboration to untangle the many strands to make up the whole network.

Key Words: Mediterranean diet, memory, Alzheimer's and Parkinson's diseases

ESSENTIAL OILS AND AROMATIC PLANTS OF CYPRUS

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Cyprus is the 3rd largest island in the Mediterranean Sea with a land cover of 9.251 sq.km. Being in eastern Mediterranean it is under the influence of the floras of Asia, Africa and Europe. Flora of Cyprus is well documented with 1.610 species and altogether 1.738 taxa. 108 species (143 taxa) are endemic plants comprising 6.7% (8.2%) of the flora. The families Asteraceae (66), Lamiaceae (39) and Apiaceae (29) are important for the aromatic flora.

We have been systematically investigating essential oils from aromatic plants of Cyprus and here I shall present our results on the essential oils of the following species: *Anthemis tricolor, Asphodelus aestivus, Chenopodium murale, Chrysanthemum coronarium, Eucalyptus camaldulensis, Eucalyptus torquata, Helichrysum conglobatum, Helichrysum italicum, Lagoecia cuminoides, Lathyrus spp., Origanum cordifolium, Origanum dubium, Origanum majorana, Phlomis brevibracteata, Phlomis cypria var. cypria, Pimpinella cypria, Sideritis cypria, Teucrium cyprium, Teucrium kyrenia, Teucrium micropodioides, Teucrium salaminium, Thymus capitatus, Zosima absinthifolia*.

Key Words: Essential oil, aromatic plants, Cyprus, endemic

ENERGY-EFFICIENT AND QUALITY-PRESERVING DRYING OF MEDICINAL AND AROMATIC PLANTS

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The global demand for high-quality medicinal and aromatic plants (MAP) is rising due to their extensive use in pharmaceuticals, cosmetics, and food industries. However, traditional drying methods often compromise product quality and consume significant energy. This presentation explores innovative drying techniques aimed at optimizing energy efficiency while preserving the biochemical integrity, color, aroma, and active compounds of MAP. Techniques such as low-temperature drying, solar drying, and condensation drying are evaluated for their performance and sustainability. Case studies demonstrate substantial reductions in energy consumption and quality degradation. The findings underscore the potential for scalable, cost-effective drying solutions that align with both environmental and quality assurance standards.

Key Words: Medicinal and aromatic plants, drying, quality, energy efficiency

MEDICINAL AND AROMATIC PLANTS: AN OVERVIEW ON ITALIAN MARKET AND MAJOR CHALLENGES FOR GROWERS AND PROCESSORS IN EU

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The Italian market for medicinal and aromatic plants (MAPs) has been characterized by a wide range of species in commerce—nearly 300—despite over 1,700 taxa being officially recognized as medicinal or aromatic. The value of finished products exceeds €10 billion, encompassing thousands of commercial brands dedicated to health and wellness, with more than 75,000 products registered as herbal remedies. National cultivation remains insufficient to meet domestic demand for raw materials, which generate approximately €1 billion in trade value. Cultivated areas are dominated by coriander and bergamot, while over 142 species are grown on relatively small scales. Domestic production comprises around 3,500 tons of dried herbs and spices and 12,000 tons of essential oils, primarily from citrus. Analysis of the ITC/UNCTAD public trade database (trademap.org) estimated the total European MAP market at €10 billion and 1.5 million tons, with Germany, France, and Italy ranking as the top three countries in both consumption and international trade. Between 2019 and 2023, trade in MAP-related agricultural products increased by 31.34% in the EU-28, with an average annual growth rate of 6.3%. Despite this robust growth, the sector faces significant, potentially transformative challenges. Chief among these are contamination risks in a context where quality control standards are stringent, yet cultivation and harvesting environments are often polluted. Product adulteration—particularly affecting processed forms such as extracts—poses a serious threat to consumer trust. Findings from the Botanical Adulteration Prevention Program reveal that while adulteration in raw plant material remains below 10%, it rises to nearly 50% in processed products.

Furthermore, the European Union's regulatory framework—driven by a precautionary principle often applied arbitrarily—poses a persistent threat to market stability. Established products are regularly subjected to reevaluation, and the introduction of new products has become nearly impossible. To ensure the sector's resilience and growth, targeted interventions are required across the entire supply chain. This includes expanding agricultural production, enhancing safety and quality standards, and preserving the integrity and authenticity of sourcing networks. It is also crucial for industry associations to unify their efforts and assert their right to engage in constructive dialogue with regulatory authorities, to prevent unjustified constraints that may harm the sector without improving consumer safety.

Key Words: Medicinal and aromatic plants, EU, commercial brand, qualiy, standards

PLECTRANTHUS: EXPLORING MEDICINAL PROPERTIES

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Aromatic plants have long been recognized for their therapeutic potential, offering diverse bioactive compounds that contribute to innovative treatments in modern medicine. Among them, the Plectranthus genus (Lamiaceae family) has been widely used in traditional medicine. However, beyond its ethnomedicinal applications, plants from this genus have attracted interest due to the presence of diterpenoids, natural compounds that have shown antitumor activity in several human cancer cell lines. These diterpenoids display a wide variety of structures and bioactive characteristics, making *Plectranthus* spp. a rich source of promising molecules for the development of anticancer therapies [1, 2]. A particularly promising advancement is the patented diterpenoid dibenzoylroyleanone (RoyBz), derived from Roy, which has been identified as a PKC-selective activator with potent anti-proliferative effects in colon cancer cells. This activity is mediated through a PKC-dependent mitochondrial apoptotic pathway. A recent study by Rijo P. focused on compounds isolated from the acetonic extract of the stems of *P. hadiensis*, a plant traditionally used in the treatment of brain tumors [1, 2].

Various abietane-type diterpenoids were identified, and their antiproliferative activity was evaluated in several glioma cell lines. These abietane-type diterpenoids exhibited significant antiproliferative and cytotoxic effects against glioma cells, with low IC50 values (half-maximal inhibitory concentration) across multiple cell lines. Additionally, a new fluorescent derivative, BODIPY-7-acetoxy-6-hydroxy-royleanone, confirmed increased intracellular fluorescence associated with the antiproliferative activity of an abietane acompound, suggesting its potential as a basis for developing new therapeutic strategies against glioblastoma [3]. These findings highlight the therapeutic potential of *Plectranthus*-derived compounds and their role in advancing novel anticancer therapies [1].

Key Words: *Plectranthus, Lamiaceae, abietane, cancer*

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MEDICINAL AND AROMATIC PLANTS BRIDGING PAST TO THE FUTURE

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Medicinal and Aromatic Plants (MAPs) have been utilized in various forms since the earliest days of mankind. They have maintained their traditional basic curative role even in our modern societies. Apart from their traditional culinary and food industry uses, MAPs are intensively consumed as food supplements (food additives) and in animal husbandry, where feed additives are used to replace synthetic chemicals and production-increasing hormones. Importantly medicinal plants and their chemical ingredients can serve as starting and/or model materials for pharmaceutical research and medicine production. Current areas of utilization constitute powerful drivers for the exploitation of these natural resources. Today's demands, coupled with the already rather limited availability and potential exhaustion of these natural resources, make it necessary to take stock of them and enrich our knowledge regarding research and development, production, trade and utilization, and especially from the viewpoint of sustainability.

In the era of national and international regulations and agreements, the presentation will cast a look into our present knowledge of this vast interdisciplinary domain: from exploration to the sourcing, ultimately the utilization of raw materials: quality, safety and efficacy have remained to be the main guiding principles, although the dominance of overregulation with its constantly evolving requirements, seems to be jeopardizing the delicate balance of health advantages and hazards. Relying on cutting edge knowledge and technologies, medicinal and aromatic plants can constitute a solid way to their sustainable, multi-faceted utilization for the benefit of our societies. This is a nice, rewarding challenge for Harmonization, Collaboration, Education.

Key Words: Medicinal and aromatic plants, culinary uses, food industry, health

BRIDGING TRADITION WITH INNOVATION AND UNLOCKING NATURE'S THERAPEUTIC POTENTIAL

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In recent decades, the global healthcare landscape has witnessed a growing interest in natural remedies and traditional medicine, driven by a desire for holistic well-being and sustainable treatment approaches. Bridging tradition with innovation offers a powerful pathway to unlocking nature’s vast therapeutic potential, where centuries-old practices can be reevaluated, standardized, and optimized through the lens of scientific rigor. Anatolian traditional medicine, shaped by the rich cultural mosaic of Anatolia and its biodiversity, offers a deep reservoir of therapeutic knowledge that dates back thousands of years. Plants, minerals, and natural extracts used in Anatolian folk medicine have historically played a significant role in preventing and treating various ailments. Today, with the advancement of pharmaceutical sciences, there is a renewed interest in integrating this traditional wisdom with modern drug development processes. Bridging this tradition with innovation is key to unlocking nature’s therapeutic potential in a scientifically validated and sustainable manner. Pharmacy, as both a health profession and a research discipline, is uniquely positioned to lead this integration. By combining ethnobotanical studies with modern techniques in phytochemistry, pharmacology, and pharmaceutical technology, researchers can isolate active compounds or standardized extracts from Anatolian medicinal plants, determine their mechanisms of action, and develop standardized dosage forms. For instance, *Ruscus aculeatus* has shown promising pharmacological profiles.

Moreover, pharmaceutical innovation, including advanced extraction technologies, nanotechnology, and controlled-release drug delivery systems, enables the transformation of traditional medicines into evidence-based phytotherapeutics. In addition, collaboration with local healers, preservation of oral knowledge, and adherence to ethical bioprospecting practices are essential to protect the cultural and ecological heritage of Anatolia. Anatolian traditional medicine provides a valuable framework for new pharmaceutical discoveries in the context of global health trends seeking natural, personalized, and holistic treatment approaches. With an interdisciplinary approach, pharmacy education and research can play a pivotal role in reviving, validating, and industrializing these ancient practices. Thus, bridging Anatolian medical tradition with pharmaceutical innovation is not only a scientific endeavor but also a cultural mission, one that holds the promise of delivering safe, effective, and culturally rooted therapeutics for future generations.

Key Words: Anatolian traditional medicine, ethnobotany, *Ruscus aculeatus*, Neoven

RESEARCH PROGRESS IN OREGANO MOLECULAR BREEDING, SYNTHESIS MECHANISM OF CARVACROL AND ANTIBACTERIAL FUNCTION

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Oregano is a medicinal and aromatic plant of value in the pharmaceutical, food, feed additive, and cosmetic industries. This paper introduces the research progress of the molecular breeding and activity function of oregano.

Breeding：

- This study confirmed that ^{60}Co-γ could be used as an effective mutagenic method, and the suitable irradiation dose was 20 Gy[1].
- we evaluated the phenotypes of 12 oregano genotypes and generated F1 progenies by hybridization. SSR markers were developed and 64 codominant SSR primers were screened on the parents of the six oregano combinations[2].

Components, antibacterial activity and antibacterial mechanism：

- we evaluated the chemical compositions of eight oregano essential oils using GC/MS and assessed their antibacterial activities[3].
- explored the antibacterial effect and mechanism of a carvacrol-rich OEO extracted from *Origanum vulgare* 'Hot & Spicy' on the inactivation of *S. aureus* [4].
- The morphologies, chemical compositions and antibacterial abilities and EO yields from the inflorescences, leaves, and stems of three oregano cultivars were evaluated[5].
- OEOs had the strong antibacterial activity against *Cronobacter sakazaki*[6].
- ^{1}H NMR–based metabolomics reveals the antimicrobial action of oregano essential oil against *Escherichia coli* and *Staphylococcus aureus* in broth, milk, and beef[7].
- The metabolite profiles and underlying biosynthesis mechanisms of elaborate tissues (stems, leaves, bracts, sepals, petals) of *Origanum vulgare* have been reported[8].

Application scenarios and technology research：

- We developed an intelligent and antibacterial packaging film using a chitosan matrix embedding oregano essential oil (OEO) and black rice bran anthocyanin (BRBA)[9].
- Aromatic EOs serve as natural antimicrobial agents with advantages of low toxicity and no residues, can be used as feed additives to replace the antibiotics for animal health[10].
- Essential oils are becoming increasingly popular as potential antibiotic alternatives for animal production based on their antibacterial properties[11].
- Dietary supplementation could improve egg-production performance, decrease feed conversion ratio, and alter the fatty acid and microbial composition of eggs from late-phase laying hens[12].

Key Words: Oregano, breeding, carvacrol, bacteriostatic mechanism, antibacterial packaging film, antibiotics

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MEDICINAL PLANTS AS PROMISING AGENTS FOR TISSUE REPAIR AND REGENERATION

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Human health depends on plants, which are also excellent candidates to be used as pharmaceutical research and discovery agents. Global demand for plant-based products is rising, and both consumers and medical professionals are looking for proof of the products' safety and advantages. Thus, preclinical and clinical studies of medicinal plants' biological activities have recently attracted a lot of interest, exposed their biological activities and validated their traditional use. To determine which phytoconstituents are entirely or partially responsible for the associated effects, bioactivity studies are required. Many medicinal plants, which have long been utilized for their therapeutic qualities, can have a major impact on the complicated biological process of wound healing. Not only external wounds, but also the healing processes of injuries that occur within the abdomen after surgeries involve common biological mechanisms. The current study investigates the ability of a number of medicinal plants to cure wounds, paying special attention to both new discoveries from our own research and the most well-known plant species that are known to be effective in wound care. In order to speed up healing and lessen scarring, these plants' antibacterial, anti-inflammatory, antioxidant and regenerative properties are essential. Based on our research, certain plant extracts demonstrate synergistic effects that enhance the rate of recovery. A comprehensive overview of these plants' possible uses in wound care is provided by a comparative study of them in experimental settings. The study's findings highlight how crucial it is to validate conventional wisdom using cutting-edge scientific methods in order to maximize wound healing treatments. To guarantee the safety and efficacy of these plant-based medicines across a range of patient populations, more study is required to determine the best dosages, formulations, and clinical uses.

Key Words: Medicinal plants, wound healing, preclinical studies

STIMULATION OF BIOSYNTHESIS OF HEALTH-PROMOTING BIOACTIVE COMPOUNDS IN THE *IN VITRO* CULTURE MODEL OF *BRASSICA OLERACEA* L. VAR. *ACEPHALA* (GREEN KALE)

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The study integrates biotechnology, nanotechnology, phytochemistry, and pharmacology to investigate the impact of nanoparticles (NPs) on the production of secondary metabolites in *in vitro* cultures of *Brassica oleracea* L. var. *acephala* (kale). The primary aim is to enhance our understanding of how non-ionic metal NPs — specifically silver (Ag), gold (Au), copper (Cu), and platinum (Pt) — affect the bioelements, secondary metabolites, and biological properties of the resulting plant extracts. *In vitro* cultures were initiated and optimized on Murashige and Skoog (MS) medium devoid of plant growth regulators to achieve high biomass yield under agitated growth conditions. The culture media were enriched with metal NPs of Ag, Au, Cu, and Pt (with dimensions smaller than 5 nm) at concentrations of 0 (control), 5, 10, and 15 ppm. The growth cycle spanned 14 days across three series (n=4). Methanolic extracts from the *in vitro* biomass were analyzed for polyphenolic compounds (phenolic acids and flavonoids by HPLC-DAD), glucosinolates (by UHPLC-HRMS), and the bioaccumulation of elements (by ICP-MS). Moreover, the cytotoxicity tests of studied extracts were done. Among the identified phenolic acids across all extracts were protocatechuic, chlorogenic, caffeic, sinapic, ferulic, and rosmarinic acids. Flavonoids quercetin and kaempferol were also present in every extract. Notably, supplementation with 15 ppm of PtNPs yielded the highest levels of phenolic compounds, with rosmarinic acid increasing by up to 618% compared to control samples. Furthermore, 12 glucosinolates were detected in extracts from cultures supplemented with 5 ppm of PtNPs, including progoitrin, 2-methylbutyl glucosinolate, and glucobrassicin. The research highlights the innovative and practical implications of NP supplementation in *in vitro* culture models of *B. oleracea* var. *acephala*. These findings enhance our understanding of NPs utilization and designate *Brassicaceae in vitro* cultures as viable models for future research that could be scaled up. Furthermore, this study suggests that biomass stimulated by NPs could serve as a promising, innovative and alternative to plant material sourced from *in vivo* conditions.

Key Words: Plant biotechnology, kale *in vitro* cultures, nanopartcels, plant stress, plant secondary metabolites

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CURRENT TRENDS IN MEDICINAL PLANTS AND HEALTH PRODUCT RESEARCH IN SOUTHERN THAILAND

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Southern Thailand has a long history of using herbal remedies for treating common ailments. Researchers are focusing on verifying the efficacy and safety of these traditional medicines through laboratory and clinical studies. Medicinal plants such as *Thunbergia laurifolia*, *Garcinia atroviridis*, and *Garcinia Magostana*, and *Myristica fragrans* are being studied for their anti-inflammatory, anti-microbial, and metabolic health benefits. Expansion of functional foods and herbal nutraceuticals derived from medicinal plants, particularly those with anti-aging, immune-boosting, and disease-preventing properties. Developing herbal teas as supplements, and food additives using local plants like *Centella asiatica*, *Moringa oleifera*, and *Curcuma longa*. These products cater to the increasing consumer preference for natural health solutions. Moreover, the herbal cosmetics and skincare development in Southern Thailand is expanding, with a focus on plant-based skincare products of herbal extracts on skin health, targeting anti-aging, skin hydration, and pigmentation control. Plants like *Aloe vera*, Rice Bran extract, and Mangosteen pericarp are commonly used in beauty formulations. Integration of modern technology in herbal research are being applied to enhance the efficacy and bioavailability of herbal compounds. Nanotechnology-based formulations allow for improved delivery of active ingredients in medicinal and cosmetic applications.

In conclusion, Southern Thailand is at the forefront of medicinal plant research, driven by its rich biodiversity, strong scientific community, and growing market demand for natural health products. Future research will focus on integrating herbal medicine into modern healthcare, developing herbal-based pharmaceuticals, and expanding the global market for Thai herbal products. With continuous support from academic institutions, government policies, and international collaborations, Southern Thailand has the potential to become a leading center for herbal medicine research and innovation.

Key Words: Medicinal plants, bioactivity, research, innovation, Southern Thailand

BIOACTIVE COMPOUNDS FROM *CASTANEA SATIVA* CHESTNUT BURRS

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Castanea sativa (Mill.), commonly known as the sweet chestnut, belongs to the family Fagaceae and is a key fruit crop in Southern Europe, bearing notable economic significance. Worldwide, its production is predominantly concentrated in the two macro-regions of Asia and Europe. Italy stands as the leading chestnut producer in Europe, with five regions specializing in cultivation. The *C. sativa*s pecimens investigated in this study originate from chestnut groves in Monte Amiata (Tuscany) and bear the “Protected Geographical Indication (PGI) Castagna del Monte Amiata” certification. The purpose of this study was to emphasize the potential for recovering added value products from PGI *C. sativa* Mount Amiata spiny burrs, which can act as an innovative, cost-effective, and readily available raw material for applications in the health and wellness sector.

Key Words: Bioactive compounds, *Castanea sativa*, sweet chestnut, Tuscany

LAMELLAR SOLIDS AS NOVEL MEANS FOR PLANT FOOD PROCESSING

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Solid-phase extraction (SPE) is a well-established and powerful technique widely applied to chemically complex matrices, including edible plant extracts, phytocomplexes, and food preparations. This approach enables the concentration and/or purification of selected bioactive compounds, active principles, and secondary metabolites, facilitating their subsequent analysis, isolation, or enrichment in food and phytopreparations. Over the past five years, we have developed an innovative plant food processing methodology that employs solid sorbents from various chemical classes, such as layered structures (e.g., inorganic and inorganic-organic hydrotalcites and zirconium phosphates), magnesium oxide and hydroxide, and phyllosilicates. This strategy has been successfully applied to the selective extraction of:

- anthraquinones from laxative plants,
- polyphenols and alkaloids from *Camellia sinensis* (L.) Kuntze (tea plant) extracts,
- curcumin from *Curcuma longa* L. (turmeric),
- bitter principles from the leaves and roots of *Gentiana lutea* L. (great yellow gentian),
- capsaicinoids from various cultivars of *Capsicum annuum* L. (pepper),
- oxyprenylated coumarins from *Citrus* fruits,
- crocetin from *Crocus sativus* L. (saffron),
- polyphenols from *Punica granatum* L. (pomegranate).

In all cases, the extraction process achieved high yields, demonstrating the efficiency of our approach. Furthermore, the solid sorbents proved to be recyclable, maintaining their adsorption capacity over multiple reuse cycles without significant loss of performance. This novel methodology represents a promising advancement in the selective extraction and enrichment of bioactive compounds from plant-based food sources.

Key Words: Clays, food plants, layered double hydroxides, secondary metabolites, selective extraction, solid phase adsorption

BRINGING VALUE TO MAPS: BIODIVERSITY, CASE STUDIES, AND FUTURE CHALLENGES

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Albania is home to a rich diversity of Medicinal and Aromatic Plants (MAPs), many of which are endemic, culturally significant, and economically valuable. This presentation explores how MAPs in Albania contribute to traditional knowledge, rural livelihoods, biodiversity conservation and the development of nature-based value chains. Through selected case studies from key actions, we examine traditional knowledge, harvesting practices, and recent innovations in sustainable cultivation, post-harvest processing, and market access. Special attention is given to the role of locally based initiatives and the importance of Good Agricultural and Collection Practices (GACP) for MAPs in enhancing product value and traceability. Despite these opportunities, the sector faces major challenges, including habitat degradation, overharvesting and climate changes. The presentation calls for integrated strategies that combine biodiversity protection with economic valorization and capacity-building at the local level, aiming to secure the long-term sustainability and competitiveness of Albania's MAPs sector in the international markets.

Key Words: Albania, medicinal and aromatic plants (MAPs), biodiversity conservation, climate changes

ESSENTIAL OILS AS ANTIOXIDANTS: CHALLENGES AND OPPORTUNITIES

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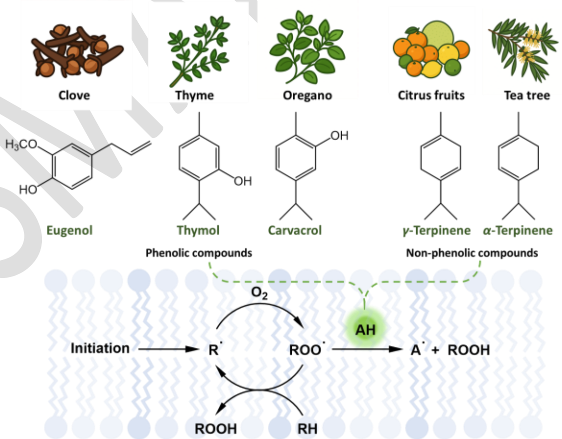
Essential oils are complex mixtures of volatile organic compounds produced by aromatic plants. They are being extensively studied as natural alternatives to artificial antioxidants, particularly in food preservation. Lipid peroxidation is an undesirable oxidative process in organic materials, leading to hydroperoxides and other oxygenated byproducts (e.g., aldehydes, epoxides), which degrade food quality and have toxic effects. Lipid peroxidation consists of a radical chain reaction, triggered by factors such as light, heat, and metal ions, that results in the extensive deterioration of lipids (see Figure). Many essential oil components are prone to oxidation, undergoing peroxidation similarly to unsaturated lipids. However, certain essential oil constituents exhibit antioxidant properties, inhibiting lipid peroxidation. Phenolic compounds, such as carvacrol, thymol, and eugenol, are well-known antioxidants,[1] but non-phenolic components like *γ*- and *α*-terpinene also display antioxidant activity due to their unique pro-aromatic structure. Unlike phenols, these compounds act as antioxidants only under specific conditions, such as in biphasic systems [2] or in the presence of other antioxidants. For instance, we have observed a synergistic effect between γ-terpinene and *α*-tocopherol in preventing sunflower oil oxidation.[3] Moreover, *γ*-terpinene exhibits antioxidant activity in cellular systems, reducing plasma membrane oxidation [2]. Understanding the radical interactions of essential oil components can help predict their pro- or antioxidant behavior, guiding their application in food preservation and other sensitive materials.



Key Words: Essential oils, *γ*-terpinene, carvacrol, thymol, lipid peroxidation, radicals

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FOLIAR APPLICATION OF BIOSTIMULANTS AS SUSTAINABLE APPROACH TO IMPROVE THE PRODUCTION OF ORGANIC MEDICINAL AND AROMATIC PLANTS

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In recent decades, an increasing interest on medicinal and aromatic plants (MAPs) has been observed. In the Mediterranean area, this group of plants is often grown using both low-input farming practices and organic farming methods and it is well-documented that their morphological, productive and chemical characteristics are affected by abiotic and biotic factors. The use of biostimulants seems to be one of the most interesting innovative practices due to fact they can represent a promising approach for achieving sustainable and organic agriculture. Despite a large application in horticulture, the use of biostimulants on MAPs has been poorly investigated. On this basis, a field experiment in a 2-year study was done to assess the effect of foliar treatments with different types of biostimulants on productive and chemical characteristics of *Origanum vulgare* L., *Salvia officinalis* L. and *Salvia Rosmarinus* Spenn. grown organically in Mediterranean environment. Productive and chemical parameters were affected by the biostimulant application. The highest fresh and dry yield were observed in biostimulated plants, in particular in those treated with fulvic acid and protein hydrolysate.

Contrasting response have been observed for the EO content and yield. No significant effects have been recorded for the EO principal components of the three species. Foliar biostimulants produced different responses considering total phenolic content, antioxidant activity and rosmarinica acid content. This study emphasizes how biostimulant applications may be used to improve MAPs production performance when produced in agricultural organic system.

Key Words: Biostimulants, yield, chemicals, medicinal and aromatic plants, organic

IN SILICO AND *IN VITRO* EVALUATION OF SELECTED RARE HERBAL PLANTS INDIA IN COMBATING ALZHEIMER DISEASE THROUGH INHIBITION OF BACE1 ENZYME ACTIVITY AND TAU HYPER-PHOSPHORYLATION

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Few endangered medicinal plants viz. *Coscinium fenestratum*, *Decalepis nervosa*, *Belosynapsis vivipara, Cinnamomum wightii,* and *Embelia adnata* were selected for anti-alzheimer activity. Various phytochemical screening was performed for each plant extract, extracted by microwave using ethanol as solvent. Berberine (from *Coscinium fenestratum*), Gallic acid (*Decalepis nervosa*), and beta sitosterol (from *Belosynapsis vivipara*), globulol (from *Cinnamomum wightii*), and quercetin (from *Embelia adnata*) were isolated based on TLC and HPLC study and further docked these compounds with Alzheimer protein (PDB-ID: 1J1B; and 4ACU) Docking of the ligand was performed using the software AutoDock 4.0, to obtain the binding energy and the possible conformations and orientations at the binding site. Thereafter, *in vitro* acetylcholine esterase (AchE) inhibition study was performed using modified Electric eel method. Physostigmine, a standard AchE inhibitor, was used as positive control, which was dissolved in ethanol. Percent acetylcholinesterase inhibition was calculated. Result showed binding affinity for all the cases below -8.0 which indicated good responses of the bioactive constituents with the Alzheimer proteins. Furthermore, Protox-II software study revealed the non-toxic nature of all the constituents. Thereafter, inhibition of AchE was significantly resulted with the all the constituents when studied in vitro. Furthermore, the inhibition of beta-site APP-cleaving enzyme 1 (BACE-1) and tau hyper-phosphorylation were also estimated in vitro and resulted remarkable reduction by the isolated herbal bioactive constituents with lesser IC_{50} value. Overall result concluded that plant isolated bioactive compounds are much useful in mitigation of Alzheimer disorder with safe administration.

Key Words: Alzheimer disorder, isolated compounds, *in vitro* treatment, molecular docking, rare plants

A STANDARDIZED EXTRACT OF *OPUNTIA FICUS-INDICA* (L.) MILL. AND *OLEA EUROPAEA* L. PROTECTS AGAINST INDOMETHACIN-INDUCED CACO-2 INTESTINAL EPITHELIAL CELLS INJURY

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Nonsteroidal anti-inflammatory drugs (NSAIDs), widely used for their antipyretic, analgesic and anti-inflammatory effects, can damage the gastrointestinal mucosa, leading to mitochondrial dysfunction, oxidative stress, apoptosis and inflammation, and altering the permeability of the intestinal barrier with consequent cytotoxic effects. Proton pump inhibitors protect against gastric damage induced by NSAIDs but are ineffective for intestinal damage. Today, research has focused on the study of natural products as protective agents for intestinal damage caused by NSAIDs. In this study, a model of intestinal epithelial cells injury induced by indomethacin (INDO) was used to evaluate the *in vitro* beneficial effects of a standardized extract (OFI+OE), rich in polysaccharides and polyphenols, obtained from cladodes of *Opuntia ficus indica* (L.) Mill. and from leaves of *Olea europaea* L. Fully differentiated Caco-2 cells were pretreated with OFI+OE (350 and 700 µg/mL) for 24 h and subsequently exposed to 1 mM INDO for 24 h. Pretreatment with OFI+OE protected cells from INDO-induced epithelial damage, as demonstrated by transepithelial electrical resistance (TEER) measurement, fluorescein permeability, and tight junctions' expression. The extract significantly reduced oxidative stress, decreasing reactive oxygen species (ROS) levels and increasing total antioxidant activity (TAA), and blocked apoptotic cell death, by modulating Bcl-2, Bax and Caspase-3. Furthermore, the extract inhibited the NF-κB inflammatory pathway.
In conclusion, these data support the use of OFI+OE extract as a natural approach for the prevention and treatment of gastrointestinal mucosal damage induced by NSAID, demonstrating its beneficial effects through modulation of oxidative, apoptotic, and inflammatory pathways.

Key Words: Anti-inflammatory activity, apoptosis; Caco-2, indomethacin, NF-κB, *Olea europaea*, *Opuntia ficus-indica*, oxidative stress, tight junctions

ORAL PRESENTATIONS

GARLIC EXTRACTS NANOLIPOSOMES AS AN ENHANCER OF THE BIOAVAILABILITY OF ABA AND THIAMINE CONTENT, AND AS AN ANTIFUNGAL AGENT AGAINST *FUSARIUM OXYSPORUM* F.SP. PISI INFECTING *PISUM SATIVUM*

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Fusarium infections in *Pisum sativum* L. crops present a major constraint to cultivation, leading to substantial yield losses. However, effective disease management strategies, particularly the implementation of biological control methods, offer promising approaches for mitigating infection severity and limiting pathogen spread. In *P. sativum* cultivation, pathogen control is particularly challenging due to the limited penetration of pesticides into the leaves. This is attributed to the dense crystalline plate structure within the leaf cuticle, which acts as a barrier, reducing the efficacy of conventional chemical treatments. Therefore, optimizing the formulation of biopesticides and plant conditioning agents is essential to improve the absorption and bioavailability of active ingredients, ensuring more effective disease management in *P. sativum* cultivation. This study examined the exogenous effects of garlic extract in different formulations, including Elice Vakcina (liposomal formulation), Garlic-lipo (liposomal formulation), and Garlic-oil (oil-based formulation), which contained high concentrations of abscisic acid (ABA) at 6.3, 81, and 80.4 µg g^{-1}, respectively. Transcriptomic profiling, including the identification of Differentially Expressed Genes (DEGs) and KEGG pathway analysis of Elice Vakcina-treated field samples, revealed a significant upregulation of stress- and defense-related genes, as well as pathways associated with thiamine metabolism and ABA signaling. Notably, key defense genes, including pathogenesis-related (PR1, PR2, PR4, PR5), and SnRK2, were overexpressed, indicating an enhanced stress response. HPLC-DAD analytical investigations confirmed the activation of the thiamine biosynthesis pathway, demonstrating a 14.3% increase in vitamin B1 content. Furthermore, the absence of Fusarium infection in the treated small-plot field cultures suggests that the tested garlic extract formulation functions as a promising preventive biostimulants against plant fungal diseases.

Key Words: Garlic Extract, nanoliposome, bioavailability, antifungal, *Pisum sativum*

THE DETERMINATION OF GENETIC RELATIONSHIPS AND DIVERSITY AMONG *MUSCARI NEGLECTUM* AND *MUSCARI ARMENIACUM* POPULATIONS BY USING ISSR MARKERS

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Muscari Miller (Asparagaceae) species have been used in folk medicine for centuries as diuretic, expectorant, anti-nausea, anti-wart and anti-rheumatic. The species are also used as food for humans and animals in Türkiye. *Muscari neglectum* Guss. ex Ten. and *Muscari armeniacum* Leichtlin ex Bakerin are the most widely distributed species within the *Muscari* genus. Species are sometimes confused with dry samples by taxonomists. In fact, sometimes populations of species overlap and it becomes very difficult to separate dried samples from each other. In this study, populations of the targeted species were characterized with ISSR markers. Information on intra- and inter-species genetic variation and differentiation was attempted to be explained. According to molecular variance analysis (AMOVA), since the variation between populations (88.21%) is higher than the variation within populations (11.79%), it is thought that the species are quite differentiated from each other in point of genetic features.

Key Words: Asparagaceae, genetic differentiation, genetic diversity, population genetic analysis, Türkiye

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BIOACTIVE COMPOUNDS IN YERBA MATE: A NEW PERSPECTIVE ON IMMUNE CELL MODULATION

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The culture of tea drinking began quite some time ago; especially herbal tea blends have a certain potential to promote health. From a scientific perspective, the influence of natural substances from tea plants on human health is of particular interest. To better understand the health-promoting effects or therapeutic and prophylactic effects of teas, it is essential to elucidate individual ingredients of teas and their potential mechanisms of action through the identification of therapeutic targets. We are investigating the molecular basis of herbal teas from Paraguay. Our first focus is on Yerba Mate, a plant traditionally used in Paraguay, which is gaining significance in Europe as well [1]. Although the antioxidative and anti-inflammatory effects of Yerba Mate are well established, there remains limited understanding regarding its possible therapeutic indication beyond these aspects [2]. Another challenge arises from the predominant focus of existing literature on the exhaustive extraction of compounds from Yerba Mate, with less attention directed towards aqueous extracts [3]. Here we focus on the investigation on concentration-dependent effects of Yerba Mate on chemokine-induced immune cell mobilization and migration. Furthermore, we developed methods to collect different active ingredients from Yerba Mate. We show that Yerba Mate decreases the chemokine-induced migration of human immune cells in a dose-dependent manner. The outcomes from our assays are very promising and indicate that we will be able to unravel potential modes of action for Yerba Mate in immune-cell dependent indications.

Key Words: Yerba Mate, *Ilex paraguariensis*, herbal tea, immune cell migration, chemokine-induced response

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GENETICALLY MODIFIED MEDICINAL AND AROMATIC PLANTS

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Medicinal and aromatic plants hold a significant position in the pharmaceutical, cosmetic, and food industries due to their secondary metabolites, which exhibit antioxidant, anti-inflammatory, and antimicrobial properties. However, the production of these plants is influenced by genetic structure, environmental conditions, and agricultural practices. Genetic engineering emerges as a crucial tool to enhance resistance to biotic and abiotic stresses, optimize secondary metabolite production, and improve agricultural efficiency. This review article examines genetic modification techniques used in medicinal and aromatic plants and their applications. Modern gene-editing technologies such as CRISPR/Cas9, transgenic technologies, RNA interference (RNAi), and cloning are discussed, focusing on their potential to enhance secondary metabolite production, improve stress tolerance, and promote plant growth. Additionally, the biosafety, ethical considerations, and regulatory frameworks surrounding genetically modified medicinal plants are critically evaluated. The findings indicate that genetic engineering plays a significant role in the cultivation of medicinal and aromatic plants; however, biosafety concerns and regulatory frameworks must be considered. This study aims to provide a scientific perspective on integrating genetic modification techniques into medicinal plant cultivation and to guide future research in this field.

Key Words: Medicinal and aromatic plants, genetic engineering, genetic modification, biotechnology, secondary metabolites, sustainable agriculture

KARYOMORPHOLOGICAL AND PALYNOLOGICAL CHARACTERISTICS OF THREE ENDEMIC *HYACINTHELLA* (ASPARAGACEAE) TAXA IN TÜRKİYE

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In this study, the karyomorphological and palynological features of three *Hyacinthella* Schur: (*H. lazulina* K.Perss. & Jim Perss., *H. venusta* K.Perss., and *H. campanulata* K.Perss. & Wendelbo), endemic to Türkiye, were examined, and the basic chromosome numbers (x) of the genus *Hyacinthella* were confirmed. Plant bulbs were collected from their natural habitats in April 2019. Root tips obtained from the bulbs were used for chromosome analysis, employing the squash method with acetocarmine staining. After pretreatment, fixation, and staining, permanent preparations were made. Metaphase cells with clear morphology were photographed under a research microscope, and karyotypic parameters (chromosome measurements, arm ratio, asymmetry indices) were determined using the KAMERAM software. For palynological studies, pollen grains were analyzed using both light microscopy (LM) and scanning electron microscopy (SEM), and interspecific comparisons were made. The somatic chromosome numbers were determined as $2n$=22 for *H. lazulina* and *H. venusta*, and $2n$=18 for *H. campanulata*, supporting the base chromosome numbers of x=9 and 11 for the genus. The karyotypes of all three taxa were similar, with exclusively metacentric chromosomes. Asymmetry index (AI) values were also closely related: 0.477 (*H. lazulina*), 0.554 (*H. venusta*), and 0.532 (*H. campanulata*). In all three taxa, pollen grains were found to be prolate in shape with tectate exine structure and reticulate-perforate sculpturing. However, muri showed interspecific variation: muri were regular and uniform in *H. venusta*, small but regularly arranged in *H. lazulina*, and appeared large and irregular in *H. campanulata*. Palynological observations further revealed distinguishing features among the species. These findings provide significant insights into the cytogenetic and palynological diversity of *Hyacinthella*, contributing to the taxonomic and evolutionary understanding of this genus.

Key Words: Chromosome asymmetry, chromosome number, Hyacinthaceae, karyotype, pollen

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DIVERSITY OF SOME CRITICAL GEOPHYTES FROM ALGERIAN FLORA EVIDENCED BY TAXONOMICAL AND ECOLOGICAL DATA

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Geophytes are a key plants comprising several rare and endemic taxa, mostly members of the petaloid monocots clade. In recent years, their underground storage organs (bulbs, corms, rhizomes and tubers) were intensively collected by the Algerian local population for its ethnobotanical uses. This anthropogenic menace, habitat degradation and climate changes, really threaten their survival. Systematic inventory with nomenclature update was carried out. Sampled material from various bioclimatic sites was subjected to macro-and micro-morphometric measurements. Data matrix including ecological parameters was subjected to multivariate statistical analyses. Results revealed distinctive groups structured in a different bioclimatic gradient emphasizing adaptations and suggesting genome plasticity. This work aimed to clarify taxonomical and ecological status of Algerian taxa and prepares systematic revision with a focus on threatened endemics. Molecular phylogenetic inferences are currently carried out.

Key Words: Geophytes, Liliales, Asparagales, Systematic, Algeria, Ecology, Evolution.

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INVESTIGATION OF GENES AND KEY FACTORS ASSOCIATED WITH DROUGHT RESISTANCE IN MEDICINAL PLANTS: INSIGHTS INTO GENETIC AND ENVİRONMENTAL INTERACTION

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Drought stress is one of the most significant abiotic factors limiting plant growth and productivity. Understanding the genetic and biochemical mechanisms underlying drought resistance in medicinal plants is crucial for enhancing their tolerance and ensuring sustainable agriculture. Recent advances in molecular genetics have led to the identification of key genes involved in drought response, including those associated with polyphenol biosynthesis, flavonoid metabolism, antioxidant enzyme activity, and glycyrrhizin synthesis. This study investigates the genetic and environmental interactions influencing drought resistance in medicinal plants. Research findings indicate that certain genes play a fundamental role in plant adaptation to drought stress and can serve as potential targets for genetic engineering and breeding programs. For example, flavonoid biosynthesis, glycyrrhizin production and antioxidant activity play a key role in mitigating oxidative stress. The findings of this research suggest that increasing polyphenol and flavonoid production, activating antioxidant defense systems, and optimizing secondary metabolite biosynthesis can enhance drought tolerance in medicinal plants. These insights provide valuable strategies for plant breeding, genetic modification, and sustainable cultivation of drought-resistant medicinal plants.

Key words: Drought resistance, medicinal plants, gene expression, antioxidant enzymes, polyphenols and flavonoids

A COMPREHENSIVELY COMPARISON OF TWO CLOSELY RELATED MUSCARI SPECIES AND THEIR CONSERVATION STATUS

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The *Muscari* Miller genus in the Asparagaceae family comprises approximately 77 species worldwide. The genus has a wide distribution in the Caucasus, temperate Europe, Africa, and north-western, south-western, and central Asia. *Muscari* species are perennial bulbous plants native to Eurasia, predominantly blooming in spring with flowers that are typically blue, purple, or violet, and resemble grape clusters. In Türkiye, the numbers of recognized species have been increasing, with 52 currently identified. *Muscari turcicum* Uysal, Ertuğrul & Dural and *Muscari vuralii* Y. Bağcı & Doğu are two morphologically similar and closely related endemic species in Türkiye. Both species were recently described based on limited and localized populations, raising questions about their taxonomic boundaries due to the use of minimal distinguishing characters. This study aims to reveal the taxonomic relationships and boundaries of *M. turcicum* and *M. vuralii* taxa by comparing them with a broad concept using morphological, anatomical, palynological, chromosomal and molecular studies. In addition, it is aimed to clarify the taxonomic status of the taxa and determine their threat status. The analyses revealed that the two taxa discussed have significant common features, except for molecular data, and do not contain any significant differences in terms of the characters discussed. A study employing the *trnL* intron region to assess the genetic diversity and differentiation of these species found that gene flow (Nm) between populations ranged from 1.71 to 9.99, and genetic differentiation (G_{ST}) varied between 0.04 and 0.22. Additionally, both species exhibited high haplotype diversity (Hd: 0.7-0.8) and low nucleotide diversity (Pi: 0.004-0.005), suggesting rapid population expansion and the emergence of polymorphic regions during demographic growth. These findings indicate significant genetic overlap between the two species. Despite of the determined different haplotype diversity between two taxa, considering the high phylogenetical similarity and limited differences, it is proposed that *M. vuralii* be treated as a synonym of *M. turcicum* and classified as a subspecies under *M. turcicum*. We hope that this approach would simplify their taxonomic status and aid in conservation efforts.

Key Words: Endemic, Grape Hyacinth, Haplotype, Maternal Hereditary, Population, Türkiye.
Acknowledgements: We are grateful to the foundation TUBITAK (the project number 117Z222) and S.U. BAP (Project numbers: 18401016 and 19401172) because of their financial support.

MEDICINAL AND AROMATIC PLANTS AND TERRITORIAL DEVELOPMENT MODELS

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In Basilicata, the cultivation of Medicinal and Aromatic Plants (MAPs) remains a niche sector, although in certain areas, structured production systems have developed within micro-supply chains. Despite the presence of successful examples of cooperation among enterprises in the sector, the local MAP supply chain remains poorly structured and highly fragmented. The main challenges identified include the dispersion of the production sector, the limited capacity for aggregation among small enterprises, and difficulties in implementing integrated and efficient organizational models. Within this context, the transnational cooperation project ME.PLA.SU.S. (MEdicinal PLAnts in a SUstainable Supply chain. Experience of land-use practices) has been launched, promoted by the Council for Agricultural Research and Economics – Research Center for Policies and Bioeconomy (CREA-PB). The aim of this study is to present how the project has analyzed, within specific work packages, the sector's dynamics in relation to aggregation processes, highlighting how cooperative models and integrated strategies can contribute to improving the overall production framework, strengthening economies of scale, and enabling the adoption of more sustainable and innovative organizational models. The research activity on the MAP sector in Basilicata has revealed, from the early stages, a significant fragmentation of data, making it necessary to adopt an integrated methodological approach. This approach combined quantitative and qualitative analyses, structuring the investigation process into multiple phases to ensure a multidimensional assessment of the sector. Specifically, an initial desk analysis of the sector was conducted, followed by the administration of a structured questionnaire to a representative sample of enterprises. Subsequently, three in-depth Focus Groups were carried out, allowing for the identification of key critical issues, business needs, and barriers to the establishment of collaborative networks. The results obtained contributed to the identification of a development model aimed at strengthening supply chain dynamics in line with the needs of local producers. In the productive context of MAPs in Basilicata - characterized by the coexistence of well-established relationships and areas where connections between operators remain weak - the Network Contract could represent a strategic solution to enhance competitiveness, foster innovation, and promote the integration of local resources. The creation of a structured network could facilitate access to new development opportunities, expand international market outlets, promote know-how exchange, and optimize the shared use of equipment and infrastructure. Moreover, a network-based organization would ease access to funding provided by regional and national Rural Development Programs, thereby enhancing the sector's sustainability and competitiveness.

Key Words: Sustainable supply chain, competitiveness, medicinal and aromatic plants.

BIOLOGICAL ACTIVITY ASSESSMENT OF DIFFERENT PARTS OF *HEDYSARUM ANATOLICUM*

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The genus *Hedysarum* L. is represented by ca. 32 species in Türkiye, 18 of which are endemic. *Hedysarum anatolicum* Amirahm. & Kaz.Osaloo is a plant species endemic to Türkiye, belonging to the family Fabaceae. This species grows naturally in temperate climate conditions in a local of the Southeast region of Türkiye. It is thought that *H. anatolicum* could be a scientific importance due to its limited distribution area. In this study, methanol extracts from the leaves and flowers of *H. anatolicum* were prepared, and the antioxidant potentials of the obtained extracts and their effects on genotoxicity and acetylcholinesterase enzyme activity were investigated. In this scope, antioxidant activity was evaluated by the DPPH radical scavenging method, genotoxicity was evaluated by the *Allium cepa* L. method and acetylcholinesterase (AChE) inhibition was measured spectrophotometrically. The results revealed that the flower extract showed stronger antioxidant activity than the leaf extract. Similarly, in agreement with the antioxidant findings, AChE inhibitory activity was higher in the flower extract. In the *Allium cepa* test, leaf extract caused a decrease in mitotic index at all applied concentrations, while flower extract caused a decrease in mitotic index only at the highest concentration. These results suggest that the flower extract of *H. anatolicum* has significant biological activity and may be a promising candidate for future pharmacological studies.

Key Words: AChE, Fabaceae, pharmacological potential, ROS, Türkiye

IS ADVANCED NUTRIVIGILANCE A MISSING PIECE IN THE EU'S BOTANICAL SUPPLEMENT SAFETY PUZZLE?

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The term 'Botanical' presents the European Union with a longstanding regulatory issue because they fall under medicinal products for curative consumption but lacks formal definition as food supplements. The EU's legislation on botanical supplements leaves several unresolved puzzles, including the lack of standardized dosages, comprehensive positive/negative lists, and recognition of traditional evidence for health claims [1,2]. This creates genuine problems as member states classify the same botanicals differently, blurring the line between supplements and drugs. Moreover, the food manufacturers bear the responsibility to prove product safety through pre-market assessments while post-market risks receive insufficient attention [3]. The Aristolochia poisonings and more recent reports of hepatotoxicity linked to green tea extract serve as historical examples of how insufficient oversight exposes consumers to dangerous risks from plant-derived products that may carry similar risks to their medicinal counterparts [4, 5]. Beyond these widely recognised issues lies a critical but underemphasised safety gap: medicinal products benefit from robust pharmacovigilance systems monitoring adverse events, whereas their food supplements lack equivalent harmonised mechanisms, relying on reactive tools like the Rapid Alert System for Food and Feed [6]. To address this, the study explores legal sytems surrounding botanical supplements, drawing on EU regulations, case law, and comparative analyses of national reporting systems. As a solution, it proposes a proportionate and tailored nutrivigilance model-featuring an EU-wide adverse event reporting platform, harmonised terminology, and minimum reporting obligations for serious incidents. In conclusion, the paper suggests that such a system may also serve as a regulatory foundation for developing unified negative or restrictive lists for borderline products-an issue that remains unresolved in EU food law. The time is ripe for the EU to prioritise a coordinated nutrivigilance framework. This would ensure that consumer safety is not subordinated to market pressures, but embedded within a shared EU responsibility for public health.

Key Words: Botanical supplements, nutrivigilance, food law, eu regulation, safety monitoring, public health

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CHEMICAL PROFILING AND BIOACTIVITY ASSESSMENT OF *SATUREJA* SPP.: INSIGHTS FROM SPME AND IN VITRO ANTIMICROBIAL ASSAY

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The genus *Satureja* (Lamiaceae) comprises aromatic species that are particularly rich in volatile secondary metabolites, many of which are linked to notable biological activities such as antimicrobial effects. Endemic to the Mediterranean region, the genus is distributed across Southern Europe, North Africa, and Western Asia. Turkey, in particular, represents a centre of diversity, with 17 species, including seven endemics. In the present study, the volatile profiles of two *Satureja (S. cilicica* and *S. cuneifolia*) species were analysed using Solid Phase Microextraction (SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS). As a result of SPME analysis, it was determined that α-Terpinene, α-Phellandrene, and dl-Limonene compounds, which are compounds typically seen in *Satureja* species and other aromatic Lamiaceae members, were present in similar proportions in the content. Additionally, the antimicrobial potential of ethanol and water extracts of plant species was evaluated in vitro against some pathogenic Gram-positive (*Staphylococcus aureus* (MRSA), *Sarcina lutea*, *Bacillus cereus*, *Listeria monocytogenes*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Proteus mirabilis*) bacterial and fungal strains (*Candida parapsilosis* and *Candida albicans*) using broth microdilution method. When the antimicrobial activity results were evaluated, it was concluded that the extracts had different MIC (Minimum Inhibitory Concentration) values on different microorganisms and that ethanolic extracts were more effective than water extracts in terms of antimicrobial activity. The outcomes support the role of specific volatiles in antimicrobial efficacy and point to *Satureja* species as promising candidates for further phytochemical and pharmacological research.

Key Words: Antimicrobial activity**,** kayakekiği, kınalıkekik, Lamiaceae, volatile compounds

EXPLORING THE ROLE OF PLANT EXTRACTS IN ANIMAL MODELS OF INTRA-ABDOMINAL ADHESION: EFFECTS OF TANNINS

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Intra-abdominal adhesions, which arise from damage to internal organs or the peritoneum, are typically induced by factors such as surgical procedures, trauma, burns, tissue ischemia, and infections. This condition imposes a substantial financial burden on healthcare systems globally, with previous abdominal surgeries being the most prevalent cause. Adhesions that form following intra-abdominal surgery can lead to severe, life-threatening complications, occasionally requiring subsequent surgical interventions. Research indicates that certain compounds, particularly those possessing flavonoid, tannin, and anthraquinone structures, may offer protective and therapeutic potential in the context of intra-abdominal adhesion formation. Consequently, the present study evaluated the *in vitro* fibrinolytic effects of methanolic extracts prepared from the roots of *Asphodeline lutea* Rchb. (Asphodelaceae), *Rheum ribes* L. (Polygonaceae), *Rubia tinctorum* L. (Rubiaceae), and *Rumex nepalensis* Spreng. (Polygonaceae). *R. nepalensis*, which exhibited the highest fibrinolytic activity, was selected for further activity-guided fractionation studies. Through methods such as Sephadex LH-column chromatography, reversed-phase VLC, and preparative HPLC, cinnamtannin B1 and epicatechin gallate were isolated. Cinnamtannin B1 (1.9911 U/mL) demonstrated a higher fibrinolytic effect than epicatechin gallate (1.3371 U/mL). The bioactivity of the most active crude extract, sub-extract, fraction, sub-fraction, and isolated compound was comparatively assessed using an *in vivo* experimental intra-abdominal adhesion model in rats. Notably, in the *in vivo* experiments, the bioactivity of the VLC1 sub-fraction, which contained both epicatechin gallate and cinnamtannin B1, was particularly pronounced, with adhesion scores of 1.30±0.49, 0.67±0.21, and 0.83±0.31, compared to those of the control group (4.00±0.00, 3.00±0.00, and 3.00±0.00). This effect is hypothesized to be due to the synergistic interaction of these two compounds. Based on the findings, preclinical studies should focus on evaluating various dosages, formulations, and administration routes, alongside toxicity profiling and bioavailability assessments, to support clinical application.

Key Words: Intra-abdominal adhesion, *Rumex nepalensis*, cinnamtannin B1, epicatechin gallate

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POTENTIAL AROMATIC PLANT IN TÜRKİYE: *IRIS GERMANICA* L.

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Iris germanica L., a perennial rhizomatous plant of the family Iridaceae, is an economically valuable species with high medicinal and aromatic value. Essential oil, absolute and tea obtained from the rhizomes of the species are used in the treatment of cough, diarrhea, anti-inflammatory, arthritis, rheumatism and sinus infections, mental problems such as distress, anxiety, fear and mental fatigue. It also has antioxidant properties. The triterpenoid iridals in iris essential oils are biosynthetic precursors of iron and are widely used in the perfume and cosmetics industries for their pleasant violet-like aroma and their ability to fix other fragrances. Iris species contain more than 250 chemical constituents, including flavonoids, isoflavonoids and their glycosides, as well as various secondary metabolites such as benzoquinones, triterpenoids and stilbene glycosides. *α*, *β*, *γ*-irones, which are the main constituents *of Iris germanica* essential oil and determine the quality of the essential oil, are formed by the gradual oxidation of structures called irides during the aging process of the rhizomes. 3-year-old rhizomes are dried after harvesting and extraction processes are carried out after the dry rhizomes are aged (2-5 years). In Turkey, *Iris germanica* is cultivated only in a limited area of 250 ha in Isparta/Kuyucak. With this review, scientific researches on the botanical characteristics, uses, contents and cultivation of *Iris germanica* were examined and it was aimed to provide a source for future studies.

Key Words: *Iris germanica*, Orris Butter, essential oil, Süsen, irons, absolute

REGIONAL VARIATION OF CASTICIN LEVELS IN *VITEX AGNUS-CASTUS* L. POPULATIONS COLLECTED FROM WEST COAST OF ANATOLIA, TÜRKİYE

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The widespread use of medicinal and aromatic plants is related to the increase in people's awareness of health, their desire to take precautions against diseases and create their own treatments. These approaches encourage the transformation of medicinal and aromatic plants into new industrial products. Vitex agnus-castus L. is one of the most widely used and commercialized medicinal plants in the world with its rich therapeutic potential caused by the bioactive compounds it contains. Aims of this study to determine the populations of the chaste tree that can meet the needs of the sector in terms of high fruit and indicator compounds casticin from the regions spread in the Aegean Region, and to provide material for variety development studies in accordance with the codex and pharmacopoeias. The study carried out at in the Aegean Agricultural Research Institute, İzmir, Türkiye between 01.01.2022 and 31.12.2024, a collection program covering 6 provinces in the Aegean Region (Aydın, İzmir, Çanakkale, Manisa, Muğla, Balıkesir) was established. Casticin analyses of samples collected from 95 points were carried out by Bezmialem Vakıf University, Faculty of Pharmacy, Bezmialem Phytotherapy Center. Casticin was detected in 54% (52) of 95 fruit samples at a rate of more than 0.08% specified in the European Pharmacopoeia (EP), the U.S. Pharmacopoeia (USP) or the WHO monographs of chaste berry, the European Pharmacopoeia. Among the samples containing casticin at rates ranging from minimum 0.02% to maximum 0.22%. Sample coded 57 from Balıkesir origin ranked first with the highest content at 0.22%.

Key Words: *Vitex agnus-castus*, genetic resources, Aegean Regio, casticin, chase

BIOACTIVE PHYTOCHEMICALS, FATTY ACIDS AND SENSORY EVALUATION OF MELON FRUIT SEED OIL

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Oil extraction from fruit seeds is a process where oils are obtained from the seeds of various fruits. These oils are often rich in nutrients and bioactive phytochemicals and have various culinary, medicinal, and cosmetic applications. This study aims to investigate melon (*Cucumis melo* L.) seed oil bioactive compounds such as phenolic compounds, phytosterols and carotenoids and to evaluate the sensorial profile of the extracted seed oil. Obtained results could promote the potential applications of melon processing by-products with respect to their thermal properties. Melon seeds were found to contain 30.65% oil (on a dry weight basis). The chromatographic analysis of phenolic compounds showed that flavonoids were the most important group with predominance of amentoflavone (32.80 µg/g). Besides, melon seed oil presented considerable amounts of phytosterols (369.37 mg/100 g) in which *β*-sitosterol was the major sterol having 206.42 mg/100 g. Linoleic acid and oleic acid accounted for 84.82% of the total fatty acids in melon seed oil. Moreover, Hedonic tests revealed that over 80% of sensory panelist appreciated the melon seed oil aroma. Indeed, there is no significant difference (p-value > 0.05) in taste, color and appearance between melon seed oil and virgin olive oil. Understanding the bioactive molecules and nutritional qualities of seed oils is an essential factor when assessing their potential applications. Therefore, melon seed oil could be introduced as rich source of bioactive compounds for food formulations.

Key Words: *Cucumis melo* seed oil, polyphenols, phytosterols, fatty acids, bioactive compounds, sensorial analysis

EFFECTS OF *IN VITRO* HUMAN DIGESTION SIMULATION ON PHENOLIC PROFILE AND ANTIOXIDANT PROPERTIES OF *GALEGA OFFICINALIS* L.

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Galega officinalis L. (Goat's rue) from Fabaceae family is a perennial herb bearing white, blue or purple flowers. In medieval Europe, the plants aerial parts were medicinally used to treat various diseases such as plague and dysuria. Its hypoglycemic activity was discovered in the early 20th century [1]. Although there are studies investigating its bioactivities, evaluating the effect of human digestion on its phenolic profile and antioxidant activity is absent [2]. Studies show that human digestion system plays a crucial role on the bioavailability of herbal drugs [3]. Hence, this study aimed to compare the antioxidant activities of *G. officinalis* methanolic extracts before and after *in vitro* gastrointestinal human digestion simulation along with enlightening its phenolic profile. After the digestion simulation DPPH radical scavenging activity, metal reducing antioxidant activities (CUPRAC and FRAP), total antioxidant capacity (ToAC), total phenolic content (TPC), and total flavonoid content (TFC) assays were performed for each phase of digestion (ND: non-digested, PG: post-gastric, IN: serum-available/bioavailable). Additionally, phenolic profile of *G. officinalis* was brought to light with high performance thin-layer chromatography (HPTLC). Rutin and quercitrin presence were detected, therefore their absorbances were measured from TLC plate to obtain quantitative data. Results showed that overall antioxidant assays and TFC exhibited decreasing activity throughout the phases of digestion simulation. On the contrary, total phenolic content is increased after gastric digestion (ND: 110.87 ± 1.81 PG: 123.72 ± 1.68) yet serum-available fraction showed less activity compared to non-digested and post-gastric fractions (ND: 110.87 ± 1.81 IN: 87.77 ± 0.79). Rutin and quercitrin amounts were not significantly changed in PG fractions compared to ND extract whereas their amounts in IN fractions were not quantified. The obtained data displays the importance of digestion in terms of bioavailability of orally consumed herbal products and suggests that further studies should consider the effect of phytochemical profile on its bioavailability.

Key Words: *Galega officinalis* L., *in vitro* human digestion simulation, phenolic profile, antioxidant activity

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ANTIFUNGAL ACTIVITY AND CHEMICAL COMPOSITION OF *THYMBRA SPICATA* EXTRACTS AGAINST *SCLEROTINIA SCLEROTIORUM* AND *MACROPHOMINA* SP.: A COMPARATIVE STUDY

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In this study, the antifungal effects of essential oil, supercritical carbon dioxide (SC-CO*2*) extract, methanol extract, and SC-CO_2+ethanol extract obtained from *Thymbra spicata* L. were investigated under *in vitro* conditions against the fungal pathogens *Sclerotinia sclerotiorum* and *Macrophomina* sp., which cause significant economic losses in cultivated plants. The chemical composition of the extracts obtained in the study was determined using a GC/MS device. Carvacrol and p-cymene were identified as the main components of the SC-CO_2 and SC-CO_2+ethanol extracts, with percentages of 86.79% and 4.7% in SC-CO_2, and 72.07% and 4.6% in SC-CO_2+ethanol, respectively. The main components of the *T. spicata* essential oil were determined to be carvacrol (89.61%) and caryophyllene (1.86%). The main components of the methanol extract were identified as carvacrol (33%) and linoleic acid (21.19%). In the study, the contact effect method was used, and trials were set up with concentrations of 0.25, 0.5, 1.00, 2.00, and 4.00 µl/ml for all applications. The results showed that *T. spicata* essential oil (1.00, 2.00, and 4.00 µl/ml) and SC-CO_2 extract applications (2.00 and 4.00 µl/ml) completely inhibited (100%) the mycelial growth of *S. sclerotiorum* in a dose-dependent manner, demonstrating fungicidal effects. Similarly, against *Macrophomina* sp., the essential oil at 2.00 and 4.00 µl/ml, SC-CO_2 at 4.00 µl/ml, and SC-CO_2+ethanol extract at 4.00 µl/ml completely inhibited mycelial growth, showing fungicidal properties. These results revealed that the inhibition rates of mycelial growth were statistically significant ($p \leq 0.05$). However, the methanol extract at all doses had no effect on inhibiting the mycelial growth of either pathogen.

Key Words: Pathogens, carvacrol, supercritical CO_2, essential oil

GC-MS IDENTIFICATION AND TLC ISOLATION OF *PEGANUM HARMALA* SHOOTS TERPENOIDS WITH ANTI-INFLAMMATORY ACTIVITIES THROUGH MODULATION OF PRO-INFLAMMATORY CYTOKINES

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Natural products are increasingly recognized as a promising source of anti-inflammatory agents due to their multifaceted bioactivities and minimal side effects. Among these, plant-derived compounds such as phytosterols and terpenoids have shown potent antioxidant and anti-inflammatory properties, offering therapeutic potential for managing chronic inflammatory and oxidative stress-related diseases. This study aimed to optimize the extraction of bioactive compounds from *Peganum harmala* shoots using ultrasound-assisted extraction (UAE), a green and efficient technology, combined with Response Surface Methodology. Key parameters—ethanol concentration, ultrasonic power, and extraction time—were systematically varied to maximize the yield of antioxidant compounds. The optimized extraction conditions (73% ethanol, 20% ultrasonic power, and 12 minutes), combined with subsequent fraction purification, yielded a fraction significantly enriched in bioactive compounds, as determined by GC-MS. The fraction contained a high concentration of β-sitosterol (19.08%) and phytol (16.51%). Biological assays demonstrated potent antioxidant activity of shoot fraction, as evidenced by its high $DPPH^{•}$ and $ABTS^{•+}$ radical scavenging capacities. Moreover, *in vitro* experiments using LPS-stimulated RAW 264.7 macrophages revealed significant modulation of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, with no cytotoxicity observed at effective concentrations. These findings highlight the therapeutic potential of *P. harmala* as a natural source of bioactive compounds for mitigating inflammation and oxidative stress. Additionally, the study underscores the utility of sustainable extraction techniques, such as UAE, in producing bioactive-rich extracts for potential applications in pharmaceuticals, nutraceuticals, and functional foods.

Keywords: Natural anti-inflammatory agents, ultrasound-assisted extraction, *Peganum harmala* L., beta-sitosterol, oxidative stress modulation

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UTILIZATION OF MORPHOLOGICAL AND CHEMOTYPIC VARIATION IN PLANT BREEDING

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Breeding and variety development studies in medicinal and aromatic plants are carried out on different species all over the world. Species found in the natural flora of each region are turned into varieties using different breeding methods. The fertilization biology and production techniques of species that spread naturally are effective in the breeding method to be selected. At the same time, the used part of the bred species is also important in determining these studies. As in other species, yield and quality come to the fore in medicinal and aromatic plants. The most obvious selection criterion affecting yield and in terms of breeding is the morphological structure of the plants. In this respect, breeding studies are carried out according to the morphological characteristics of different types of plants in our studies. Plant height, branching status, leaf shape, size, fruit color, size, thorniness, hairiness, tuber shape, size, and flowering are some of the features used in these selections. On the other hand, in the evaluation made in terms of quality, selection and evaluation were also made according to the active ingredient contents of the plants. In this method, breeding studies were carried out according to chemotypic variation, especially in species containing essential oils. Because of the evaluation made according to the main components in the essential oils of plants, it was determined that different main components were prominent in different plants within the same species in essential oils. Thus, an important source was created in the selection of plants that can be used for different purposes and variety candidates were determined. In the studies we have conducted to date, 74 varieties belonging to 24 different species have been registered in medicinal and aromatic plants in Turkey. The most important species developed in this context are oregano, sage, rosemary, bay laurel and rosehip.

Key Words: Medicinal plants, breeding, variation, morphology, chemotype

RESEARCH OF SOME CHARACTERISTICS OF THE *CERINTHE MINOR* PLANT

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Cerinthe minor L. (Boraginaceae) is among worth-mentioning traditionally used plants in Turkey. *C. minor* belongs to the tribe Lithospermeae Dumort, and genus *Cerinthe* L., which is known as a small genus that includes approximately ten species distributed in the circum-Mediterranean region and Central Europe, more specifically, from the Atlantic region of Morocco to the western parts of the Irano-Turanian region As of today, four Cerinthe species (*C. minor, C. retorta* Sm., *C. major* L., and *C. glabra* Mill.), and two *C. minor* subspecies (*C. minor* subsp. *auriculata*, and *C. minor* subsp. *minor*) have been identified in Turkey. The above-ground parts of the plants were collected from Van Yuzuncu Yil University, Medicinal and Aromatic Plants Garden in 2022. In this study; total ash and dry matter ratio, macro-micro and heavy metal contents were examined. As a result of the study; total ash content was determined as 16.02% and dry matter content as 92.14%. Some of the macro and micro mineral contents were determined as potassium (K), 38.58 g kg^{-1}, calcium (Ca), 35.25 g kg^{-1}, magnesium (Mg), 3.57 g kg^{-1}, iron (Fe), 505.51 mg kg^{-1}, zinc (Zn), 31.98 mg kg^{-1} and copper (Cu) content was determined as 11.32 mg kg^{-1}. In terms of heavy metal content was determined as arsenic (As) as 0.707 mg kg^{-1}, lead (Pb) as 1.13 mg kg^{-1}, chromium (Cr) as 1.44 mg kg^{-1} and cobalt (Co) content was determined as 0.017 mg kg^{-1}.

Key Words: *Cerinthe minor* L., medicinal plant, chemical content, mineral elements

ESSENTIAL OIL YIELDS OF SOME *ORIGANUM* SPECIES DISTRIBUTED IN THE FLORA OF TÜRKİYE

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The flora of Türkiye is quite rich in terms of *Origanum* L. species and includes 32 taxa belonging to 27 species. 15 of these species are endemic. *Origanum* L. species, which have very different areas of use, are generally evaluated as spices and essential oils. The aim of this study is to determine the essential oil yields of some species of the *Origanum* L. genus, which naturally spread in different regions and provinces in Türkiye. 153 populations, including 6 species of the *Origanum* L. genus and 4 subspecies of these species, were used in the study. These populations were collected from natural flora in 29 provinces in 7 different regions of Türkiye. The Aegean and Mediterranean regions were the regions with the highest number of samples. When examined based on species, the highest number of samples belongs to the *Origanum onites* L. species with 102 samples. When the essential oil contents were evaluated according to species, *Origanum onites* L. varied between 0.2-8.5%, *Origanum majorana* L. 2.3-6.8%, *Origanum saccatum* P.H.Davis 2.8%, *Origanum sipyleum* L. 0.1%, *Origanum syriacum* L. 1.3-5.2%, *Origanum vulgare* L. 0.1-5.9%, *Origanum syriacum* ssp *bevanii* (Holmes) Greuter & Burdet 1.3-4.8%, *Origanum vulgare* ssp. *gracile* (K.Koch) Ietsw. 1.2-6.0%, *Origanum vulgare* ssp. *hirtum* (Link) Ietsw. 1.5-7.8%, *Origanum vulgare* ssp. *viridulum* (Martrin-Donos) Nyman 0.1-4.8%. The highest essential oil yields in *Origanum onites* L. were obtained from samples collected from the Mediterranean (8.5%) and Aegean (7.3%) regions. The highest essential oil yield in *Origanum majorana* L. was determined in samples from the Mediterranean region (6.8%). The higher essential oil content of these species, which are also widespread in other regions, in the Aegean and Mediterranean regions is closely related to the climate characteristics of the region, especially the high temperature.

Key Words: *Origanum* L., regions, species, populations, essential oil

DEVELOPMENTAL AND ENVIRONMENTAL FACTORS INFLUENCING ACCUMULATION OF PHENOLICS IN WOOD BETONY (*BETONICA OFFICINALIS* L.)

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Wood betony (*Betonica officinalis* L.) is a perennial species, used in Traditional European Medicine since ancient times. Although not included in the European Pharmacopoeia, it's herb (*Betonicae herba*) is still applied against some skin and digestive disorders. The raw material is collected exclusively from wild-growing plants. The extracts thereof reveal anti-inflammatory, antihemmorhagic and antireumatic activity. They are rich in phenolic substances. The aim of the study was to evaluate the accumulation of phenolics in betony herb during its developmental stages and to assess the influence of shading and environmental factors (geographical location) on the content of theses substances in the raw materials.
In the first experiment the raw materials (aboveground organs) were collected in the second year of cultivation, during five successive development stages (from vegetative phase in the spring to seed-setting). In the second, the herb was obtained from plants cultivated under shading nets and at full sunlight. The plants were grown in two locations (lowlands and mountain regions) simulating natural conditions in which betony occurs. The raw materials were evaluated in the content of five flavonoids (luteolin-8-C-glucoside, luteolin-7-glucoside, apigenin-7-glucoside, apigenin-3-glucoside¸ apigenin) and four phenolic acids (chlorogenic, caffeic, ferulic, and rosemary acids), using HPLC. Among analysed compounds apigenin and chlorogenic acid were the dominants. The highest content of both, flavonoids and phenolic acids was detected at the beginning of flowering. The plants cultivated under shading nets were chracterised with distinctly lower content of flavonoids but higher content of phenolic acids in comparison to those grown in full sunlight. Here, the geographical location influenced the quality of herb, too. The content of flavonoids and phenolic acids was distinctly higher in the raw materials obtained from plants grown in the mountain region than in lowlands. The obtained results show that the cultivation of bettony in mountain regions may be a promising method to obtain good quality, standardized raw material.

Key Words: Herb, phenolic acids, flavonoids, developmental stages, shading, geographical location

INSECTICIDAL ACTIVITY OF *ARTEMISIA ABSINTHIUM* L. ESSENTIAL OIL AGAINST TRIBOLIUM CONFUSUM DU VAL

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Essential oils and their bioactive constituents have garnered considerable attention worldwide for their potential in pest management. *Artemisia absinthium* L., also known as wormwood, is a bitter, aromatic medicinal plant traditionally used in various healing practices. The present study focuses on analyzing the chemical profile and assessing the insecticidal properties of *A. absinthium* essential oil (AEO) against *Tribolium confusum* Du Val, a significant pest of stored food products. Insecticidal assays were conducted using contact toxicity, fumigation, and repellency methods to evaluate the oil's effectiveness. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that the oil is predominantly composed of (+)-2-Bornanone (Camphor) (29.82%), followed by Beta-Myrcene (9.58%), Beta-Thujone (8.76%), Gamma-Terpinene (7.72%), Alpha-Pinene (7.67%), and Camphene (7.11%). These findings indicate that Algerian *A. absinthium* is a rich source of biologically active compounds. The observed insecticidal activity suggests that this essential oil could be developed as a promising natural alternative for controlling *T. confusum* infestations in stored-product environments.

Key Words: *Artemisia absinthium*, essential oil, *Tribolium confusum*, insecticidal activity, GC-MS, natural bioinsecticide

INFLUENCE OF UNSATURATION LEVELS ON THE EFFICACY OF ROSEMARY-DERIVED ANTIOXIDANTS IN STABILIZING WOODY EDIBLE OILS

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The development of woody oil crops in China has been prioritized to address supply-demand imbalances. However, specialty woody edible oils are highly susceptible to oxidation, posing significant preservation challenges. Plant-derived antioxidants offer advantages such as safety, efficacy, and environmental sustainability. Among the few food-grade plant antioxidants permitted in China, rosemary extract (RCE) is one of the three approved. However, its antioxidant effects, along with its main components, collectively termed Natural Products derived from Rosemary (NPR), remain understudied in Chinese specialty woody edible oils. To assess NPR's impact on oxidative stability, three oils from Camellia, Xanthoceras, and Paeonia seeds were selected and categorized based on their predominant fatty acid composition: n-9, n-6, and n-3. RCE, carnosic acid (CA), α-Tocopherol (α-T), and tert-butylhydroquinone (TBHQ) were tested for their effects on oxidative volatile formation and antioxidant performance. CA exhibited broad-spectrum inhibition of oxidative volatiles, surpassing both RCE and synthetic antioxidants. In Coil, oxidative volatile compounds (OVCs) of CA is 55.97 μg/g, which is lower than 66.66 μg/g of TBHQ. For Xoil, OVCs of CA is 49.2 μg/g， which is lower than 74.9 μg/g of TBHQ. In Poil, oxidative OVCs of CA is 20.68 μg/g, which is lower than 139.17 μg/g of TBHQ. Differential oxidative volatiles among oil types and those modulated by different antioxidants were identified using OPLS-DA and machine learning models, providing preliminary insights into oxidative aroma variances and antioxidant effects. Furthermore, MDA and FFA measurements confirmed NPR's antioxidant effects, while real-time oxygen consumption kinetic analysis demonstrated CA's superior antioxidant efficacy under high-temperature oxidation. In Poil, FFA inhibition rate of CA is 92%， which is higher than 84% of TBHQ. In Poil, MDA inhibition rate of CA is 95%, which is higher than 90% of TBHQ. This study highlights NPR's role, particularly CA, in mitigating oxidative deterioration, offering a promising green antioxidant strategy for enhancing specialty woody edible oil stability.

Key Words: Specialty woody edible oils, rosemary, carnosic acid, volatiles, antioxidant

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DIVERSITY OF COMMON VALERIAN ORIGINATING FROM POLAND

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Common valerian (*Valeriana officinalis* L.) is one of the most important medicinal plants cultivated in Poland. The plant provides a pharmacopoeial raw material (rhizome with roots; Valerianae radix) used for the production of sedative and anxiolytic preparations. As specified by the European Pharmacopoeia (monograph 04/2017:0453) dried, whole or fragmented underground organs of the plant are standardized on the content of sesquiterpenic acids (minimum 0.17%) and essential oil (4 mL/kg DW). The aim of the study was to investigate the diversity of common valerian originating from Poland in terms of: 1) differences between wild-growing populations and cultivated forms of the species; 2) the influence of bolting (pre-mature flowering) on the mass of underground organs and accumulation of active compounds; 3) chemical differences between underground and aboveground organs. The research was carried out using 10 wild-growing populations of common valerian originated from Eastern Poland and four forms of 'Lubelski' landrace cultivated in three regions of the country. The influence of bolting on the mass and quality of raw materials was investigated using plants with bolts kept (a) and with bolts removed (b). The diversity was also investigated on the level of plant organs. In the study underground organs, i.e. rhizome, thick root, thin root and aboveground organs, i.e. flowers, were compared. The raw materials obtained in the three experiments were analysed in the content of sesquiterpenic acids (HPLC) and essential oil (EO). The composition of the EO was determined using GC-MS. The wild-growing populations (diploids) were characterised with distinctly higer content of EO, but very low content of sesqiuterpenic acids in comparison to cultivated forms (tetraploids). Bolting in valerian cultivation resulted in a distinct reduction in the mass of underground organs. Among underground organs, thick roots are the primary source of EO and sesquiterpenic acids. The chemical composition of valerian rhizome, root, and flower EO is diverse. Bornyl acetate clearly dominates in underground organs EO, whereas in flower EO hexadecanoic acid is the major compound.

Key Words: Valerian, diversity, sesquiterpenic acids, essential oil, underground organs, flowers

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MASS SPECTROMETRY-BASED PROTEOMICS REVEALS MOLECULAR MECHANISMS UNDERLYING THE ANTICANCER ACTIVITY OF *SALVIA OFFICINALIS* ESSENTIAL OIL IN HUMAN PROSTATE CANCER

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Salvia officinalis L. (sage) is a medicinal and aromatic plant traditionally known for its rich in bioactive components such as thujone, camphor, and 1,8-cineole. These components are suggested to possess anti-inflammatory, antioxidant, and anticancer properties. This study was designed to explore the anticancer potential of *S. officinalis* essential oil (SOEO) in human prostate cancer (DU-145) cells through integrated proteomics and bioinformatics strategies. DU-145 cells were exposed to varying concentrations of SOEO and cytotoxic effects were assessed using the MTT assay. Apoptotic cell death was evaluated using dual staining with Annexin V-FITC and PI, followed by flow cytometry analysis. Proteomic alterations were analyzed by 2D-SDS-PAGE, and differentially expressed proteins were identified using MALDI-TOF/TOF-MS. Identified proteins were analyzed using multiple bioinformatics platforms and databases including STRING, OMIM, GeneCards, Swiss-Prot, HPA, GO, and KEGG. Western blot analysis was conducted to validate key targets identified in the proteomic screening. SOEO significantly inhibited cell proliferation in DU-145 cells in a concentration-dependent manner, with IC_{50} values ranging from 20 to 80 μg/mL. Apoptosis analysis showed increased phosphatidylserine exposure, caspase-3 activation, and modulation of the Bax/Bcl-2 ratio, indicating intrinsic mitochondrial apoptotic signaling. Proteomic profiling identified several differentially regulated proteins post-SOEO treatment. Key downregulated proteins included HSP90AA1 (involved in protein folding and cancer cell survival), GAPDH (a glycolytic enzyme with roles in apoptosis regulation), and HNRNPK (a multifunctional RNA-binding protein linked to oncogenesis). In contrast, pro-apoptotic proteins such as GRP78 (an ER stress marker) and p53-related proteins were upregulated. Bioinformatics analyses demonstrated that these proteins were enriched in pathways related to p53 signaling, ER stress response, PI3K/AKT signaling, oxidative stress regulation, and TNF-mediated apoptosis. Notably, suppression of proteins involved in DNA repair (e.g., RAD51) and metastasis (e.g., MMP-2 and MMP-9) was observed, suggesting impaired cellular repair mechanisms and reduced invasive potential. In conclusion, SOEO promotes apoptosis via mitochondrial and ER stress-related pathways, disrupts oncogenic signaling, and impairs cell proliferation and invasion. Accordingly, these findings support the potential application of SOEO as a promising phytotherapeutic agent in prostate cancer treatment.

Keywords: *Salvia officinalis* L., essential oil, prostate cancer, apoptosis, proteomics, MALDI-TOF/TOF-MS

TEA TREE OIL AGAINST DERMATOPHYTES AND YEASTS

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Onychomycosis is defined as infection caused by nondermatophytic molds and yeasts: tinea unguium is caused by dermatophytes. Within this study, hydroxyethyl cellulose (HEC) as an important non-ionic, water-soluble cellulose derivative was chosen to develop formulations containing tea tree oil as active antifungal agent were developed and evaluated for their potential in the treatment of onychomycosis. Two polymeric films based on HEC (HEC-B-04 and HEC-E-10) were obtained by solvent evaporation method and characterized in terms of appearance, disintegration, stickiness, elongation, rheological behavior and adhesiveness. Moreover, different strains of dermatophytes such as *Trichophyton rubrum* and yeasts as *Candida albicans* were treated with polymeric films containing tea tree oil (0.5 – 2 % v/v) in order to determine their antifungal potential by the inhibition zone assay. Results: HEC-B-04 and HEC-E-10 were investigated by SEM measurements resulting in confluent surface morphology. HEC-B-04 and HEC-E-10 showed disintegration after 32.7 min and 34.0 min, respectively. Furthermore, HEC-E-10 revealed a moisture index of 1.74 and underpinned adhesive properties in terms of required detachment force with 4.86 N. HEC-E-10 pointed to the most antifungal one among the others against *Trichophyton rubrum* and *Candida albicans.* Taking these findings in consideration, promising adhesive onychial formulations were developed as forthcoming approach in treatment of nail infections.

Key Words: Essential oils, natural cosmetics, tea tree oil, *Candida albicans*

PHYTOPHARMACEUTICAL POTENTIAL AND BREEDING CHALLENGES OF SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES* L.): A REVIEW OF AN UNDERUTILIZED HORTICULTURAL RESOURCE

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Sea buckthorn (*Hippophae rhamnoides* L.), a deciduous and dioecious shrub native to Europe and Asia, has garnered significant attention due to its rich phytochemical profile and broad spectrum of medicinal applications. The berries, seeds, and leaves of sea buckthorn contain high concentrations of bioactive compounds, including vitamins C and E, flavonoids, carotenoids, phytosterols, and essential fatty acids such as omega-3, -6, -7, and -9. These constituents offer antioxidant, anti-inflammatory, immunomodulatory, and wound-healing properties, positioning sea buckthorn as a valuable phytopharmaceutical crop. Despite its therapeutic promise, the crop remains underutilized in modern horticultural systems, particularly in countries like Türkiye, where its natural habitat suggests potential for adaptation and cultivation. This review highlights the current understanding of sea buckthorn's medicinal value while emphasizing the major challenges limiting its widespread breeding and commercialization. Key breeding constraints include dioecy, long juvenile phase, thorniness, and the lack of early sex identification markers. Moreover, limited genomic resources and inadequate propagation methods hinder the selection and improvement of elite cultivars. Recent advances in biotechnology such as tissue culture, molecular marker-assisted selection, and transcriptomic tools, offer promising strategies to overcome these challenges. Integrating conventional and biotechnological approaches could accelerate the development of high-yielding, thornless, and nutraceutical-rich cultivars. By combining current knowledge, this review aims to support repositioning sea buckthorn as a valuable medicinal and horticultural resource, especially in arid and semi-arid regions where it can contribute to human health and sustainable agriculture.

Key Words: Sea buckthorn, phytopharmaceuticals, breeding challenges, biotechnology, underutilized medicinal plant

INTEGRATED APPROACHES FOR THE OPTIMIZATION OF BIOACTIVE *THYMUS* PRODUCTS

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Species from the genus *Thymus* are of global phytochemical interest due to their diverse biological activities and potential applications in the pharmaceutical, cosmetic, and food industries. In Bulgaria, 21 species of *Thymus* are found, including two Bulgarian endemics, four Balkan endemics, and three subendemics restricted to the Balkan Peninsula. The taxonomic complexity of the genus and the difficulty in accurately identifying species have limited species-specific phytochemical studies worldwide. In industrial practice, various *Thymus* species are often collected under the general label *Thymus* sp. (Thyme), without consideration for the species-specific phytochemical profiles that directly influence their biological efficacy. The aim of the study is to develop an integrated and sustainable production model for high-quality *Thymus*-based products, building upon extensive taxonomic, phytochemical, and ecological research on the genus. As a result, the project will deliver a complete product package consisting of: (1) three distinct thyme teas with well-defined essential oil profiles, rich in valuable secondary metabolites and expected to exhibit strong antioxidant and antibacterial activities, derived from cultivated plants with enhanced biomass yield; (2) seedlings of the selected *Thymus* species; and (3) a practical cultivation guide promoting propagation through environmentally sustainable agricultural practices.

Out of 283 natural populations studied across the country, three promising species were selected and successfully introduced into cultivation: *T. longedentatus*, *T. zygioides*, and *T. pannonicus*. The essential oil of *T. longedentatus* is rich in oxygenated monoterpenes (78.7%), especially the citral isomers neral (27.5%) and geranial (30.3%), which give it a strong lemon aroma. *T. zygioides* has a thymol-dominant oil, with concentration significantly increasing after cultivation (51.2%). T. pannonicus exhibits the most complex oil profile and was classified as a germacrene D/β-caryophyllene chemotype. To preserve the unique characteristics of the selected species, they will be vegetatively propagated via hydroponics from cuttings. The project also incorporates biodegradable seedling containers and organic farming methods, reinforcing its commitment to environmental sustainability. Through its innovative methods and sustainable practices, the project has the potential to serve as a long-term model for high-quality herbal product development, contributing positively to both the economy and ecology of the region.

Key Words: Functional herbal products, green biotechnology, Thyme, sustainable cultivation

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UNVEILING CHEMOTYPE-SPECIFIC ANTIBACTERIAL ACTIVITY IN CUPRESSACEAE ESSENTIAL OILS: INTEGRATING MACHINE LEARNING WITH BIOLOGICAL INSIGHTS

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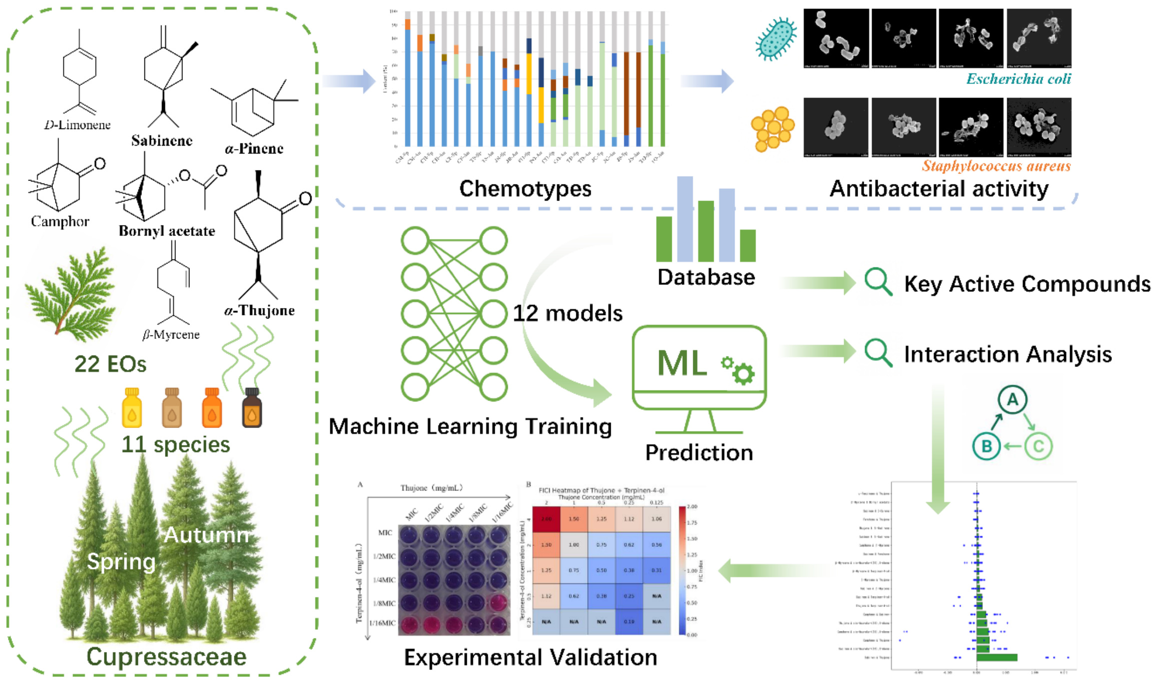
Cupressaceae essential oils (CEOs) are gaining interest for their potential as natural antibacterial agents in food applications. In this study, 22 CEOs from 11 species across 8 genera were analyzed to evaluate chemical variation influenced by genetic origin and harvesting season. The CEOs were classified into four major chemotypes, with significant seasonal effects on key components. Antibacterial assays revealed that CEOs exhibited stronger activity against *Escherichia coli* than *Staphylococcus aureus*, which was associated with membrane disruption and the leakage of nucleic acids and proteins. Among the chemotypes, thujone-type CEOs showed the highest activity, particularly in spring-harvested samples, correlating with elevated thujone content. Thujone was also identified as one of the most active monoterpenes. A chemical–activity database was constructed and used to train multiple machine learning models to explore constituent interactions. Experimental validation confirmed a synergistic antibacterial effect between thujone and terpinen-4-ol, partially explaining the enhanced efficacy of some CEOs. This study highlights the influence of seasonal and genetic factors on CEO composition and activity, identifies key antibacterial constituents, and demonstrates the value of machine learning in predicting synergistic interactions. These findings provide a scientific basis for the development of CEOs as effective natural preservatives in food systems.

Key Words: Cupressaceae essential oils, Antibacterial activity, Seasonal influence, Synergistic interaction, Machine learning



CARBOHYDRATE ENCAPSULATION OF FRAGRANCES IN *PARROTIA PERSICA* LEAVES

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Starting from the assumption that different carbohydrates can encapsulate fragrances and influence the dynamic release of them, a study was conducted to determine the carbohydrate composition of *Parrotia persica* leaves. After solvent extraction, the extract obtained was analyzed by GC-MS. The GC-MS chromatograms were recorded in splitless mode. The 1,2:4,5-di-O-isopropylidene-*α*-D-fructopyranose was present in 21.20%. The diacetoneglucose was present in 9.81%, while 2,3:4,5-bis-O-(1-methylethylidene)-*β*-D-fructopyranose in 2.12% and 1,2:3,4:5,6-tris-O-(1-methylethylidene)-D-mannitol in 1.77%. The most dominant compound present in 24.37% was of non terpenoid structure and was not identified. Further investigations can reveal the influence of carbohydrates present in *P. persica* leaves on the fragrance's encapsulation, dynamic release and impact on fragrances release profiles.

Key Words: Carbohydrates, *Parrotia persica*, leaves, GC-MS

THERAPEUTIC POTENTIAL AND BIOACTIVE PROPERTIES OF POMEGRANATE PEEL EXTRACT FROM ISFAHAN VARIETIES IN VETERINARY MEDICINE

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Pomegranate peel extract, especially from the Isfahan variety, has garnered significant attention in veterinary medicine due to its rich bioactive composition and numerous therapeutic potentials. This study explores the phytochemical profile of pomegranate peel and evaluates its therapeutic effects, particularly its antioxidative, antimicrobial, and anti-inflammatory properties. Four different samples of pomegranate peel were analyzed, and the results revealed varying levels of bioactive compounds. The total phenolic content (TPC) ranged from 12.8 to 23.3 mg GAE/ml, while the total flavonoid content (TFC) varied between 27.6 to 61.9 mg RU/ml. Notably, the antioxidant activity exhibited strong inhibition values ranging from 90.4% to 95.7%, indicating the extract's powerful potential in countering oxidative stress. These findings suggest that pomegranate peel extract could be effectively used in veterinary applications to support animal health, particularly in oral hygiene products and as a hepatoprotective agent. However, further research is required to optimize extraction techniques and evaluate the long-term safety and efficacy of the extract in animal models. If this research progresses, pomegranate peel could become a valuable and sustainable alternative in veterinary medicine.

Key Words: Pomegranate peel extract, antioxidant activity, phenolic content, flavonoid content, veterinary medicine

STUDYING WILD *THYMUS* SP. (L.) CHEMOTYPE DIVERSITY ACROSS PISTOIA MOUNTAINS: A KEY FOR OBTAINING TAILORED THYME-DERIVED PRODUCTS WITH ANTIFUNGAL EFFICACY

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With more than 500 species, *Thymus* sp. (L) is an aromatic plant populating the whole Mediterranean area, up to 1800 meters above sea level. The Pistoia Apennines (Tuscany, IT) hosts a great variety of aromatic species, including *Thymus* sp. (L) belonging to the *Serpyllum* group. A thyme population was sampled from five different locations (Butale, Donagaccia, Melo, Osservatorio, and Pracchia) within this area, and subjected to a chemical characterization of the foliar terpene profile through GC-MS analysis. Key information was obtained studying the chemotype diversity of this population, which helped to select the plant material for thyme essential oil (EO) extraction. Based on the prevailing terpenes, seven distinct chemotypes were found analyzing 94 leaf samples by GC-MS, with the thymol-chemotype being the most represented across all the considered locations. Thyme EO was steam-distilled from the fresh biomass collected in the area of Osservatorio in July 2023, and the hydrolate obtained as byproduct of the distillation process was also recovered and chemically characterized. The main terpene was thymol plus its precursors, representing about 75 % (in EO) and 62 % (in hydrolate) of the total terpenes. An additional objective of this work involved the experimentation of thyme EO and its hydrolate as antifungals on two mould species found to degrade paper, *i.e.*, *Alternaria alternata* and *Cladosporium cladosporioides*. The tests were performed *in vitro* on Whatman paper inoculated with a $1x10^5$ conidia/mL fungal suspension. For both fungi, a significant growth reduction was observed with 4 mL and 10 µL of hydrolate and thyme EO, respectively. Further research is necessary to explore the applicability of such thyme-derived products, and an accurate selection and chemical characterization of the plant material is an essential prerequisite to start with.

Key Words: *Thymus* sp., chemotype, GC-MS, essential oil, hydrolate

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CHIRAL CONSTITUENTS OF SEA FENNEL ESSENTIAL OIL

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Sea fennel (*Crithmum maritimum* L.) is one of the most widespread perennials, facultative halophytes with a great ability to survive in saline environments, and well-developed mechanisms of adaptation to the Mediterranean climate. Due to its resilience to harsh environmental conditions and climate changes, sea fennel is being studied for its potential applications in sustainable agriculture and functional foods. In addition, it is used for medicinal purposes, as numerous nutritive and health-beneficial components have been detected and identified in all parts of the plant, such as ascorbic acid, minerals, carotenoids, organic acids, fatty acids, phenolic compounds and volatiles [1]. The dry sea fennel stems were hydrodistilled in a Clevenger-type apparatus for 3 h and the analysis of the obtained essential oil was performed by gas chromatography coupled with mass spectrometry (GC-MS) equipped with a standard non-polar (HP-5MS) and chiral (CycloSil-B) columns. Essential oil constituents were identified by comparison of their retention indices and matching the mass spectra with commercial databases, literature data and comercial standards [2]. Moreover, the main chiral constituents were determined by the comparison with reference standards. Sea fennel essential oil was characterised by a high content of limonene (almost 50%) and significant amounts of sabinene (16%), *γ*-terpinene (12%), *p*-cymene (7 %) and terpinen-4-ol (5%). GC-MS analysis using the chiral column revealed that only (*R*)-(+)-limonene was present while (*S*)-(–)-limonene was not detected. Similarly, only a single enantiomer was detected in the case of sabinene, and *γ*-terpinene. On the contrary, both enantiomers of terpinen-4-ol were identified, with *S*-(+)-terpinen-4-ol being more abundant than the subsequently eluting (*R*)-(–)-terpinen-4-ol. This is the first study to investigate the chiral chemical composition of *C. maritimum* essential oil.

Key Words: sea fennel (*Crithmum maritimum* L.), essential oil, chiral composition, limonene, sabinene, *γ*-terpinene, terpinen-4-ol

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STUDY OF THE CHEMICAL COMPOSITION AND CHEMOTYPES OF THE ESSENTIAL OILS OF THYMELAEACEAE FROM ALGERIA AND TUNISIA

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Natural plant-derived substances are highly sought-after due to their diverse biological activities, which offer significant health benefits. These bioactive properties make them valuable across various fields including pharmaceuticals, perfumery, cosmetics and food processing for their therapeutic, organoleptic and aromatic attributes. Algeria and Tunisia are rich in aromatic and medicinal plants with significant interest, particularly in valorizing of phytogenetic resources. The genus Thymelaea (Thymelaeaceae) comprises approximately 31 species with eight species recorded in Algeria (Quezel and Santa, 1962-1963). Several species of this genus have shown biological activity, leading to several phytochemical investigations that have revealed a wealth of bioactive secondary metabolites. Among the species, *Thymelaea hirsuta* (L) Endl has been the focus of this study, aiming to contribute to its valorization by establishing a correlation between its chemical composition and geographical distribution. Essential oils of *T. hirsuta* were extracted using a Clevenger-type apparatus, and their chemical composition was analyzed via GC/MS. The essential oil yield averaged 0.3 ± 0.12 % per 100 g of plant material. Gas chromatography analysis identified 45 chemical components, with the major constituents being nonanal-n (10.39 ± 3.21%), hexadecanoic acid (9.77 ± 2.81%), nonanoic acid (9.13 ± 6.49%), triacontane (7.2 ± 3.34%), isopropyl tetradecanoate (6.16 ± 1.99%) and tridecane (4.87 ± 3.1%). UPGMA clusters analysis and the chemical variability assessment of *T. hirsuta* essential oil led to the identification of multiple chemotypes, resulting in the classification of studied populations into two distinct clades. The first clade includes two Algerian populations, characterized by high nonanoic acid content (9.13% on average). The second clade encompasses populations with an elevated triacontane concentration (7.2%). Similarly, Tunisian populations were divided into two clades; the first is distinguished by the presence of a high proportion of heptane, suggesting a unique heptane- germacrene-D Tunisian chemotype, while the second clade includes both Algerian and Tunisian populations with dominant components such as hexadecanoic acid, stylopsal and 4,8-dimethylhecosane, indicating a second distinct Tunisian chemotype. In conclusion, the chemical differentiation among *T. hirsuta* populations confirms significant intra- and interspecific variations, highlighting the species' potential for further biochemical and ecological studies

Keywords: Essential oils, *Thymelaea hirsuta*, chemical identification, chemotype, Algeria, Tunisia

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MORPHOLOGICAL, MICROMORPHOLOGICAL, ANATOMICAL AND PALYNOLOGICAL STUDIES ON *GALIUM PEPLIDIFOLIUM* BOISS. AND ENDEMIC *GALIUM PENDULIFLORUM* BOISS. SPECIES FROM TÜRKİYE

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In this study, two closely related species of *Galium peplidifolium* Boiss. and the endemic *Galium penduliflorum* Boiss. in the *Kolgyda* section of the genus *Galium* L. belonging to the family *Rubiaceae* were investigated for their morphological, micromorphological, anatomical and palynological characteristics. The morphological studies described detailed descriptions of the species and the deficiencies in the Flora of Turkey were filled. Morphologically, *G. peplidifolium* differs from *G. penduliflorum* by its shorter stature, curved and geniculate stem with about 1/2 purplish color from the base, 4-5(-6) circumferential leaves, the corolla shape is cup-shaped, the apex is not aristate and the pedicels are not somewhat nodding. In micromorphological studies, stem and leaf hair characteristics and fruit surface structures were examined. Both plants have simple, short or long unicellular hairs. There are more hairs on the upper side of the leaf than on the lower side. The surface of the mericarps is partially depressed-split or folded. The surface of the mericarp epidermis of *G. peplidifolium* is striated, especially in the middle parts, whereas in the other it has a small granulate-striated structure. Stem and leaf structures of the plants were described in anatomical studies. The stems of both species are square in cross-section and densely hairy. A denser layer of chlorenchymatic parenchyma was found in *G. penduliflorum*. The leaves of the plants are dorsiventral. Both species have hairs and raphytids on the upper and lower surfaces of the leaves, while sand crystals were found only in *G. peplidifolium*. Stomata are parasitic type and were observed on both epidermis. In palynological studies, pollen characteristics of the plants were examined by scanning electron microscopy. Pollen grains were hexa-heptacolpate in *G. peplidifolium* and hexa-octacolpate in *G. penduliflorum*. In both species, the shape of the pollen grains is prolate and the ornamentation is microechinate-perforate.

Key Words: *Rubiaceae*, *Galium*, morphology, micromorphology, palynology, anatomy

THE POTENTIAL OF SOME WILD PLANTS COMMONLY CONSUMED IN THE EASTERN BLACK SEA REGION FOR MIXED CULTIVATION IN HAZELNUT ORCHARDS

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A soil survey was conducted in the Eastern Black Sea region of Turkey Ordu province Gölköy district) to evaluate soil characteristics relevant to intercropping wild edible plants sakarca (*Ornithogalum umbellatum* L.), melocan (*Smilax excelsa* L.), and galdirik (*Tra*chystemon *orientalis* L.) within hazelnut orchards. Twenty-two soil samples were collected from Gölköy (Ordu), and their coordinates were recorded. Soil texture, pH, electrical conductivity (EC), lime content, and organic matter (O.M) were analyzed. The results showed a range of soil textures including clay loam, clay, sandy loam, and loamy sand. Soil pH was moderately acidic to near-neutral. EC values were generally low, indicating a lack of salinity. Lime content was low across all samples. O.M varied, suggesting differing levels of fertility. Based on these soil properties, the suitability for intercropping sakarca, melocan, and galdrik varies. The moderate acidic pH is generally favorable for these plants, which often thrive in slightly acidic conditions. Low lime content is also beneficial, as high lime levels can inhibit nutrient availability. While specific requirements for each plant need to be considered, the range of soil textures observed suggests that with appropriate management, including organic matter amendments where necessary, intercropping these wild plants could be viable in the studied hazelnut orchards.

Key Words: Aromatic plants, hazelnut orchards, soil analysis, soil fertility

PROTECTIVE EFFECTS OF *SALVIA OFFICINALIS* L. HYDRODISTILLATION WASTEWATER IN *IN VITRO* MODELS OF INFLAMMATION

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The essential oil production from *Salvia officinalis* L. generates, alongside an exhausted biomass, a variable amount of wastewater rich in water-soluble compounds, with potential biological activity, that are often discarded without further application. This study aimed to characterize the polyphenolic composition of *S. officinalis* hydrodistillation wastewater and to evaluate its antioxidant and anti-inflammatory properties using *in vitro* models. Phenolic compounds were identified by HPLC-DAD and HPLC/ESI-MS analysis. Antioxidant capacity was assessed through DPPH, TEAC, and SOD-like activity assays. Inflammatory markers were evaluated in Raw 264.7 and Caco-2 cells using qPCR and western blot, while barrier integrity in Caco-2 cells was measured by TEER analysis. The phenolic HPLC profiles of wastewater revealed the presence of organic acids, flavones and flavonols. The extract showed also a strong antioxidant capacity, confirmed by DPPH, TEAC and superoxide dismutase (SOD)-like activity assays. Regarding the anti-inflammatory activity, the phytocomplex (50 and 100 µg/mL) prevented *E. coli* LPS-induced inflammation in Raw 264.7 cells, suppressing NF-κB activation and downregulating its target genes (*IL-6*, *IL-8*, and *TNF-α).* Additionally, a co-culture model was developed to better simulate the intestinal environment. In this system, cells were pre-treated with the extract (100 and 200 µg/mL) for 24 hours and subsequently exposed to E. Coli for 2 hours to mimic *in vitro* pro-inflammatory conditions. In *E. Coli*-challenged Caco-2 intestinal cells, pre-treatment with the extract preserved epithelial barrier integrity by increasing TEER values in a dose-dependent manner. This effect was also associated with claudin-1 and occludin restoration and reduced COX-2 expression. These findings highlight the presence of active antioxidant and anti-inflammatory compounds in *Salvia officinalis* L. hydrodistillation wastewater. Its reuse offers, therefore, a sustainable strategy for developing bioactive products with potential applications in the prevention of intestinal inflammation, promoting a circular approach within the medicinal and aromatic plant supply chain.

Key Words: *Salvia officinalis* L., hydrodistillation wastewater, flavonoids, *E. coli*, intestinal inflammation

MORPHOLOGICAL, MICROMORPHOLOGICAL AND KARYOMORPHOLOGICAL FEATURES OF MEDICINAL *SILYBUM MARIANUM* (ASTERACEAE) IN TÜRKIYE

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Silybum marianum is a well-known medicinal plant traditionally used for its hepatoprotective properties. It has been employed in the treatment of various liver conditions, including those involving functional impairment or degenerative necrosis. The plant's hepatoprotective effects are multifaceted, acting through antioxidant and anti-inflammatory mechanisms, regulating cell permeability, stabilizing membranes, promoting liver regeneration, and inhibiting collagen deposition, which could potentially lead to cirrhosis. While much of the research on *S. marianum* has focused on liver diseases, recent studies have also highlighted its potential benefits for a range of other conditions. These include renal protection, hypolipidemic and anti-atherosclerotic effects, cardiovascular health, prevention of insulin resistance (especially in cirrhosis patients), and potential roles in cancer and Alzheimer's disease prevention. Additionally, *S. marianum* is also utilized as a food remedy. After being collected in 2023 from the Gaziantep province of Türkiye, *S. marianum* was studied in detail in terms of its morphological, karyomorphological, and micromorphological characteristics. This study presents the first detailed report on various characteristics of *S. marianum*, including stem diameter, measurements of upper stem leaves, bract series, shapes and dimensions of phyllaries, and floret traits. Additionally, the chromosome number was determined to be 2n=34, and the seed surface pattern was identified as reticulate.

Key Words: Chromosome, Compositae, milk thistle, plant

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SOME BIOACTIVE COMPOUNDS OF *ACHILLEA* SPP. AND THEIR MEDICINAL USES

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Achillea L. is one of the important medicinal plants, commonly known as Yarrow, belongs to the Asteraceae family. It is a perennial plant that spreads in temperate regions such as Asia, Europe and North America. The flower's colors such as white, red, pink and yellow and the plant can reach up to 1 meter in height. Flowering of plants starts from May, depending on the climate. Plants can be propagated by seed, cuttings, stolons, rhizomes and tissue culture methods. While approximately 130 species of *Achillea* spp. have been recorded in the world, 52 species, 12 subspecies and 2 varieties have been identified in the Türkiye flora. 33 of the 66 taxa of *Achillea* species distributed in Türkiye have been registired endemic species. *Achillea millefolium* L. can grow up to 3500 meters above sea level. From the past to present the flowers and leaves of the plant are collected by the local people and it has been used for wounds, respiratory infections, skin and digestive problem as a mild sedative. As a result of some researches, it was stated that the essential oil ratio in the *Achillea* species varies between 0.1% and 1.2%. *Achillea millefolium* L. species is used as a medicinal raw material in the world. 1.8 cineole, camphor, terpineol, borneol, sabinen and *ß*-pinen, and camazulen were found as the most important components in *Achillea* species. *Achillea* species are rich in essential oil, flavonoids, phenolic acids and some other substance groups such as amino acids and fatty acids. 1,5-di-O-caffeoylquinic acid, Caffeic acid, Chlorogenic acid, Neochlorogenic acid are some phenolic compounds isolated from *Achillea millefolium* L. species. Some flavonoids isolated from *Achilllea millefolium* L. are 5-hydroxy-3,6,7,4'-tetramethoxyflavone, Apigenin, Artemetin, Cosmosin, Luteolin, Rutin, Sinaroside. According to research conducted, Quercetin which is a one of the chemicals found in the yarrow plant, it has given positive results when used as a dye.

Key Words: Yarrow, *Achillea* spp., *Achillea millefolium* L., medicinal uses, bioactive compounds

GREEN VETERINARY THERAPIES BASED ON POMEGRANATE *(PUNICA GRANATUM)* FOR THE CONTROL OF GASTROINTESTINAL NEMATODES OF SHEEP TO IMPROVE ANIMAL WELFARE AND HEALTH

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Gastrointestinal nematodes (GINs) are a serious problem in sheep, causing disease with often subtle symptoms throughout the flock, with serious consequences for animal health and welfare. Currently, the control of these helminth infections is mainly based on the use of synthetic drugs, which, when used improperly, have led to increasing development of resistance to one or more classes of anthelmintics. In Calabria region (soutern Italy) pomegranate *(Punica granatum)* is used in ethno-veterinary for helmintiasis control in sheep. A study on the therapeutic activity of an ethno-veterinary aqueous macerate pomegranate fruits-based, used for the control of GIN in sheep, is presented. For the study, 30 sheep were selected and divided into 2 groups, homogeneous for eggs per grams of faeces (EPG mean: 735): TG-treated with *P. granatum* macerate (50 mL/sheep/orally) and CG-untreated. The timing was: on day 0, group assignment, treatment and faecal sampling for parasite intensity assessments were performed; on days 7, 14 and 21, faecal sampling was repeated, and the anthelmintic efficacy was evaluated. The macerate was examined using liquid chromatography electrospray ionization mass spectrometry (LC/MS-ESI). The FLOTAC technique, was used to estimate the GIN faecal egg count (FEC). Formula 100×(1-[T2/C2]) was used to evaluate the anthelmintic efficacy (T2 represents the mean TG post-treatment FEC, C2 represents that of the CG). The results showed a good anthelminthic efficacy (52%) of the macerate. This efficacy can be attributed to the chemical composition of the phytocomplex, which mainly includes alkaloids, tannins, flavonoids, glycosides and phenols, especially gallic and ellagic acids. Therefore, the use of this green preparation as an alternative therapeutic remedy for the control of GIN in sheep is strongly recommended to improve the welfare and health of animals. In this way, there would also be a decrease in drug treatments and a reduction in the development of anthelmintic-resistance phenomena.

Key Words: Green veterinary therapies, Etno-veterinary medicine, pomegranate fruit, *Punica granatum*, anthelmintic activity, animal welfare and health.

TARRAGON (*ARTEMISIA DRACUNCULUS* L.): CHEMISTRY AND BIOLOGICAL ACTIVITIES

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Artemisia dracunculus L., commonly known as Tarragon, belongs to the Asteraceae family [1]. This plant, native to the Eurasian and North American continents, is cultivated all over the world and used as a spice to flavor sauces and dishes thanks to its fresh aroma and its herbaceous, bitter and sour flavor with notes of anise and basil [1-2]. Several beneficial and therapeutic properties are attributed to the plant: digestive, carminative, laxative, choleretic, calming, antiepileptic, analgesic, anti-inflammatory, antibacterial, diuretic, insect repellent [1-3]. In this work an essential oil (EO) of *A. dracunculus* from Southern Italy was obtained by steam-distillation. The composition, studied by gas chromatography-mass spectrometry, was dominated by estragole (72.56%), *trans*-β-ocimene (11.07%) and *cis*-β-ocimene (11.07%). The EO showed good antioxidant activity, with IC_{50} values of 2.15 ± 0.05 mg/mL in the DPPH assay, 305.23 ± 10.56 µmol of Fe^{2+} equivalents/g in the RAP) assay and 396.42 ± 11.71 µmol/g in the ABTS assay. The anti-enzymatic activity of the EO was tested on α-amylase and α-glucosidase enzymes; the EO showed good IC_{50} values: 7.34 ± 0.69 mg/mL on α-amylase and 5.72 ± 0.74 mg/mL on α-glucosidase. Finally, the phytotoxic activity of different concentrations of EO on germination and radical growth of two crops (*Hordeum vulgare* L. and *Raphanus sativus* L.) and two weeds (*Sinapis alba* L. and *Lolium multiflorum* Lam.) was evaluated. The EO was more able to reduce the radical elongation, with greater activity on *S. alba* (11.50-44.50% based on concentrations) and *R. sativus* (14.73%-37.30%). The complex of data shows that *A. dracunculus* EO could find application as a potential alternative biocontrol product against weeds and may have a role in the treatment of metabolic diseases.

Key Words: *Artemisia dracunculus*, antioxidant activity, anti-enzimatic activity, phytotoxicity

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PHENOLIC AND FLAVONOID CONTENT OF SOME MUTANT *CHRYSANTHEMUM* VARIETIES IN TÜRKİYE

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*Chrysanthemum*s belong to the Asteraceae family, encompassing 160 species and 22478 varieties worldwide. They originated in China and were first cultivated as a medicinal plant. Their cultivation began in Korea and Japan after China and expanded globally. While primarily known as ornamental plants, they have served as medicine, insecticide, food, and raw materials for cosmetics for 2000 years. *Chrysanthemum indicum* and *Chrysanthemum morifolium* are widely used in traditional medicine, particularly in East Asia, due to their diverse pharmacological properties. Recent scientific studies confirm their potential benefits, primarily attributed to their rich composition of flavonoids, phenolic acids, polysaccharides, carotenoids, and essential oils. These compounds contribute to their antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory effects. As bioactive components are crucial for human health, total phenolic and flavonoid analyses were conducted to evaluate the flowers of newly developed mutant varieties. The flowers of five new mutant varieties Bademler Beyazi, Ege Meltemi, Kaan, Ozan, Bayram2023, and two mutants obtained through *in vitro* mutation techniques by irradiating 20 Gray of gamma radiation, were used as materials which were compared with the Bacardi chrysanthemum variety as the control group. The total phenolic and flavonoid contents of the flowers were determined using the UV-Vis spectrophotometric method. The results indicated that the highest phenolic (3.29 g/100 g dried plant) and flavonoid (2.13 g/100 g dried plant) content was found in the Ozan variety. Light pink flowered mutant chrysanthemum samples ranked next in terms of phenolic content. Bademler Beyazi exhibited the lowest values for both phenolic (1.64 g/100 g dried plant) and flavonoid (0.93 g/100 g dried plant) contents. The control group had lower phenolic (3.13 g/100 g dried plant) and flavonoid (1.86 g/100 g dried plant) contents than the Ozan variety but higher concentrations than the other varieties. In conclusion, the higher phenolic and flavonoid content in Ozan compared to other varieties may be due to its dark pink color.

Key Words: *Chrysanthemum*, phenolic, flavonoid, mutant

THE EFICIENCY AND SAFETY OF BIOAPIFIT® WOUND CARE OINTMENT IN THE TREATMENT OF WOUNDS

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The objective of this study were efficacy and safty assessment of Bioapifit® wound care ointment for the treatment of surgical wounds. Bioapifit wound care ointment is homogeneous, greasy, viscous mass of characteristic herbal odor and olive green color. It is composed of honey (certified organic), *Cera flava*, glycerin, the oil macerate of *Plantago major* L., *Achilea millefolium* L., *Quercus robur* L., Salvia *officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Symphytum officinale* L., *Calendula officinalis* L. *and Matricaria chamomilla* L.; essential oils*:* Melaleuca *alternifolia*, *Thymus vulgaris* ct. Thymol and *Origanum vulgare.* 50 patients with several wounds were treated 3-10 days (twice a day) with Bioapifit® wound care ointment applied on conventionally cleaned wound and covered with bandage during the whole course of the study. At the end of treatment with Bioapifit wound care ointment, 84% of the wounds were completely closed, which also represents the clinical cure rate. Compared to baseline, the total wound surface area decreased by 96.8%. All wounds were completely dry. The score for the type of tissue also decreased by 96.8%, and wound pH by 18%. All wounds were sterile. None of the patients in Bioapifit treated group or control group experienced any adverse effect (worsening of the existing symptoms, appearance of new symptoms, irritation or allergic reaction) during the treatment and follow up period. The application of Bioapifit® wound care ointment resulted in complete closure of 84.8% of the wounds and a reduction in their surface area by 98.7%, with an average healing time of 3-7 days.

Key Words: Wounds, surface area, herbal macerate

STUDY OF THE CHEMICAL COMPOSITION AND PROPERTIES OF WHEAT SPROUTS

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The main aim of this work was to examine the chemical properties of extracts obtained from wheat germ, with particular emphasis on the content of phenolic acids, that have antioxidant properties. This study focuses on examining the content of many nutrients in cereals, which potentially increase the functional qualities of food and enrich the daily human diet. During these investigations for the extraction of components from wheat germ as well as for the quantitative and qualitative analysis such chromatographic and spectroscopic techniques have been applied. Cereal grains are the main source of many nutrients that humans provide in their diets. However, sprouted cereal grains are considered to have exceptional nutritional value. During germination of cereal grains, a number of chemical changes occur, resulting in increased activity of hydrolytic enzymes and improves the quality of nutrients and bioactive compounds of cereals, increasing thus the content of proteins, amino acids, sugars and vitamins. Cereal sprouts are special case as a source of antioxidants, which show numerous beneficial effects and functions for the human body. Biologically active compounds found in sprouts participating in metabolic processes, they strengthen the immune and antioxidant systems, affect the cardiovascular, digestive, nervous and respiratory systems. In our study the method development for separation and determination of biological active compounds from wheat sprouts (*Triticum aestivum* L.) have been presented. The most effective extraction has obtained, when 96% ethanol is solvent applied for the extraction. Although the total content of polyphenolic compounds was similar in all extracts, the extraction efficiency was differed, depending solvent type use. Applied spectroscopic technique UV-Vis allowed for analysis of the content of β-carotene, chlorophyll a and chlorophyll b. Applications of TLC showed the presence of many polyphenolic compounds (including chlorogenic acid) in wheat sprouts. Moreover, the presence of 1-octacosanol in tested sprouts has been confirmed.

Key Words: wheat sprouts, antioxidant activity, polyphenols, 1-octacosanol

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ORAL MICROBIOTA IN INFLAMMATORY PERIODONTAL DISEASES AND PROSPECTS OF ITS CORRECTION USING SUBSTANCES OF PLANT ORIGIN

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Our long-term research has demonstrated that dysbiosis of the microbiota in periodontitis is associated with the formation of dominant microbial associations consisting of periodontopathogenic and opportunistic microorganisms. These microorganisms possess pathogenicity and virulence factors, demonstrate the ability to form biofilms, and exhibit increased resistance to antimicrobial agents. The growing issue of antimicrobial resistance among microorganisms underscores the relevance of identifying alternative antimicrobial agents. To investigate the antimicrobial activity of biologically active plant-derived compounds, 38 extracts from 18 medicinal plants were obtained at the laboratory of the Department of Pharmaceutical Technology, Pharmacognosy, and Botany at the University of Veterinary Medicine and Pharmacy in Košice. Ethanol and methanol extracts (Sigma, Germany) were prepared in accordance with the requirements of the State Pharmacopoeia of Ukraine. The crude solutions were placed in a coated dry ice condenser rotary evaporator to obtain purified alcoholic extracts at a temperature of 50 °C and a rotation speed of 82 rpm.

The sensitivity of microorganisms to the plant extracts was assessed using the agar diffusion method and determination of minimum inhibitory concentrations (MICs), as described by Balouiri M. et al. (2016) and Kozlovska I. et al. (2017). The study utilized reference strains from the American Type Culture Collection (ATCC, USA): *Candida albicans* ATCC 885-653, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* ATCC 19615, and *Pseudomonas aeruginosa* ATCC 27853. In addition, clinical strains isolated from the oral cavity of patients with inflammatory periodontal diseases were studied, including *Candida albicans*, *S. aureus*, *E. coli*, *S. pyogenes*, *E. faecalis*, *Hafnia alvei*, *Klebsiella rhinoscleromatis*, *Porphyromonas gingivalis*, *Prevotella spp.*, *Schaalia odontolytica*, and *Slackia exigua*. The study showed that the following plant extracts exhibited antimicrobial activity against oral clinical isolates: *Vaccinium vitis-idaea* L. (leaves) (MIC 2.08–9.33 mg/mL), *Vaccinium vitis-idaea* L. (berries) (MIC 2.33–11.42 mg/mL), *Potentilla erecta* L. (rhizome) (MIC 10.50–15.17 mg/mL), *Equisetum arvense* L. (shoots) (MIC 5.42–16.00 mg/mL), and *Arnica montana* L. (inflorescences) (MIC 5.08–15.33 mg/mL). The evaluation of anti-biofilm activity revealed that the most potent effects were observed with extracts of *Vaccinium vitis-idaea* L. (leaves), *Potentilla erecta* L. (rhizome), *Equisetum arvense* L. (shoots), *Chamaenerion angustifolium* (L.) Scop. (leaves), *Crataegus laevigata* (Poir.) DC. (berries), *Betula pendula* L. (leaves), *Symphytum officinale* L. (root), and *Achillea millefolium* L. (inflorescences). These findings indicate the potential of plant-derived substances for application against oral microorganisms under conditions of inflammatory periodontal disease.

Key Words: Antibioticresistan bacteria, plantextracts, moicrobiota of oral cavity

IN SILICO ANALYSIS OF SAFETY AND EFFICACY OF HERBAL SKIN-LIGHTENING AGENTS: A TOXICOLOGICAL PERSPECTIVE

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Skin-lightening products represent a multi-billion-dollar global industry driven by complex sociocultural factors and beauty standards. While synthetic agents like hydroquinone, mercury compounds, and topical corticosteroids dominate this market, their documented toxicity profiles have raised significant safety concerns. This study critically evaluates the historical context, usage patterns, and toxicological implications of conventional skin-lightening agents, with a focused analysis of plant-derived alternatives. Natural compounds including glabridin (*Glycyrrhiza glabra*), aloesin (*Aloe vera*), mulberroside F (*Morus alba*), and ellagic acid (various plant sources) demonstrate tyrosinase inhibition with potentially improved safety profiles compared to synthetic alternatives. However, comprehensive assessment of their pharmacokinetic parameters and toxicity profiles remains inadequate. This research addresses this gap through comprehensive in silico analysis examining the drug-likeness of selected herbal compounds using computational models to predict Log Kp (skin permeation) values, passive intestinal absorption, and potential brain penetration as functions of lipophilicity and apparent polarity. Additionally to assess bioavailability, the active efflux by P-glycoprotein is evaluated. Beyond established mechanisms, ligand-based virtual screening by similarity and target prediction algorithms are employed to identify both therapeutic targets and potential off-target interactions responsible for toxicity. This multifaceted computational approach provides critical insights into the safety and efficacy of herbal skin-lightening agents, offering a foundation for developing safer alternatives to conventional products with established toxicity concerns. The findings contribute to evidence-based selection of herbal compounds for skin-lightening applications and establish methodological frameworks for safety assessment of cosmeceutical ingredients derived from medicinal and aromatic plants. The computational methodology we use is applicable to all classes of herbal bioactives across various therapeutic domains, delivering crucial drug-likeness parameters, comprehensive ADME profiles, and toxicological risk assessments as demonstrated in our extensive work with diverse medicinal plants including *Reynoutria japonica* [1].

Key Words: Skin-lightening, herbal compounds, tyrosinase inhibitors, in silico toxicology, pharmacokinetic prediction, medicinal plants

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PHYTOCHEMICAL SCREENING AND BIOLOGICAL ACTIVITIES OF *MENTHA SPICATA*

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The main objective of our work is the determination of phytochemical screening and the evaluation of the anti-inflammatory and anti-oxidant activity of extracts (water and hydromethanolium) of *Mentha spicata* from the region of Ouled Dris (Souk Ahras). The phytochemical screening of *Mentha spicata* L. extract indicates the presence of tannins and mucilage while the absence of saponosides, leukoanthocytes, starch, anthocyanin and cumarin in the aquatic extract. The hydro-methanol extract shows the presence of reducing compounds, catechic tannins, quinones, phlobatanins, essential oils, glycosides, steroids and flavonoids. The results obtained from hydro- distillation show a yield of 0.89% for mint and rosemary. The anti-inflammatory potency is stronger in mint where a rate of inhibition of denaturation (0.49%) was recorded with the lowest concentrations (0.2 and 0.4 mg/ml) and a zero rate (0.007%) with the raw extract. On the other hand, the evaluation of the antioxidant potential of the extract by the DPPH test revealed a considerable anti-oxidant response based on the prepared concentrations of Ascorbic acid.

Key Words: Phytochemical screening, biological activity, antioxidant, anti- inflammatory, medicinal plant, mint

CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM SOME LAMIACEAE SPECIES GROWING IN ALBANIA

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Albania possesses a rich and diverse flora, with approximately 3,600 plant species, of which a significant part are medicinal aromatic plants. Medicinal aromatic plants, in addition to their medicinal value, have economic, social, and environmental significance. Among Albania's traditionally used plants are those of the Lamiaceae family, from genera *Thymus*, *Origanum*, *Satureja*, *Melissa* etc. This study aims to analyze the chemical composition of essential oils from two *Thymus spp.* and *Melissa officinalis L.*, contributing to the quality assessment of plant materials vital to the pharmaceutical industry. The areial parts of wildly grown *Thymus capitatus*, *Thymus longicaulis* and *Melissa officinalis* were gathered during flowering stage, from different regions of Albania. The essential oils (EO) were extracted by hydrodistillation using a Clevenger-type apparatus. Their chemical composition was analyzed using GC-FID method. Kovats retention indices (RI) for detected components were determined by injecting a hexane solution containing a homologous series of *n*-alkanes (C8-C26). The compounds were identified by comparing their retention indices with those obtained from the literature. The concentrations of compounds were calculated by integrating the area under peak and presented as percentage. The highest essential oil yield was found in the *Thymus longicaulis* sample (1.25%). GC/FID analysis revealed that the main component of *Thymus capitatus* EO was carvacrol (58.7%). *Th. longicaulis* essential oil's main components were p-cymene (20.45%), carvacrol (17.24%) and carvacryl acetate (9.6%). Analysis of the essential oil of *Melissa officinalis* revealed the presence of citral (29.86%), citronellal (24.69%) and linalool (6.8%) as the major components. In conclusion, this study indicates the presence of a high percentage of oxygenated monoterpenes in the essential oils extracted from two *Thymus spp.* and *Melissa officinalis L.*, emphasizing their potential use in the pharmaceutical, cosmetic and food industry.

Key Words: Essential oils, chemical composition, GC/FID analysis, Albanian flora

INVESTIGATION OF ANTIPROLIFERATIVE AND ENZYME INHIBITORY EFFECTS OF THE PEELS OF FOUR POTATO (*SOLANUM TUBEROSUM* L.) GENOTYPES

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Potato (*Solanum tuberosum* L.) is one of the most important crops in the world that contributes to food security on a global scale due to its high yield. This study focuses on the comparative antiproliferative and tyrosinase enzyme inhibitory effect of peels of 4 different potato genotypes (Ata 25, Can, Kaan, Mete). The antiproliferative activity was evaluated using the MTT assay against the cell lines L929, A549, Hep3B and PC3. According to the results of inhibition of the mushroom tyrosinase enzyme using L-tyrosine as a substrate, the highest effect was observed in the methanol extract of the Kaan genotype with a value of 48.80% at 200 µg/ml. For the Ata, Can and Mete genotypes, these values were determined to be 38.36%, 32.16% and 34.20% respectively. Enzyme inhibition was found to be very low in aqueous extracts. No cytotoxicity was observed in healthy L929 cells in the concentration range of 20-400 µg/ml. The methanol extract of the Ata 25 genotype showed the highest antiproliferative effect against PC3 (IC_{50}:306.24 µg/ml) and Hep3B (IC_{50}:284.26 µg/ml) cells. However, no significant cytotoxic effect against cancer cells was observed in the aqueous extracts. Potato peels, which are considered waste, can be a new, sustainable and safe natural resource for the food, pharmaceutical and cosmeceutical industries.

Key Words: *Solanum tuberosum* L., genotypes, tyrosinase, MTT, antiproliferative

Acknowledgements: The author would like to thank the Eastern Anatolia Agricultural Research Institute Directorate (TAGEM) for providing the plant materials.

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INVESTIGATION CYTOTOXICITY AND WOUND HEALING OF *ACHILLEA BIEBERSTEINII* EXTRACTS

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The *Achillea* genus, named after the Greek mythological figure Achilles and often known as Yarrow, consists of 115 species found across Europe, Asia, North Africa, North America, Australia, and New Zealand, with 50 distinct species identified in Türkiye according to the List of Turkish Plants (Bremer, 1994; Guner & Aslan, 2012; Eruygur et al, 2020). *Achillea biebersteinii* Afan. has been traditionally used as a snake repellent, wound healing, and treatment for abdominal and stomach aches (Sezik et al, 2001; Eruygur et al, 2020). Previously, n-hexane extract of *A. biebersteinii* aerial parts was studied *in vivo* incision and excision wound model (Akkol et al, 2011). In this study, chloroform, ethanol, and water fractional extracts were prepared from *A. biebersteinii* flowers using Soxhlet apparatus. Phenolic compounds carried by the obtained extracts were determined by LC-MS/MS analysis among 32 different standard compounds. According to the analysis results, the extracts carry 20 different compounds, including Isoquercitrin, chlorogenic acid, and kaempferol, in different proportions. Anticancer effects of the extracts against SH-SY human neuroblastoma cells were investigated by the MTT assay. Accordingly, IC_{50} values of chloroform, ethanol and water extracts were 217.7, 333.2, and 381.4 µg/mL, respectively. The cytotoxicity test indicated that the chloroform extract exhibited superior anticancer activity compared to the ethanol and water extracts. The therapeutic effects of ethanol and water extracts with low IC_{50} values on wound healing in L929 cell lines were examined. No significant results were recorded as a result of the wound healing investigation. This study is the first original investigation into the wound healing effects of polar extracts from *Achillea biebersteinii*, which is among the Achillea species known for its wound healing abilities. Furthermore, plant extracts have been recognised for their efficacy against neuroblastoma cancer cells for the first time.

Key Words: wound healing, anticancer, *Achillea*, LC-MS/MS analysis

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EFFECTS OF DIFFERENT EXTRACTION METHODS ON TOTAL PHENOLIC CONTENT AND BIOACTIVITY IN *TRIBULUS TERRESTRIS* L.

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Tribulus terrestris is an annual medicinal plant belonging to the Zygophyllaceae family, widely recognized for its diverse pharmacological properties and its extensive use in both traditional and modern medicine [1]. Flavonoids, alkaloids, saponins and lignins are the primary active compounds found in this plant [2]. This study aims to investigate the impact of various extraction methods on the yield and bioactivity of *T. terrestris* extracts, with the goal of supporting the development of effective therapeutic agents and herbal supplements derived from this plant. Solvent extraction methods including Soxhlet, maceration, ultrasound-assisted extraction and microwave-assisted extraction were applied for varying durations to obtain *T. terrestris* extracts. The study focused on evaluating the antioxidant and cytotoxic activities of *T. terrestris* extracts through *in vitro* assays. Antioxidant activity was assessed using the DPPH radical scavenging assay, while cytotoxic effects on different cell lines were determined via the MTT assay. In addition, the total phenolic content (TPC) of the extracts was quantified using the Folin–Ciocalteu (FC) method [3]. The findings indicate that advanced extraction methods are significantly more effective than conventional methods in maximizing the phenolic content of *T. terrestris* extracts and this was also consistent with the bioactivity results. The observed antioxidant activity is likely attributed to the presence of these phenolic compounds. These results highlight that modern extraction methods offer advantages not only by reducing extraction time and increasing yield but also by requiring less solvent and being more environmentally friendly.

Key Words: *Tribulus terrestris*, extraction methods, total phenolic content, antioxidant activity, MTT

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ULTRASOUND-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM *PORTULACA OLERACEA*: EXPLORING NATURAL ANTI-INFLAMMATORY AGENTS AND THEIR IMPACT ON SIGNALLING PATHWAY

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Natural anti-inflammatory agents derived from plants have gained significant attention for their potential therapeutic effects in managing chronic inflammatory conditions. These compounds can modulate inflammatory pathways and provide protective effects against oxidative stress, making them valuable in developing nutraceuticals and pharmaceuticals. This study investigates the extraction and purification of antioxidant-rich fractions containing steroids and triterpenes from Purslane (*Portulaca oleracea*) using ultrasound-assisted extraction (UAE). By optimizing extraction parameters such as ethanol concentration, extraction time, and ultrasonic power, the research aims to maximize the antioxidant agents and assess their biological properties. The optimization process revealed that the optimal conditions for UAE were 75% ethanol concentration, 30% ultrasonic power, and a 10-minute extraction time, resulting in a high $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ inhibition. The extracted fraction underwent bio-guided fractionation, leading to the identification of key bioactive compounds via gas chromatography-mass spectrometry (GC-MS), including β-sitosterol, β-Amyrin, α-Amyrin, and Uvaol. The biological evaluation demonstrated significant anti-inflammatory activity, with reductions of IL-6, IL-1β, and TNF-α level in LPS-stimulated RAW 264.7 macrophages. Additionally, the fraction effectively modulated calcium-signalling pathways, indicating its potential therapeutic applications. This research highlights the effectiveness of UAE in extracting valuable bioactive compounds from purslane, displaying its potent antioxidant and anti-inflammatory properties.

Keywords: Natural anti-inflammatory agents, ultrasound-assisted extraction, *Portulaca oleracea*, beta-sitosterol, signaling pathway

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ENDOPLASMIC RETICULUM STRESS AND REDOX MODULATION BY COFFEE SILVER SKIN EXTRACTS IN UVA-INDUCED KERATINOCYTES AND ZEBRAFISH

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The silver skin of coffee (CSS) is one of the by-products of the industrial processing of coffee. It is a highly available waste product and more stable due to its low water content. It is known that the bioactive compounds in silver skin have potential protective action on the skin, counteracting skin aging and inflammation. Skin inflammation can result from the deleterious effects of extrinsic factors, such as overexposure to UV solar radiation, which over time lead to pathological conditions such as erythema, edema, hyperpigmentation, premature skin aging, and cancer. UV radiation induces an overproduction of ROS, resulting in oxidative stress; for this reason, the use of compounds with antioxidant effects could be a promising approach to inhibit skin damage induced by their irradiation. This study aimed to chemically and biochemically characterize an extract of CSS and its purified fractions, obtained through green extraction technologies, and to evaluate their dermo-protective potential in a UVA-induced damage model using both human keratinocytes (HaCaT) and zebrafish larvae. In vitro, CSS treatment counteracted UVA-induced oxidative and proteotoxic stress by reducing unfolded protein response (UPR) markers and cell death. Mechanistically, CSS enhanced the PERK-dependent activation of the Nrf2-HO-1 axis, suggesting a central role for ER stress signaling in mediating the antioxidant and cytoprotective effects of the extract. Inhibiting PERK impaired Nrf2 activation by CSS and reduced cell viability, further supporting this functional link. *In vivo*, CSS significantly decreased ROS levels, tyrosinase activity, and melanin accumulation in zebrafish, alongside reduced protein aggregation, indicating mitigation of ER stress. Collectively, our findings highlight the potential of CSS as a natural skin-protective agent that modulates redox and ER stress responses, opening new perspectives for preventing or attenuating UV-induced skin damage.

Key Words: oxidative stress; natural products; in-cell studies; tyrosinase inhibition; unfolded protein response; endoplasmic reticulum stress; zebrafish model

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LICHEN EXTRACTS DISRUPT *CANDIDA ALBICANS* GROWTH AND BIOFILM FORMATION VIA INHIBITION OF THE cAMP/PKA and CEK1 MAPK SIGNALING PATHWAY

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This study aimed to investigate the antifungal activity of extracts from *Dermatocarpon miniatum* (*D. miniatum*) and *Parmelia saxatilis (P. saxatilis*) lichen extracts against *Candid*a spp., focusing on their effects on *C. albicans* biofilms. Methanol (ParM, DerM) and aqueous (ParW, DerW) extracts were prepared, with antifungal activity assessed using the CLSI M27-A3 microdilution method. The biofilm formation ability of *C. albicans* isolates was assessed using the crystal violet test, and the inhibitory effects of lichen extracts on biofilms were analyzed by CCK-8 assay compared with fluconazole (FLC). Gene expression related to biofilm formation in the cAMP-PKA and CEK1 MAPK pathway was examined by qRT-PCR and morphological changes were evaluated by FESEM. Statistical analyses were performed using ANOVA with Bonferroni post hoc test. Only ParM and DerM demonstrated antifungal activity, with concentrations ranging from 160 to 2500 µg/mL for ParM and 320 to 2500 µg/mL for DerM. The aqueous lichen extracts (ParW, DerW) showed no antifungal activity, so methanol extracts (ParM and DerM) were used in subsequent analyses. Biofilm formation by seven *C. albicans* strains was evaluated using crystal violet staining, identifying three strong biofilm producers. The inhibitory effects of ParM, DerM, and fluconazole (FLC) on biofilms were analyzed via the CCK-8 assay. Results showed significant biofilm reduction at 1250 µg/mL for ParM, 2500 µg/mL for DerM, and 4 µg/mL for FLC. Gene expression analysis revealed that ParM downregulated key genes in the cAMP-PKA pathway (ALS3, HWP1, ECE1, UME6, HGC1) and the CEK1 MAPK pathway (CEK1, HST7, STE11, TPK2, CST20, CDC42), which are critical for hyphal growth and biofilm maintenance ($p<0.05$). FESEM analysis confirmed reduced hyphal networks and impaired cell surface integrity following treatment with Parm ve DerM. This study represents the first demonstration of the effects of lichen extracts on the hyphal growth and biofilm formation of *C. albicans* through the cAMP-PKA and CEK1 MAPK pathways by qRT-PCR, confirmed by FESEM analysis. These findings highlight the therapeutic potential of lichen extracts, although further research is needed to fully understand their antifungal and antibiofilm mechanisms.

Key Words: *Candida albicans*, lichen, *Dermatocarpon miniatum*, *Parmelia saxatilis*, antifungal activity, cAMP-PKA pathway

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PHYTOCHEMICAL VALORIZATION OF TWO PARTS OF A MEDICINAL PLANT: PEPPER TREE

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Traditional medicine employed *Schinus molle* for its antibacterial and antiseptic qualities to treat a range of wounds and illnesses. Moreover, it has been used as a diuretic, antidepressant, and for toothaches, rheumatism, and menstrual disorders. Recent research in mice may confirm its antidepressant properties. It has also been hypothesized that *S. molle* is a viable substitute for synthetic chemicals in pest control due to its insecticidal qualities. The presnt study aims to evaluate the phytochemical composition of a medicinal plant *Schinus molle* in two different parts: the leaflets and the branches. Phytochemical screening has proven that both studied parts are rich in catechin tannins; however, anthocyanins are present in the branches and absent in the leaflets. The saponins showed a few quantities in both parts of the studied species. These preliminary results have allowed us to distinguish that the leaflets have antioxidant and anti-inflammatory properties, which are currently being determined through biological activity tests.

Key Words: Medicinal plants, *Schinus molle*, phytochemical composition, antioxidant activity, anti-inflammatory properties

EVALUATION OF COMBINED ANTIBACTERIAL EFFECT OF SOME *CITRUS* SPECIES WITH HONEY

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The failure of antibiotics has prompted humans to seek other sources of natural and effective products from plants and bees. Lemon, lime and honey present these criteria. Sometimes, the treatment of certain diseases requires the combination of two or more antibacterial agents.
This study contributed to evaluate the combined antibacterial effect of some species of *Citrus* with honey on two pathogenic bacterial strains (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). For this work, we selected two *Citrus* species (*Citrus limon* and *Citrus aurantiifolia*) and two honey samples (mono and polyfloral). Our research was based on the evaluation of the antibacterial effect using the incorporation and dilution methods to determine the various inhibition parameters (MIC, MBC). The obtained results showed that lime contains a high efficacy compared to lemon. However Polyfloral honey presents a strong antibacterial activity in comparison with monofloral honey. The evaluation of the combined effect of polyfloral honey and lime shows a very high efficacy against the two tested bacterial strains with a MIC ($0.6*10^{-6}$ mL of honey and $3.5*10^{-6}$ mL of lime) in the mixture and a CMB ($0.6*10^{-4}$ mL of honey and $3.5*10^{-4}$ mL of lime). From the above, it is suggested the possibility of using honey and lime as natural antimicrobial agents for the cure of some diseases affecting human health and as an alternative to the use of antibiotics.

Key Words : *Citrus limon*, *Citrus aurantiifolia*, honey, antibacterial effect, combined effect, pathogenic bacteria.

SOME RESEARCH ON *POLYGONUM ORIENTALE* L. *(≡PERSICARIA ORIENTALIS*; POLYGONACEAE) PLANT

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The Polygonaceae family, which has the highest distribution in the Northern Hemisphere, is represented by 52 genera and an estimated 1552 taxa in the world. These family members are defined as herbs, shrubs, rarely climbers or trees. There are 10 genera under the Polygonaceae family in Turkey. Poligonum orientale, which is located under Polygonum, one of the largest genera of the Polygonaceae family, is accepted in many international references under the name *Persicaria orientalis*. The plants were collected from Van Yüzüncü Yıl University, Medicinal and Aromatic Plants Garden in 2020. In this study; antioxidant activity, total phenolic and flavonoid substance amounts and dualex values (nitrogen balance index (NBI), chlorophyll content, flavonol and anthocyanin content) were examined. In the results of working; The amount of antioxidant substance was determined as 137.73 µmol TE/g, total phenolic substance (202.87 mg GAE/g) and total flavonoid substance amount was determined as 7.82 mg QE/100 g. The data obtained in terms of Dualex values (dx) such as Nitrogen Balance Index (NBI), chlorophyll content, flavonol and anthocyanin content are respectively; It was determined as 13.70 mg/g, 23.86 mg/cm2, 1.74 dx and 0.08 dx.

Key Words: *Polygonum orientale* L., medicinal plant, antioxidant, phenolic, flavonoid, dualex value

A NWFP NATURAL FLAVOR AS A SOLUTION TO PREVENT ACB (TAB) SPOILAGE OF CLEAR BEVERAGES

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Spoilage represents a huge challenge for the beverage industry. Whereas pasteurization eliminates most organisms, it actually activates the spore-forming *Alicyclobacillus acidoterrestris* (ACB) pertaining to the group of thermophilic acidophilic bacteria (TAB) to begin germinating, spoiling beverages in a matter of days. ACB spoilage in the beverage industry, is characterized by the volatile, unpleasant odorous compound named guaiacol. Whilst not specifically harmful for humans, the spoilage caused by ACB spores is a matter of quality and results in losses for the industry, such as quarantine periods, statistical ACB tests and ultimately, the need to recall or reimburse for spoiled drinks, compounded by the company's damaged reputation. Here we present FLAVORWATCH™, a NWFP ingredient natural flavor, based on a plant extract, which in addition to its flavor properties inherently eliminates ACB germinating spores, thus, efficiently protecting acidic beverages from TAB spoilage during their entire shelf life. To investigate the effect of FLAVORWATCH™ on TAB, ACB spores were introduced into commercial 10% apple-based beverage and followed by time-lapse microscopy. After eight hours of incubation, aberrations in spore layers were observed, and about 4 hours later, the spores burst leaving only damaged cell wall behind. FLAVORWATCH™ eliminates ACB by disintegrating the spores' protecting layers. Adding the developed NWFP FLAVORWATCH™ as an ingredient kills TAB spores even during pasteurization, probably by penetrating the spore protecting layers due to the high temperature. Seven days post production, positive control bottles displayed apparent haziness while bottles supplemented with FLAVORWATCH™ remained clear for their entire shelf-life (180 days) and beyond (420 days). This haziness was a result of TAB growth as cells reached a number of almost 1 million per ml. TAB were not detected in bottles supplemented with FLAVORWATCH™ for the entire experiment timeline. This natural flavor also efficiently kept the organoleptic properties of the beverages.

Key Words: TAB: (Thermophilic Acidophilic Bacteria), ACB: (Alicyclobacillus spp), beverages, spoilage, NWFP

BIOSYNTHESIS OF ELEMICIN AND ISOELEMICIN IN *DAUCUS CAROTA* LEAVES

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Volatile phenylpropenes comprise one of the largest groups of plant phenylalanine-derived volatiles that not only possess ecological roles but also exhibit numerous pharmacological activities. Despite their wide distribution in the plant kingdom, biosynthesis of only a small subset of these compounds has been discovered. Here, we elucidated yet unknown steps in the biosynthesis of isoelemicin and elemicin using carrot (*Daucus carota* subsp. *sativus*), which produces a wide spectrum of volatile phenylpropenes, as a model system. Comparative transcriptomic analysis combined with metabolic profiling of two carrot cultivars producing different spectrums and levels of phenylpropene compounds revealed that biosynthesis of isoelemicin and elemicin could proceed via the (iso)eugenol-independent pathway, which diverges from the lignin biosynthetic pathway after sinapyl alcohol. Moreover, *in planta* results showed that two different NADPH-dependent reductases, a newly identified 5-methoxy isoeugenol synthase (DcMIS) and previously characterized (iso)eugenol synthase (DcE(I)GS1), both of which use sinapyl acetate as a substrate, are responsible for the biosynthesis of immediate precursors of isoelemicin and elemicin, respectively. In contrast to penultimate reactions, the final steps in the formation of these phenylpropenes are catalyzed by the same newly characterized methyltransferase, *S*-adenosyl-L-methionine:5-methoxy(iso)eugenol *O*-methyltransferase, that methylates the *para*-hydroxyl group of their respective precursors, thus completing the (iso)eugenol-independent route for the biosynthesis of isoelemicin and elemicin.

Key Words: Carrot, biosynthesis, Elemicin, Isoelemicin

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PHYTOCHEMICAL ACTIVITY AND DESIGN OF FILM-FORMING SPRAY FORMULATIONS OF *TANACETUM PARTHENIUM* L. EXTRACT

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Tanacetum parthenium L., a member of the Asteraceae family, is a medicinal plant traditionally utilized in the form of infusions, decoctions, hydroalcoholic extracts, or direct topical applications for the management of various conditions, including dermatitis, arthritis, insect bites, and inflammatory skin disorders. In line with the growing interest in plant-based dermal delivery systems, this study aimed to obtain the ethanolic extract of *Tanacetum parthenium* and subsequently develop film-forming spray formulations to improve ease of application, dose uniformity, and skin retention of phytopharmaceutical compounds. The aerial parts of dry *T. parthenium* were powdered and then subjected to continuous extraction with ethanol in a Soxhlet apparatus for 2 hours. The resulting ethanol extract was removed from the solvent under low pressure at low temperature (40-50°C), and concentrated crude extract was obtained. DPPH and total phenolic content of ethanol extract were analyzed. A series of formulations containing the extract were systematically developed by varying the concentrations of key components, including ethanol, film-forming polymers (HPMC, PVA, PVP), plasticizers (propylene glycol, glycerol) and purified water, using Ultra-Turrax (IKA T25) at 14,000 rpm for 5 minutes. Each formulation was characterized in terms of organoleptic properties, pH, density, viscosity, single actuation content, film-forming ability and spray pattern. In DPPH analysis of ethanol extract, IC_{50}: 0.1326 mg/mL standard gallic acid was determined as 0.0007745 mg/mL. Total phenolic content was 66.329 mg GAE/g extract. Organoleptic evaluations demonstrated that all formulations were homogeneous, with no evidence of phase separation. pH values ranged from 4.71 ± 0.08 to 5.06 ± 0.02, while the density measurements were between 1.639 and 1.651 g/mL. Viscosity values varied from 13.66 to 132.43 cP. The single actuation content remained consistent across all formulations, with an average of approximately 130 mg per spray, indicating dose uniformity. Formulations containing PVA and PVP successfully formed cohesive films upon application. However, HPMC-based formulation did not demonstrate comparable film-forming ability. Spray pattern analysis of PVA-based systems showed a fine and uniform mist, suggesting efficient spray performance. These findings confirm the feasibility of developing film-forming spray formulations containing *T. parthenium* extract.

Key Words: *Tanacetum parthenium*, ethanol extract, film-forming spray, topical formulation

STRATEGIES FOR INCREASING SECONDARY METABOLITE YIELD IN MEDICINAL AND AROMATIC PLANTS

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The enhancement of secondary metabolite production in medicinal and aromatic plants is an important research topic to increase the biological activity and commercial value of these plants. Secondary metabolites play a critical role in protecting plants against environmental stressors and in carrying out various biological functions. To increase the production of these compounds, various methods such as plant growth regulators, tissue culture techniques, polyploidy, and mutation are used. Plant growth regulators (PGRs) are chemical substances that control plant growth and development and stimulate secondary metabolite production. In particular, the use of plant growth regulators at correct concentrations and timings facilitates the synthesis of higher amounts of secondary metabolites. Tissue culture techniques are another significant method used to optimize the biological and chemical properties of plants, as they enable the controlled production of secondary metabolites under in vivo conditions. Polyploidy is the presence of more than two sets of chromosomes in somatic cells and is achieved by increasing the chromosome number in plants. This condition enhances the metabolic capacity of plants, thereby promoting the production of greater quantities of secondary metabolites. Therefore, polyploidy is an effective method used to enhance secondary metabolite production in plants. Mutation, which involves sudden and permanent changes in the genetic structure of plants, can also be employed to produce new and desired metabolites. Mutations in plants can be induced by chemical or physical mutagens, leading to an increase in both the diversity and quantity of secondary metabolites. These methods collectively contribute to the enhancement of secondary metabolite production in medicinal and aromatic plants, thereby ensuring more efficient and effective extraction of these compounds for use in pharmaceutical and cosmetic industries. This review aims to summarize the approaches used to enhance secondary metabolite production in plants.

Key Words: Medicinal and aromatic plants, secondary metabolites, plant growth regulator, tissue culture, polyploidy, mutation

THE ROLE OF SECONDARY METABOLITES IN MEDICINAL AND AROMATIC PLANTS

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Secondary metabolites are natural compounds other than primary metabolites such as proteins, carbohydrates, and fats that are not essential for plant life. Unlike primary metabolites, which are essential for plant growth and development, secondary metabolites are not directly involved in these processes but serve more specialized functions. These natural compounds synthesized by plants as a defense against abiotic and biotic stress factors are also known as efficient or active substances in plants. They exhibit various bioactive properties such as antimicrobial, anti-inflammatory, antioxidant, and anticancer activities. Secondary metabolites can be classified into three main groups: alkaloids, terpenoids, and phenols. Generally, alkaloids play a significant role in the development of pharmaceuticals, while flavonoids contribute to tranquilizers and anti-inflammatory effects. Terpenoids are known for their sedative properties. Plants with a high content of secondary metabolites are typically classified as medicinal and aromatic plants. These plants contain varying amounts of secondary metabolites in different organs. Medicinal and aromatic plants are categorized according to their active compounds, which determine their uses, such as perfume plants, spice plants, dye plants, and medicinal plants. For example, jasmine (*Jasminum*) is classified as a perfume plant due to its benzyl acetate content, mint (*Mentha spicata*) is classified as a spice plant due to its menthol content, madder (*Rubia tinctorum*) is classified as a dye plant due to its alizarin content, and poppy (*Papaver somniferum* L.) is categorized as a medicinal plant due to its morphine content. These compounds generally play a defense role in plants, protecting them from herbivores, pathogens, and environmental stressors. The diverse roles of secondary metabolites make them essential for plant survival and offer significant potential for developing natural drugs and products in the pharmaceutical and cosmetic industries. Consequently, secondary metabolites in medicinal and aromatic plants not only contribute to their ecological success but also form the basis for many herbal medicines and commercial products. In this review, it is aimed to summarize the functions of secondary metabolites secreted by plants and the uses of some plants according to their active ingredients.

Key Words: Medicinal and aromatic plants, secondary metabolites, alkaloids, terpenoids, phenol compounds

POSTER PRESENTATIONS

ANTIOXIDANT ACTIVITY AND ANTI-ANGIOGENIC POTENTIAL OF GLYCYRRHIZA GLABRA L. HYDROGELS FOR TOPICAL USE

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Glycyrrhiza glabra L., also known as licorice, has long been valued for its diverse therapeutic properties, largely attributed to its important glycyrrhizin and flavonoid content [1]. Its bioactive constituents have been proven effective in addressing multiple skin conditions, such as dermatitis, eczema, and acne, mainly through their potent antioxidant and anti-inflammatory actions [2]. The present study aimed to evaluate the antioxidant activity, the irritation potential and vascular effects on the chorioallantoic membrane of two hydrogel formulations containing 2% *Glycyrrhiza glabra* methanolic extract (LRME).The first formulation (S1) included propylene glycol as a penetration enhancer, while the second (S2) used Labrasol. Antioxidant capacity was assessed using two *in vitro* methods: DPPH and FRAP assays, while the *in vivo* evaluation was performed using the HET-CAM and the angiogenesis CAM protocols. DPPH results showed that both hydrogels exhibited antioxidant activity in a concentration-dependent manner, with an IC50 of 355.46 µg/mL for the S1 hydrogel, and 431.13 µg/mL for the S2 hydrogel. Furthermore, at the concentration of 1000 µg/mL, both hydrogels had an antioxidant activity close to that of ascorbic acid. The FRAP assay further confirmed the antioxidant activity of the hydrogels, showing the maximum FRAP (%) antioxidant capacities at 1000 µg/mL of 56.42% for S1 and 68.94% for S2. Hydrogel samples containing *Glycyrrhiza glabra* methanolic extract induced a moderate reduction in vessel density on the chorioallantoic membrane. Both hydrogel formulations were found to be non-irritant according to the HET-CAM assay results. In conclusion, the hydrogel formulations demonstrated significant antioxidant activity, with S2 exhibiting slightly stronger antioxidative effects compared to S1 at maximum concentration. Both hydrogel formulations were found safe for topical application, as shown by the HET-CAM assay, and led to a moderate reduction in vessel density, suggesting their potential for addressing skin conditions linked to inflammation and vascular abnormalities.

Key Words: Glycyrrhiza, antioxidant, angiogenesis

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SOME BIOLOGICAL ACTIVITIES AND TOTAL PHENOLIC CONTENTS OF THREE *PAPAVER* SPECIES FROM TÜRKİYE

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The *Papaver* L. genus, which is in the *Papaveraceae* family and whose Turkish name is "Gelincik", contains 49 taxa according to the work titled "Türkiye Bitkiler Listesi" written by Turkish botanists in 2012. *Papaver* species have been used as antidepressants in some regions of Anatolia, as well as in the treatment of many diseases such as inflammation, diarrhea and sleep disorders. The *Papaver* genus has important alkaloids (such as morphine, papaverine, thebaine) as well as rich phenolic compounds such as anthocyanins, flavonols and characteristic indole derivatives nudicaulins and essential oils. Therefore, we initiated a study to screen the ethanol extracts prepared from herba of species *P. clavatum* Boiss. & Hausskn. ex Boiss., *P. rhopalothece* Stapf and *P. syriacum* Boiss. & Blanche grown in Turkey. The antioxidant activity and total phenol content of the prepared ethanolic extracts were investigated using spectrophotometric techniques with ELISA microplate reader at different concentrations. Also The antimicrobial activity of the extracts was tested against some pathogenic bacteria and *Candida* strains. The antimicrobial effects of the extracts were conducted according to a slightly modified version of the Clinical and Laboratory Standards Institute (CLSI) protocol. *P. rhopalothece* resulted in the highest phenolic content from Serik/Antalya (73.89 ± 5.39 mg GAE/g extract), followed by *P. syriacum* from Antakya/Hatay (69.30 ± 2.25 mg GAE/g extract) and *P. clavatum* from Yayladağ/Hatay (61.56 ± 0.81mg GAE/g extract) measured by the Folin–Ciocalteu reagent method. When antioxidant activities were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, compared to the positive control gallic acid, *P. syriacum* and *P. clavatum* showed moderate levels of activity, while *P. rhopalothece* showed lower levels of activity (46.48 ± 3.16 mg/mL, 40.13 ± 3.47 mg/mL and 21.70 ± 4.99 mg/mL respectively). The total phenolic content and antioxidant capacity were shown to be significantly correlated, suggesting that phenolic chemicals play a role in these plants antioxidant qualities. None of the tested bacteria were inhibited by the extracts even at the maximum concentration used in the assay (2000 µg/mL). However, extracts E-05 and E-07 exhibited effectiveness particularly against Candida parapsilosis (MIC 1000 µg/mL).

Key Words: *Papaver sp.*, antioxidant activity, phenolics

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RHODIOLA ROSEA AND ITS MAJOR METABOLITES REDUCE INFLAMMATION IN AN INDUCED RHEUMATOID ARTHRITIS MOUSE MODEL

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Rheumatoid arthritis (RA) is a severe chronic inflammatory autoimmune disease leading to progressive joint destruction and deformity. In the etiology of RA, there is a strongly expressed immunological component, playing a key role in the development of the disease. The main manifestations of the immune response in RA are the formation of autoantibodies of the IgM class directed at the Fc region of immunoglobulin G or the so-called rheumatoid factor (RF), as well as antibodies against citrullinated proteins. The most commonly used therapeutic agents for the treatment of RA are conventional synthetic disease-modifying antirheumatic drugs which come with a range of side effects for patients, and place a burden on national budgets. The search of new biological therapeutics offers new opportunities for improving patient well-being. Phytochemical analysis of *Rhodiola rosea* (golden root) rhizome extract was conducted by nuclear magnetic resonance (NMR)-based metabolic profiling. The obtained spectra showed the presence of phenylethanoids (p-tyrosol and salidroside) and phenylpropanoids (rosin, rosavin and rosarin) in the rhizomes of *R. rosea*. The anti-inflammatory activity of *R. rosea* extract and its characteristic metabolites was investigated in a collagen-antibody induced arthritis (CAIA) mouse model. C57Bl/6 mice were used for the experiment. Treatment with the tested extract, salidroside, or rosavin began the day after arthritis induction and continued daily for 14 days. Arthritic animals treated with methotrexate served as a control group. Clinical evaluation of the disease symptoms in the experimental animals showed an improvement in the degree of paw swelling of the treated mice compared to the induced arthritis group. Preliminary histopathological examination of joint samples from treated and control groups of animals showed an improvement in synovial inflammation as a result of the therapy. At the next stage of the research, it is planned to conduct a flow cytometric analysis of synovial cells isolated from the experimental animals.

Key Words: Golden root, Rheumatoid arthritis, NMR-based metabolomics, collagen-antibody induced arthritis, flow cytometry, Synovial inflammation

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HEPATOPROTECTIVE POTENTIAL OF CURCUMIN AGAINST AROCLOR 1254-INDUCED TOXICITY IN HEPG2 CELLS

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Curcuma longa has been traditionally used for centuries due to its anti-inflammatory, antioxidant, and hepatoprotective properties. Curcumin, the principal bioactive polyphenolic compound isolated from turmeric rhizomes, exhibits remarkable pharmacological activity. This study investigated curcumin's protective potential against hepatotoxicity induced by Aroclor 1254 (A1254) that persists as an environmental contaminant despite being banned decades ago. In our phytopharmacological investigation, HepG2 cells were exposed to A1254 (12.5 μM) with or without curcumin (1 μM) pretreatment. A1254 exposure significantly increased intracellular reactive oxygen species (ROS) production (135.18±5.21% compared to control) and disrupted the cellular antioxidant defense system. A1254 significantly depleted reduced glutathione (GSH) levels and increased lipid peroxidation, indicating oxidative damage to cellular membranes. The total antioxidant capacity (TAOC) was markedly reduced in A1254-exposed cells (4.14±0.88 nmol/mg protein) compared to control cells (8.45±1.05 nmol/mg protein). Oxidative DNA damage was evident through elevated 8-OHdG levels in A1254-treated cells (4.17±0.88 ng/ml) compared to control (1.21±0.18 ng/ml). Furthermore, A1254 induced apoptotic cell death, as evidenced by increased caspase-3 (14118±1514 μnmol pNA/min/mg protein) and caspase-8 (15.14±2.21 nmol pNA/min/mg protein) activities. Phytotherapeutic intervention with curcumin effectively mitigated oxidative stress by preserving glutathionr (GSH) levels, significantly improving TAOC (7.03±0.96 nmol/mg protein), and reducing 8-OHdG levels (2.21±0.14 ng/ml). Curcumin also demonstrated potent protective effects against protein oxidation, reducing protein carbonyl groups from 8.24±1.16 nmol/mg protein in A1254-exposed cells to 4.04±0.39 nmol/mg protein. Curcumin significantly attenuated caspase-3 (10021±1143 μnmol pNA/min/mg protein) and caspase-8 (10.01±0.78 nmol pNA/min/mg protein) activities, highlighting its robust cytoprotective and anti-apoptotic potential. These findings provide scientific evidence for the traditional hepatoprotective use of *Curcuma longa* and suggest that curcumin may serve as a promising natural therapeutic agent through its potent antioxidant, anti-genotoxic, and anti-apoptotic properties. The study highlights the importance of readily cultivated medicinal plants as accessible source of bioactive compounds with significant health-promoting effects against environmental toxicants.

Key Words: *Curcuma longa*, curcumin, oxidative stress, antioxidant capacity, DNA damage, hepatoprotection

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EXPLORING THE MULTIFACETED POTENTIAL OF CAROB: FROM TRADITIONAL USES TO MODERN APPLICATIONS IN FOOD, HEALTH, AND INDUSTRY

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Ceratonia siliqua L., commonly known as the carob tree, is an evergreen species native to the Mediterranean region that has played a significant role in human history. Numerous studies have demonstrated the therapeutic potential of carob, including antioxidant, anti-inflammatory, and gastrointestinal health benefits. Its potential role in diabetes management and cardiovascular health has also been explored. In food technology, carob serves as a cocoa substitute, gluten-free ingredient, and functional food component. Despite its potential, inconsistencies in phytochemical composition data reveal the need for further research. In light of these considerations, the VALO.RE. I.N. CA.M.P.O. project, funded by the Italian Ministry of Agriculture, Food Sovereignty and Forestry, aims to highlight and subsequently increase the national production of carob for officinal purposes. This study presents an analysis of the ethnobotanical applications of the plant and outlines a strategy for the valorisation of both fresh and processed carob. The approach emphasises diversification, multifunctionality and sustainability in commercial agricultural production, potentially encouraging the cultivation of this ancient fruit in currently unexplored regions. Given the ongoing climate changes and the resilience of the species, these areas may be well suited for an expansion of carob cultivation.

Key Words: *Ceratonia siliqua* L., botanicals, bioactive compounds, polyphenols

PHYTOCHEMICAL INVESTIGATION OF *SALVIA MULTICAULIS* VAHL EXTRACTS OF DIFFERENT POLARITY

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The Lamiaceae family, recognised as one of the oldest and most widespread plant families worldwide, includes about 250 genera and more than 3200 species. Lamiaceae species, which are generally aromatic, perennial herbaceous plants, shrubs and rarely trees, produce essential oils used in perfumery, cosmetics and traditional medicine. In Turkey, the Lamiaceae family is represented by 758 taxa and shows about 45% endemism. The genus *Salvia* is a member of the Lamiaceae family and includes many important medicinal and aromatic species. *Salvia* species are commonly used in Anatolia for colds, stomach complaints and sore throats. It is also used for inflammatory skin conditions, as an antiseptic for wounds and to stop bleeding. *Salvia multicaulis* Vahl is used in our country for diabetes, colds, flu, indigestion problems, tonsillitis, sedative and inflammation. Monoterpenes such as 1,8-cineole, camphor, camphene, α-pinene, borneol, caryophyllene and bornyl acetate were determined as major compounds in the essential oil. In this study, 10 g of the aerial parts of *S. multicaulis* species, which were dried and powdered in the shade, were weighed and extracted 3 times with ethanol, acetone and dichloromethane (50 ml each) 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 analysed according to an LC-MS/MS method (Shimadzu 8040 model) previously developed and validated by our research group. By the developed method, 56 phenolic and flavanoid-derived compounds were detected in *S. multicaulis* extracts at 3 different polarities. According to LC-MS/MS results, cyranoside (41472 µg compound/g extract), cyranoside (21728 µg/g extract) and acacetin (11227 µg/g extract) were determined as the major components of ethanol, acetone and dichloromethane extracts of *S. multicaulis* species, respectively.

Key Words: *S. multicaulis*, LC-MS/MS, cyranoside, acacetin

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BIOTECHNOLOGICAL POTENTIAL OF *MELILOTUS OFFICINALIS* SHOOT CULTURES FOR BIOACTIVE METABOLITE PRODUCTION

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Melilotus officinalis L. (yellow melilot, Fabaceae) is a pharmacopoeial plant widely recognized in traditional medicine and endorsed by regulatory bodies such as the EMA and EFSA. The aerial parts of melilot (*Meliloti herba*) are considered a valuable herbal substance, with a required minimum coumarin content of 0.3% (dry weight). This study aimed to develop biotechnological protocols for *M. officinalis* by initiating and optimizing shoot cultures and evaluating the chemical composition of the resulting plant tissue. *In vitro* shoot cultures were established from seeds and maintained on Murashige and Skoog (MS) medium supplemented with plant growth regulators (PGRs), specifically cytokinins and auxins in the following combinations: BA + IBA, BA + NAA, 2iP + IBA, and 2iP + NAA (each at 1 mg/L). Control cultures were maintained on MS medium without PGRs. Both agar-based stationary and agitated cultures were tested over a 21-day growth cycles (three independent series, n=5). Methanolic extracts from *in vitro* biomass and parent plant herb were analyzed via LC-HRMS/MS for phytochemical profiling, while quantitative analysis of major compounds was performed using LC-DAD. LC-HRMS/MS analysis identified over 30 compounds across all extracts, revealing significant qualitative and quantitative differences between *in vivo* and *in vitro* samples. In the herb extract, the predominant metabolites were coumarin (786.64 mg/100 g DW), rutin (223.67 mg/100 g DW), and ononin (57.48 mg/100 g DW). Notably, coumarin, rutin, quercetin, and astragalin were absent in *in vitro* cultures. Instead, the dominant metabolites in shoot extracts were isoflavones, including ononin, daidzein, daidzin, calycosin, and formononetin. Daidzin was exclusive to *in vitro* cultures. The composition and concentration of metabolites varied depending on the PGRs and cultivation mode. The highest metabolite levels were observed in shoots grown on MS medium with 1 mg/L 2iP and 1 mg/L IBA, yielding maximum ononin (188.57 mg/100 g DW, agitated) and formononetin (96.49 mg/100 g DW, agar). This study highlights significant metabolic differences between *in vivo* herb and *in vitro* shoot cultures, demonstrating the potential of *M. officinalis in vitro* biomass as an alternative and sustainable source of bioactive compounds.

Key Words: Plant biotechnology studies, isoflavones, yellow melilot *in vitro* shoot cultures

ANTIOXIDANT AND ANTIMALARIAL ACTIVITIES OF FIVE *ARTEMISIA* L. SPECIES GROWN IN TÜRKİYE

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Artemisia L. genus is represented by 27 taxa, including 21 species, 3 subspecies and 3 varieties in Türkiye [1]. *A. annua* L. is a source of artemisinin and semi-synthetic artemisinin derivatives (dihydroartemisinin, artesunate etc.) included in the "Artemisinin-Based Combination Therapies (=ADCT)" for malaria treatment [2,3]. Chinese researcher Tu Youyou won the Nobel Prize in "Physiology or Medicine" for the discovery and purification of artemisinin and dihydroartemisinin [4]. In this study, the antioxidant activities of distilled water, ethanol, hexane and acetone extracts prepared with the aerial parts of *A. absinthium* L., *A. annua* L., *A. abrotanum* L., *A. incana* (L.) Druce and *A. tournefortiana* Rchb. were determined using four different methods (DPPH, CUPRAC, ABTS, FRAP). Additionally, the antimalarial activities of the same extracts against *P. falciparum* D6 and W2 strains were evaluated. In our antioxidant activity studies, *A. annua* was found to have the highest antioxidant capacity among the studied species. The ethanol extract of the *A. annua*, which had the lowest IC50 value (58.29µg/ml), showed the highest activity against DPPH radical. Besides this, the highest activity for all solvents used in the ABTS assay was *A. annua*; the lowest activity was seen in *A. absinthium*. In the CUPRAC method, the highest value measured is 150.35 mg GAE/g and belongs to the aqueous extract of the *A. annua* prepared by decoction. *In vitro* antimalarial activity study showed that the highest activity against both strains belonged to the hexane extracts of *A. annua* (IC50 values for D6 and W2 strains, 1861.8; 739.4, respectively). It was expected to observe high activity in *A. annua* which contains artemisinin. However, the fact that antimalarial activity has been detected in other species, albeit at very low levels, suggests that there may be other compounds responsible for the activity. Further analysis is required to detect these components.

Key Words: Antimalarial, Antioxidant, *Artemisia*, Türkiye

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PHYTOCHEMICAL STUDIES, ANTIOXIDANT POTENTIAL AND INHIBITION OF TYROSINASE PROPERTIES OF *LATHYRUS LATIFOLIUS* L. MICROSHOOT CULTURES AND PARENT PLANT EXTRACTS

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Lathyrus latifolius L. (Fabaceae) is climbing perennial occurs in eastern and southern Europe and northwestern Africa. The specie is rare and endangered in Poland with unexplored chemical composition and biological activity. The purpose of this work was assesment of the phytochemical profile and biological activity of extracts from leaves, flowers, herb and agar microshoot cultures. The parent plant material leaves, flowers and herb was obtained from the Botanical Garden in Kielce, in June 2023. *L. latifolius* microshoot cultures were maintained on standard MS medium with addition of 1 mg/L BA (6-benzyladenine) and 1 mg/L NAA (1-naphthaleneacetic acid), cultivated under continuous LED lighting in 14-day cycles. The ethanol/water extracts (70/30,v/v) were conducted by HPLC-DAD method. The antioxidant potential were performed using DPPH and ABTS methods. The tyrosinase inhibitory assay was conducted using commercially available mushroom tyrosinase and murine tyrosinase present in the lysate of murine melanoma cell B16F10 (ATCC CRL-6475). The phytochemical studies confirmed presence of six phenolic acids, two irydoids and catechin. The dominant compund of irydoids was werbascoside (517.67 mg/100 g DW, leaves). Among phenolic acids the domonant compounds were 1.5-dicaffeoylquinic acid (364.25mg/100 g DW, leaves) and isochlorogenic acid (350.19 mg/100 g DW, leaves). The content of catechin varied from 407.82 mg/100 g DW (leaves) to 107.15 mg/100 g DW (microshoot cultures). In the DPPH method, the highest antioxidant potential was obtained for flower extracts (53.17% of inhibition), while for the ABTS method, the highest capacities were obtained for leaf extracts (141.49% of inhibition). For the herb extract the highest % inhibition of fungal tyrosinase (121.89%) was obtained. On the other hand, the flower extract showed the highest inhibition of mouse tyrosinase (104.44%). Studies confirm the presence of bioactive compounds in extracts from leaves, flowers, herbs and microshoot cultures and their potential use in the pharmaceutical and cosmetic industries.

Key Words: *Lathyrus latifolius* L., plant *in vitro* cultures, chemical composition, antioxidant potential, inhibition of tyrosinase properties

ENDOCRINE DISRUPTING EFFECTS OF RESVERATROL, ROSMARINIC ACID AND EPIGALLOCATECHIN GALLATE ON 3T3-L1 CELLS

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Today, obesity has become a serious health problem in both developed and developing countries. Natural endocrine disrupting chemicals (EDCs) are widely found in many plants. Phytoestrogens (such as daidzein and genistein) are the most studied natural EDCs. However, in recent years, some plant-based compounds such as resveratrol (RV), rosmarinic acid (RA), and epigallocatechin gallate (EGCG) have also been suspected of having endocrine disrupting effects although studies are limited on these compounds concerning their effects on endocrine system. The aim of the present study was to determine the effects of RA, RV and EGCG on adipogenesis and endocrine disrupting effects in 3T3-L1 cells. An adipogenesis model was created by a differentiation procedure of 3T3-L1 cells. The MTT method was used to examine the effects of RA, RV and EGCG substances on cell viability. The changes in aromatase, peroxisome proliferator activated receptor alpha (PPARα), peroxisome proliferator activated receptor gamma (PPARγ), fatty acid binding protein 4 (FABP4) and CCAAT/Enhancer binding protein (CEBPβ) were measured at the highest doses that did not cause loss in cell viability. We found that there were significant increases in aromatase (24.44%), PPARα (22.73%), PPARγ (23.90%), FABP4 (27.17%) and CEBPβ (32%) levels only in the EGCG group compared to the control. The changes in the other groups were not significant compared to the control group. In conclusion, it appears that EGCG has the potential to have obesogenic effects as a natural endocrine disruptor whereas RV and RA did not show the potential for endocrine disruption.

Key Words: Endocrine disruptor, obesity, rosmarinic acid, resveratrol, epigallocatechin gallate

SALVIA ROSMARINUS IN TUNISIA: PHYTOCHEMISTRY, ANTIOXIDANT, AND ANTI-INFLAMMATORY PROPERTIES

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Due to their diverse biological activities and low toxicity, bioactive compounds are gaining distinction as a valuable source of antioxidant and anti-inflammatory therapies. This study investigated the bioactive properties of two Tunisian rosemary varieties, *Rosmarinus officinalis* var. typicus (R.AS and R.T) and *Rosmarinus officinalis* var. troglodytarum (R.M), to explore their potential as sources of valuable compounds. The research focused on antioxidant (DPPH, FRAP, total antioxidant capacity) and anti-inflammatory (Griess nitric oxide assay) activities, along with phytochemical profiling and the purification of carnosol. The results revealed that R.M exhibited the highest total phenolic content (151 mg GAE/gDW) and the strongest ferric reducing antioxidant power (FRAP). Carnosol, a key antioxidant compound, was successfully purified from R.M using centrifugal partition chromatography (CPC). Two rosemary extracts demonstrated dose-dependent anti-inflammatory activity by inhibiting nitric oxide (NO) production. Notably, R.AS showed the most significant inhibition, reaching 50% at a concentration of 3.84 µg/mL without inducing cellular toxicity. The aqueous sub-extract of R.AS displayed the strongest anti-inflammatory effect, likely attributed to its high rosmarinic acid content, as identified by HPLC analysis. This study highlights the potential of these Tunisian rosemary varieties as rich sources of bioactive molecules, suggesting their suitability for biotechnological applications.

Keywords: Anti-inflammatory, CPC, rosemary, carnosol, antioxidant capacity

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BIOCOSMETOLOGY: A MOUTHWASH BASED ON ESSENTIAL OILS

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Cosmetic products have become mass-market products. The synthetic substances used in their manufacture make them very dangerous for health and particularly carcinogenic. In order to demonstrate the importance and benefits of organic cosmetic formulation (based on essential oils), a mouthwash prepared from organic ingredients was tested and compared to two mouthwashes based on iodine (sample 1) and chlorhexidine (sample 2). The organic mouthwash is prepared from several essential oils followed by quality control of the appearance and pH. The microbiological effectiveness on the oral flora is evaluated by inoculating sputum from 10 people on cooked blood agar before and after 30 minutes of use. Our sample is fluorescent green, refreshing, and slightly spicy. Mouthwashes with a pH lower than 7.0 (samples 1 and 2) can cause problems such as weakening of tooth enamel. It is therefore preferable to use a neutral (sample 3) or basic mouthwash. The microbiological analysis on cooked blood agar after using the three mouthwash samples showed an effectiveness of 70% of the organic mouthwash, a reduction in the culture compared to the initial culture with sputum without using sample 2 while no change was observed with or without using sample 1.
It is therefore recommended to promote the use of organic cosmetic products for the well-being of our health but also to protect our planet.

Key Words: Formulation, ingredients, biocosmetology, quality

BIODIVERSITY AND CONSERVATION OF MEDICINAL PLANTS ALONG THE BULGARIAN DANUBE RIVERBANK

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The Bulgarian section of the Danube River represents a unique and ecologically diverse corridor, home to a rich variety of medicinal plant species with both traditional and contemporary significance. This study provides an overview of the current status of these species in the region, emphasizing their diversity, distribution, and importance for local communities and biodiversity conservation. A combination of field observations, herbarium data, and literature sources was used to compile a comprehensive list of medicinal plant species occurring in riparian and adjacent habitats. Special attention was given to taxa of high ethnobotanical value and those listed in national or international conservation frameworks. Notable representatives include species from the genera *Thymus*, *Salvia*, *Achillea*, and *Hypericum*. The analysis highlights the exceptional botanical richness of the region, shaped by its ecological heterogeneity and diverse habitat mosaic. The Bulgarian Danube corridor encompasses a range of interconnected ecosystems, including nutrient-rich alluvial soils, seasonally flooded wetlands, riparian forests, meadows, and transitional forest-steppe zones. This habitat diversity supports a high concentration of medicinal plant species, many of which are rare, endemic, or relict, and play essential roles in local ecological processes and cultural traditions. Nevertheless, this natural wealth is increasingly under pressure from a combination of anthropogenic and environmental threats. Habitat fragmentation due to expanding agricultural practices and infrastructure development disrupts ecological connectivity and alters species composition. Industrial activities and pollution degrade soil and water quality, further limiting suitable conditions for sensitive species. Overharvesting of wild medicinal plants – often driven by commercial demand without proper regulation – leads to population decline and local extinctions. The aggressive spread of invasive alien species outcompetes native flora, while climate change introduces new uncertainties through altered hydrological regimes, temperature fluctuations, and increased frequency of extreme weather events. These cumulative pressures pose a serious risk to the long-term viability of medicinal plant populations in the region. Despite the ecological and cultural value of these plant resources, conservation efforts remain limited. In situ and ex situ measures are underdeveloped, and there is a lack of coordinated strategies for their sustainable use. Addressing these challenges requires an integrated approach that combines scientific research, conservation policies, community participation, and the incorporation of traditional knowledge. The findings aim to inform and support future initiatives focused on the preservation and sustainable use of medicinal plant resources along the Bulgarian stretch of the Danube River.

Key Words: Medicinal plants, Danube River, Bulgaria, conservation, habitat loss, sustainable use

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PHYTOCHEMICAL INVESTIGATION BY LC-MS/MS OF *MENTHA LONGIFOLIA* (L.) L. SUBSP. *TYPHOIDES* (BRIQ.) HARLEY EXTRACTS

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Medicinal and aromatic plants are one of the product groups that are increasing their importance and popularity in the world because they have fewer side effects compared to synthetic drugs. One of the plants used for medicinal purposes is *Mentha* genus plants belonging to Lamiaceae family. *Mentha* species are popularly used for antibacterial, antispasmodic, antiviral, emenagogue, colds, flu, cough, gastrointestinal problems, ulcerative colitis and flatulence. Similarly, in Anatolian folk medicine, *M. longifolia* has been used in anti-parasitic, anti-helminthic, antimicrobial, emenagogue, carminative, nausea, bronchitis, ulcerative colitis and liver disorders. Volatile compounds such as linalol, carvone, piperitone oxide, menthone, pulegon were determined in the essential oil of *Mentha longifolia* (L.) L. subsp. *typhoides* (Briq.) Harley. In this study, 10 g of the aerial parts of *M. longifolia* subsp. *typhoides* species, which were dried and powdered in the shade, were weighed and extracted with ethanol, acetone and dichloromethane (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 analysed according to an LC-MS/MS method (Shimadzu 8040 model) previously developed and validated by our research group. By the developed method, 56 phenolic and flavanoid derivative compounds were determined in *M. longifolia* extracts at 3 different polarities. According to LC-MS/MS results, rosmarinic acid (67315 µg compound/g extract), acacetin (16126 µg/g extract) and acacetin (17652 µg/g extract) were determined as the major components of ethanol, acetone and dichloromethane extracts of *M. longifolia*, respectively.

Key Words: *M. longifolia*, LC-MS/MS, rosmarinic acid, acacetin

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BIOLOGICAL BASIS FOR THE CULTIVATION OF THREE ECONOMICALLY IMPORTANT THYMUS SPECIES

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Plant reproduction is a crucial biological process, not only for the propagation of a species but also for the productivity and quality of crops. Studying reproductive biology is key to the successful *ex situ* cultivation of plants, as it provides valuable insights into their reproductive potential. The present study investigates the reproductive biology and success of three *Thymus* species with established economic importance – *T. pannonicum*, *T. zygioides*, and *T. longedentatus*—in relation to their potential for agricultural cultivation. Embryological observations were conducted using the classical paraffin method on flowers and flower buds collected from natural populations in Bulgaria. The male and female reproductive structures and the processes of gametogenesis, pollination, embryo, and endosperm development were examined. Pollen and seed viability were assessed using acetocarmine and tetrazolium tests, respectively. The male and female reproductive structures were as follows: tetrasporangiate anthers with a four-layered dicotyledonous-type wall consisting of an epidermis, fibrous endothecium, ephemeral middle layer, and secretory tapetum; tetrahedral tetrads; and 2-celled mature pollen. The ovary was bilocular, each locule containing one anatropous, tenuinucellate ovule with a unicellular archesporium. The embryo sac was of the Polygonum type, the embryo followed the Onagrad type, and the endosperm was nuclear. All developmental processes proceeded normally. Pollen viability ranged from 71.47% to 75.94%, and seed viability from 70% to 72%. The established embryological characteristics identify the studied *Thymus* species as sexually reproducing, with normally functioning reproductive processes. The high pollen and seed viability indicate strong reproductive potential, supporting their effective cultivation. These findings provide a foundation for developing strategies for the successful agricultural propagation of these valuable medicinal plants.

Key Words: *Thymus*, male and female gametophyte development, reproductive capacity, *ex situ* cultivation

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LAVANDULA STOECHAS L.: A REVIEW ON BIOLOGICAL ACTIVITIES AND TRADITIONAL USES

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The flora of the Mediterranean area includes many aromatic and medicinal species. Plants belonging to *Lavandula* genus of the Lamiaceae family, which naturally distributed in the Mediterranean basin, are represented by 39 species. One of them *Lavandula stoechas* L. is an evergreen, densely branched, fragrant, semi-woody perennial shrub. It is naturally distributed in Africa, Southern Europe and Western Asia. In Türkiye, it is distributed in regions close to the Aegean, Marmara and Mediterranean coasts. This plant cultivated in France, Italy and Spain, is often referred to as French or Spanish Lavander. Due to the wide distribution area of the plant in Türkiye, it has not yet been cultivated. It is used in the pharmaceutical, food and cosmetic industries. Also, since it has a long flowering period, it is a good source of nectar for honeybees. For this reason, it is a preferred plant in honey production. Besides it uses as ornamental plant in parks and gardens. These plants produce a large number of secondary metabolites. The two main groups of non-essential compounds are flavonoids and terpenes. Also, they contain coumarins and phenolic acids. Besides, camphor, fenchone and 1-8 cineol are the main components of the essential oils. They show many biological activities like antioxidant, antimicrobial, anti-inflammatory, anticonvulsant and antispasmodic effect due to the phytochemical components they contain. The plant especially used as herbal tea, is collected during the flowering period, dried and used to relieve various health problems. It is generally used against colds, headaches and stomachaches, muscle pain, arteriosclerosis and epilepsy. In this perspective the scientific studies conducted to date and the areas of this plant by the local people overlap with each other.

Keywords: *Lavandula stoechas* L., lavander, biological activities, traditional uses

CYMBOPOGON CITRATUS (D.C.) STAPF PLANT MATERIAL AND IN VITRO MICROSHOOT CULTURES AS RICH SOURCES OF NATURAL PHENOLIC ANTIOXIDANTS AND THEIR POTENTIAL USE IN PHARMACY AND COSMETOLOGY

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Lemongrass [*Cymbopogon citratus* (D.C.) Stapf] is a perennial and medicinal herb belonging to the Gramineae family, widely cultivated in tropical and subtropical countries to produce lemon grass oil with multiple uses in the pharmaceutical, food industry and cosmetology. In this study, the polyphenol composition, cytotoxicity, antioxidant and antimicrobial activity, enzyme inhibition capacity: elastase and tyrosinase of *Cymbopogon citratus* methanolic extracts obtained from the natural state and in vitro microshoot cultures were evaluated. *In vitro* microshoot propagation of lemongrass has been successfully achieved using Murashige and Skoog (MS) medium supplemented with growth regulators like 1 mg/L 6-benzyloadenine (BA) and 1 mg/L 1-naphthylacetic acid (NAA). This method has been effective in inducing shoot proliferation and root development. The antioxidant properties of the tested extracts were determined spectrophotometrically using DPPH, FRAP, and ABTS assay. Total polyphenols content (TPC) and total flavonoids content (TFC) were also determined. The antimicrobial activity of extracts was characterized by evaluation of MIC and MBC. The antimicrobial activity of was assessed against *E. coli, P. aeruginosa, S. aureus, S. epidermidis, C. acnes P. acnes, S. mutans* and *S. sanguinis*. The study also compared the polyphenol composition in plant material obtained from the botanical garden and *in vitro* microshoot cultures using the HPLC/UV-VIS /DAD method. Lemongrass is a source of many compounds with high antioxidant potential, including: caffeic acid, ferulic acid, p-coumaric acid, luteolin, apigenin and their derivatives. The strong antioxidant properties of methanol extract of lemongrass were correlated with TPC and TFC. This study confirms that's lemongrass extracts have potential use as pharmaceutical and cosmetic skincare ingredients. Thus, *Cymbopogon citratus* can be considered a promising natural source of readily available, low-cost extracts rich in antioxidant, skincare, and antimicrobial compounds that might be suitable for replacing synthetic compounds in the cosmeceutical industry.

Key Words: *Cymbopogon citratus* extracts, *in vitro* microshoot cultures, antioxidant activity, total polyphenol content

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METABOLITE PROFILES OF *THYMUS LONGEDENTATUS* FROM NATURAL AND CULTIVATED AREAS

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Thymus longedentatus (Degen & Urum.) Ronniger is a Balkan endemic species with a pleasant lemon aroma due to its high content of citral compounds [1]. The essential oil and extracts of the species have demonstrated important biological activities [2, 3], making it not only a pleasant tea ingredient but also a valuable material for agriculture and pharmacy. However, its relatively limited distribution requires exploring possibilities for cultivation. This study aims to compare the metabolic profiles of *T. longedentatus* collected from wild populations and cultivated areas. Plant material was gathered from natural localities in the Eastern Rhodopes (ER), the southern Black Sea coast (BSC), and cultivated areas near Sofia. Compounds were identified in essential oils and alcoholic extracts using GC/MS and TLC analysis. Citral isomers (neral and geranial) were identified as the main components in essential oil profiles from all studied origins. Triterpene acids, fatty acids and flavonoids aglycones were determined as the main bioactive compounds in the exudates. Rosmarinic acid, chlorogenic acid, sugar alcohols, flavonoid glycosides and monosaccharides were found in the methanolic extracts. While the metabolic profiles from different origins were similar, significant quantitative differences were observed in some individual compounds. Significantly higher quantities of chlorogenic acid, geranic acid and others were found in the sample from the cultivated area, whereas arbutin, hydroquinone and others were more abundant in the sample from the natural locality (ER). A high degree of similarity was observed between plant material from the cultivated area and the ER population, which served as the source material for cultivation. This indicates that the metabolic profile of the starting material is well preserved during cultivation.

Key Words: citral isomers, flavonoids, chlorogenic acid, triperpene acids, thymoquinone, geranic acid

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METABOLITE COMPOSITION OF VARIOUS PARTS OF *CICHORIUM INTYBUS*

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Cichorium intybus L. (chicory) is a medicinal plant known as coffee substitute with important pharmacological activities such as antioxidant, anti-inflammatory, antidiabetic, gastroprotective anticancer, antihelminthic, hepatoprotective, and many others. The most commonly used parts of the plant are the roots and leaves [1]. Recently, it has been reported that the aqueous extract of flowering stems of *C. intybus* showed significant α-glucosidase inhibitory activity [2]. That is why the present study aims to compare the metabolic profiles of four parts of the species – leaves, flower heads, stems, and roots. The plant material of *C. inthybus* was collected from the protected area "Kaylaka" in the vicinity of Pleven (Bulgaria) in June 2021. Methanolic extracts of the various parts of the species were analyzed using GC/MS and HPTLC. Total phenolic content was determined spectrophotometrically by Folin-Ciocalteu's reagent. The antioxidant potential was assessed using the DPPH method.
Total phenolic content was the highest in the flower heads, followed by the leaves, stems, and roots. The antioxidant properties of the extracts from various plant parts follow the same sequence. Flower heads extract was found to be the richest in caffeic, hydroxycinnamic and ferulic acids. Chlorogenic, neochlorogenic, 3,5-dicaffeoylquinic acids were the most abundant in the roots, whereas the chicoric acid was in the leaves. The flower and stem extracts have the highest content of sugar alcohols (polyols): myo-inositol isomers, erythritol, mannitol and xylitol. Flavonoid glycosides were found in the largest quantity in the leaf and stem extracts. Sugar alcohols have been reported to have antidiabetic properties [3]. Their high content in the stems of the species characterizes their potential for use as an antidiabetic agent, in addition to the fact that the stems are the easiest to collect and the most abundant plant substance. Future research in this direction is forthcoming.

Key Words: Sugar alcohols, polyols, phenolic acids, DPPH, myo-inositol

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ESSENTIAL OILS IN VAPOUR PHASE: ANTIMICROBIAL EFFECTS AND MECHANISM OF ACTION AGAINST PATHOGENIC MICROORGANISMS

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This study aimed to evaluate the efficacy of selected essential oils against five bacterial and two fungal strains using thevapour-phase diffusion method. Fifteen essential oils were tested in their pure and vapour forms. Carvacrol, known for its broad-spectrum antibacterial and anticandidal activity, was used a standard natural antimicrobial agent. The chemical composition of essential oils was analyzed using a SHIMADZU GC-2010 Plus GC/MS system. CP-Sil 5CB column was used. Mass spectra were identified using the Wiley and Adams-LIBR(TP) databases. The RRI was obtained by applying an oil sample with a C9-C40 linear hydrocarbons mixture [1, 2]. Essential oils and major consituents were tested against *Staphylococcus aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *Escherichia coli* NRRL B-3008, *Pseudomonas aeruginosa* ATCC 9027, *Serratia marcescens* NRRL B-2544, *Candida albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019. Essential oils (10 µL) were applied to sterile filter paper discs placed on Petri dish lids. Plates were incubated at 37°C for 24 hours [3, 4]. For anticandidal evaluation, essential oils were tested on PDA plates for 24 and 48 hours [5]. After incubation period pigment production of *S. aureus* significantly inhibited by *O. basilicum* and *C. sativum* EOs. *C. sativum* exhibited strong efficacy against *S. aureus* (10 µL-28 mm ∅) and *P. aeruginosa* (10 µL- 21 mm ∅). Furthermore, *O. basilicum* was particularly effective against *E. coli*. The most efficient oils against *C. albicans* were *E. caryophyllus* (5µL- 20 mm ∅) *M. piperita* (5µL- 33 mm ∅) and *C. sativum* (5µL- 13 mm According to bioimagings several structural damages were demonstrated on cell membrane, protoplasm and nuclei. This study shows that essential oils in vapour phase have significant antimicrobial effects, especially against *Candida* species. These findings highlight their potential a natural antimicrobials with broad-spectrum activity against bacterial and fungal pathogens. In this context, the vapour phase of essential oils plays a key role in reducing microbial load on surfaces and in the air, with significant potential to prevent contamination.

Key Words: Vapour phase, essential oils, SEM/TEM analysis, pathogenic microorganisms, mechanism of action

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EFFECT OF HARVEST TIME AT DIFFERENT VEGETATIVE GROWTH STAGES ON YIELD AND QUALITY OF *MELISSA OFFICINALIS*: PRELIMINARY INSIGHTS

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Lemon balm (*Melissa officinalis* L.) is a perennial herbaceous plant native to the Mediterranean region and Western Asia, currently cultivated under diverse ecological conditions in temperate climate zones. The growth, development, and active compound content in medicinal plants can be significantly influenced by multiple factors, including endogenous and exogenous factors. Among these, the plant's developmental stage is a key factor influencing agronomic and qualitative parameters and it is closely linked to the harvest time. Numerous studies agree that, in the case of *M. officinalis*, the optimal harvest time occurs shortly before flowering. The aim of this study was to evaluate how the agronomic and qualitative characteristics of *M. officinalis* vary across different vegetative stages, in order to optimize the harvest time. Three harvest times were defined: one at the commonly accepted as optimal harvest time (defined according to practical experience), one earlier (approximately 2 weeks before the optimal vegetative stage), and one later (approximately 2 weeks after the optimal stage). The experiment was conducted in Merano (South Tyrol, Italy) using a randomized complete block design with three replicates. Results of the first study year showed that the average annual yield did not significantly differ between harvest times, despite variations in the number of cuts. The annual leaf yield ranged from 224.06 to 239.86 g DW/m². Harvesting at earlier vegetative stages led to the highest average essential oil content (0.22 %), characterized by high concentrations of neral (36.58 %) and geranial (47.34 %). By contrast, harvesting at more advanced vegetative stages ensured a higher essential oil yield (0.21–0.25 ml/m^2) with a profile rich in caryophyllene and caryophyllene oxide, as well as an increased total phenolic content. The intermediate vegetative stage demonstrated a good balance between quality and yield. Plant extracts obtained at this stage exhibited high antioxidant activity. Considering our preliminary results, the optimal harvest time can be determined based on the specific needs and production objectives.

Key Words: lemon balm, harvest time, yield, essential oil, total phenolic content, antioxidant activity

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EFFECTS OF ELICITOR TREATMENTS ON THE METABOLISM OF *MELISSA OFFICINALIS* CULTIVATED IN A CONTROLLED ENVIRONMENT

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The aim of this research is to evaluate the effect of elicitor treatments on the active compound of lemon balm (*Melissa officinalis* L.). The study was conducted in a controlled environment and elicitors, Chitosan Hydrochlorid and Salycilic Acid, were sprayed on plant leaf biomass. Plant's extarct were evaluated for polyphenols,flavonoids and antioxidant activity along with rosmarinc acid concentrations.The results suggest that the use of Chitosan Hydrochloide can represent a valid help in increasing the acculuation of secondary metabolites,thus affecting the quality of medicinal and aromatic species and their economic value. The biological properties of medicinal and aromatic plants (MAPs) have always been a source of fascination for humankind. Lemon balm (*Melissa officinalis* L.) is known since ancient times to be an ally in cases of anxious states thanks to its sedative and antispasmodic activity [1]. The biological activities of *Melissa officinalis* are attributed to the ability of plant species to produce active compounds. Among the main factors that can affect the content and quality of active ingredients in MAPs, there are elicitors: natural substances that induce in the plant the biosynthesis of metabolite involved in defensive response in plants [2]. The purpose of this research is to evaluate the efficacy of elicitor treatments in affecting the production of *Melissa officinalis*'s secondary metabolites. In particular, the study focuses on the application of elicitors such as Chitosan and Salicylic acid, alone or in combination, and their effects were evaluated in terms of total polyphenol, flavonoids, rosmarinic acid content and antioxidant activity. Among the elicitor tratments administered , chitosan hydrochloride, turns out to be the most effective in promoting the target seondary metabolites due to its ability to activate metabolic pathways crucial for the production of phenolic compounds. The results shows that the treatment with Chitosan Hydrochloride increase the polyphenols and flavonoids content respectively of the 53% and 49%, while the concentration of rosmarin acid is more that six time highter respect to the control. In conclusion the preliminary results provided by the present research suggest the use of natural elicitors in sustainable agriculture systems as a key strategy in reducing the use of pesticides and for improving the quality of MAPs final products.

Key Words: Elicitor treatments, lemon balm, secondary metabolites, Chitosan, Salicylic acid

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A COMPARATIVE STUDY OF ANTIOXIDANT ACTIVITY OF *PRUNUS DIVARICATA* LEDEB. VAR. *DIVARICATA* (ROSACEAE) FRUIT EXTRACTS

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Prunus divaricata Ledeb. var. *divaricata* belonging to the Rosaceae family is a deciduous tree or shrub that is widely distributed across Asia, Europe, and Anatolia. In addition to being used as food, the fruits of this plant have also been employed in folk medicine for the treatment of various ailments, including diabetes, hypercholesterolemia, atherosclerosis, hypertension, constipation, and asthma in Anatolia. This study aims to investigate the potential antioxidant activity of *P. divaricata* var. *divaricata* fruit extracts using various *in vitro* methods. In this context, nine extracts were prepared using water, 70% ethanol, and methanol from three traditionally consumed forms of ripe fruits, including fresh fruit, dried fruit, and dried layers of fruit pulp prepared using a traditional method called *pestil* in Turkish. The antioxidant activity of the extracts was evaluated using DPPH radical scavenging capacity, ABTS radical cation scavenging capacity, and cupric ion reducing antioxidant capacity (CUPRAC) assays. Additionally, the total phenolic, flavonoid and condensed tannin contents of the extracts were measured by the Folin-Ciocalteu, aluminum chloride, and vanillin-HCl colorimetric methods. The 70% ethanol and methanol extracts of fresh fruits, which possessed the highest total phenolic (153,27 and 134,61 mg gallic acid equivalents/g extract) and condensed tannin (77,32 and 91,38 mg catechin equivalents/g extract) contents, demonstrated greater scavenging activity against the DPPH radical (IC_{50}=116,31 and 125,78 µg/mL, respectively) and ABTS radical cation (161,09 and 161,36 mg Trolox equivalents/g extract, respectively). These extracts also showed higher activity in the CUPRAC assay (89,79 and 99,94 mg gallic acid equivalents/g extract, respectively) compared to the other tested extracts.

Key Words: *Prunus divaricata*, antioxidant, total phenolic content, total condensed tannin content

PROFITABILITY AND STRUCTURE OF ITALIAN MEDICINAL AND AROMATIC PLANT FARMS

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The increasing impacts of climate change and economic instability have led to a growing interest in alternative agricultural systems that provide resilience and profitability. Medicinal and aromatic plants (MAPs) are gaining attention due to their adaptability to diverse environments, high market value, and potential for economic diversification. Despite these advantages, the sector remains understudied, with limited standardized data on economic performance and structural characteristics. This study analyzes the profitability and cost structures of Italian farms cultivating MAPs, using data from the Farm Accountancy Data Network (FADN). The analysis focuses on key economic indicators such as gross output, variable costs, and net farm income per hectare to assess economic sustainability. Farms are classified based on utilized agricultural area (UAA) and their level of specialization in MAP production. The study examines the most widely cultivated species in Italy, including saffron, rosemary, lavender, oregano, and sage, to determine their respective profitability and associated cost components. The results indicate that saffron is the most profitable crop, with a gross margin of €57,600 per hectare and a gross output of €66,200 per hectare. Despite the high initial investments required, particularly for bulb propagation, its market value remains significantly higher than that of other MAPs. Rosemary follows with a gross margin of €22,000 per hectare, proving to be a stable option for medium-scale farms. Lavender, oregano, and sage show moderate profitability, although they require targeted marketing strategies to maximize returns. Cost distribution varies among species, with propagation costs representing 50% of saffron's variable expenses, while fertilization and soil amendments constitute major costs for sage and rosemary. Oregano has the highest marketing costs, accounting for 40% of its variable costs, highlighting the importance of branding and differentiation strategies. Farm size and specialization also influence economic performance. Small farms (less than 10 hectares) with a high degree of MAP cultivation achieve the highest net farm income per hectare (€4,100), benefiting from intensive management and niche market access. Medium-sized farms (10–50 hectares) balance labor efficiency and input costs, while large farms (over 50 hectares) leverage economies of scale, increasing net income per worker despite lower per-hectare profitability. These findings underscore the economic potential of MAPs and the need for strategic investments in market integration, sustainable practices, and technical innovation to enhance sectoral competitiveness and resilience.

Key Words: Medicinal and aromatic plant, farm accountancy data network, on-farm diversification, economic performance indicators

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TECHNICAL EVALUATION OF A MODULAR DRYER FOR MEDICINAL AND AROMATIC PLANTS IN PRACTICAL GERMAN CONDITIONS

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A modular batch dryer with partial recirculation of outlet air to save thermal energy was developed for small-scale medicinal plants producers in Germany. Different operational modes were tested for energy consumption and the quality of the dried product using lemon balm leaves. Fresh air mode, partial recirculation-controlled flap mode and full recirculation-controlled flap mode alternated depending on the progress of drying and the set relative humidity at the inlet. Experiments consisted of comparing two modes of controlled flap modes with relative humidity varying from 80 to 30 % for one mode and fixed at 40 % for the other mode. A total mass of 500 kg of lemon balm leaves were dried in an average of 23 hours to reduce moisture content from 72 % to 7 %. The throughput ranged from 19.7 to 23.7 kg·h-1 for lemon balm drying. Air recirculation significantly reduced the thermal energy consumption where a specific thermal energy consumption of 3540 kJ·kg-1 H_2O was achieved by controlling the inlet air humidity to 40 % when drying lemon balm compared to the 4075 kJ·kg-1 H_2O achieved under the 80-30 % mode. No significant difference in essential oils content was observed between the two humidity control modes. This research confirmed the energy-efficient attributes of the dryer and recommends the implementation of air recirculation as one of the methods to reduce energy consumption in medicinal plants drying.

Key Words: MAP drying, partial air recirculation, specific thermal energy, lemon balm, essential oils

GINKGO (*GINKGO BILOBA* L.) CALLUS INDUCTION FROM DIFFERENT PLANT PARTS

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The *in vitro* plant culture method enables the controlled cultivation of plant cells, tissues, and organs under sterile conditions. It is based on the totipotency of plant cells, which allows the development of a whole organism from a single cell or plant fragment. This rapid and controlled cultivation method serves as an important and useful tool in biotechnology and pharmacy, as it enables the synthesis of specialized plant metabolites, key factors for health, and the development of new drugs. One of the plants well-known in traditional and modern medicine is ginkgo (*Ginkgo biloba* L.), recognized for its medicinal properties. In this study, we present the development of callus, an undifferentiated and proliferating mass of cells, from ginkgo leaves. We compare the results of two culture establishment methods, covering plant material sterilization, medium preparation (Murashige and Skoog Medium and McCown's Woody Plant Medium), and the application of different concentrations and combinations of cytokinins (kinetin and BA) and auxins (IAA, 2,4-D, and NAA). The study was conducted on different parts of ginkgo – leaf petioles, leaf blades, and buds. The largest and most well-developed calluses were obtained from leaf blades, while petiole-derived calluses were smaller, and no callus formation was observed in buds.

Key Words: Callus, *in vitro* culture, ginko, leaves, phytohormones

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BIOLOGICAL ACTIVITY STUDIES AND ESSENTIAL OIL ANALYSIS ON *THYMUS* CILICICUS

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Thymus L., an important genus among aromatic plants in the *Lamiaceae* family, is represented by 60 taxa and 40 species in Türkiye. 18 of these species are endemic [1,2]. Infusions and decoctions of the leaves and flowering parts of *Thymus* have been used traditionally to prevention and treatment of numerous digestive and respiratory disorders including colds, flu, indigestion, nausea, and dysentery [3]. In this study, the antioxidant and antimicrobial properties and total phenolic content of 70% ethanol and ethyl acetate extracts of *Thymus cilicicus* Boiss. & Balansa were investigated. The chemical composition of the essential oil was analyzed. Antioxidant activity was evaluated using the DPPH method; 70% ethanol extract showed 96% inhibition, while ethyl acetate extract exhibited 36%. Total phenol content was determined using the Folin-Ciocalteu method, revealing that the 70% ethanol extract contained a higher phenolic content (113.6±0.103 mg GAE/g) compared to the ethyl acetate extract (23.23±0.04 mg GAE/g). Antimicrobial activity was determined by the microdilution method and MIC value was calculated against microorganisms. 70% ethanol extracts were more effective than ethyl acetate extracts. Antimicrobial activity was observed against standard bacterial species (*Bacillus subtilis* NRRL B478, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213) at a concentration of 1040 µg/mL and against yeast species (*Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258) at a concentration of 4160 µg/mL. Ethyl acetate extracts were effective at concentrations of 1040-4160 µg/mL. As a result of the analysis performed using the GC/GC-MS method, 55 compounds representing 98.9% of the essential oil were identified. The main compounds are α-Terpineol 18.5% and Geraniol 13.7%. As a result, the antioxidant and antimicrobial activity, and total phenol content of 70% ethanol extract were higher. In this study on *T. cilicicus*, an aromatic and medicinal plant collected from Eskişehir province, contributes valuable information to the existing literature.

Key Words: *Thymus cilicicus*, essential oil analysis, antioxidant activity, antimicrobial activity, total phenolic content

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DETECTION OF HUMAN H ANTIGEN BY LECTIN ISOLATED FROM SEEDS OF *PSOPHOCARPUS TETRAGONOLOBUS*

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Psophocarpus tetragonolobus (winged bean) is a common legume plant native to South and Southeast Asia. Legumes are rich in lectins, with specific binding properties to different cell surfaces, and can be used in medical applications such as blood typing, immunology, cancer research, and cell activation. *P. tetragonolobus* may contain lectins with specific properties, so this research aims to isolate its lectin and test with human red blood cells and secretions. Seeds of *P. tetragonolobus* were powdered, extracted with normal saline and isolated by alcohol fractionation. Crude extract and protein fractions were tested by reacting with human red blood cells and saliva to study the specificity of the lectin. The highest lectin concentration was found in fraction 2 of the crude extract. Lectin from *P. tetragonolobus* agglutinated red blood cells with specific reaction strength of O > A > B > AB cells. The results were comparable with the activities of commercial lectin from *Ulex europaeus*. These show the specificity of lectin from *P. tetragonolobus* to H antigen on the red blood cells. The lectin also reacted with the H antigen found in human saliva, giving the hemagglutination-inhibition reaction. With the comparable activities with lectin from *U. europaeus*, these show that the lectin from *P. tetragonolobus* can be used to test the H antigen secretor status of individuals. This study expanded the applications of a common plant, *P. tetragonolobus*, for the use in medical analytics. Lectin from *P. tetragonolobus* was specific to H antigen on the surface of red blood cells and in saliva. The lectin can be used, as a substitute of lectin from *U. europaeus*, for the diagnosis of Bombay and Para-Bombay phenotypes.

Key Words: *Psophocarpus tetragonolobus*, H antigen, Hemagglutination

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BIOLOGICAL ACTIVITIES AND ESSENTIAL OIL COMPOSITION OF *ORIGANUM VULGARE* SUBSP. *VIRIDULUM*

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The genus *Origanum* L. (*Lamiaceae*), commonly known as oregano, has medicinal and culinary significance [1]. Türkiye represents 27 *Origanum* taxa, of which 14 are endemic [2]. *Origanum vulgare* L. contributes to its traditional use in treating respiratory, digestive, and inflammatory disorders [3]. In this study investigates the biological activities of %70 ethanol and ethyl acetate extracts of *O. vulgare* subsp. *viridulum*, focusing on antioxidant and antimicrobial properties, total phenolic content (TPC), and essential oil composition. TPC was determined using the Folin–Ciocalteu method, as higher in the 70% ethanol extract (105.85 ± 0.03 mg GAE/g) than in the ethyl acetate extract (58.45 ± 0.015 mg GAE/g). Antioxidant potential was assessed using DPPH radical scavenging activity, where the 70% ethanol extract exhibited 93.40% inhibition at 125 µg/mL, while the ethyl acetate extract showed 47.56%. Essential oil analysis by GC/GC-MS identified 28 compounds, with carvacrol (65.6%) as the major constituent, followed by *p*-cymene and γ-terpinene. Antimicrobial activity was determined by microdilution method and MIC value of ethyl acetate extract was found to be effective against standard bacteria species (*Bacillus subtilis* NRRL B478, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853) and *Candida krusei* ATCC 6258 yeast species at a concentration of 260 µg/mL, *Candida albicans* ATCC 90028 yeast species at a concentration of 1040 µg/mL. 70% ethanol extract was effective against bacterial species at a concentration of 520 µg/mL, *Candida albicans* ATCC 90028 yeast species at a concentration of 2080 µg/mL and *Candida krusei* ATCC 6258 at a concentration of 1040 µg/mL. These results highlight the high antioxidant activity and phenolic content of *O. vulgare* subsp. *viridulum*, suggesting its potential as a natural source of bioactive compounds for pharmaceutical and food industry applications. Further studies, including antimicrobial evaluation, will provide a comprehensive understanding of its biological activities.

Key Words: *Origanum vulgare* subsp. *viridulum*, antioxidant activity, antimicrobial activity, total phenolic content, essential oil composition, GC-MS analysis

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INTEGRATION OF GENOMIC AND EST-SSR MARKERS FOR A SYSTEMATIC APPROACH TO *HELICHRYSUM ITALICUM* (ROTH) G. DON GENETIC RESOURCES EVALUATION

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Helichrysum italicum (Roth) G. Don, commonly known as immortelle, is one of the Mediterranean's most valuable medicinal and aromatic plants. Its antiaging and bioactive properties make it highly appreciated in the perfume, cosmetics and healthcare industries. Despite extensive chemical studies on its essential oil and extracts, systematic evaluation of its genetic resources has been hindered by the lack of efficient DNA markers. This challenge is further compounded by the complex taxonomic classification (the presence of subspecies, interspecies hybrids, and extensive cross-pollination), which contributes to high genetic and phenotypic diversity. In Slovenia, systematic selection of *H. italicum* began in 2017. Plant material was collected and propagated from family gardens, and the first *ex situ* collection was established in 2020, including both the original samples and their spontaneous hybrids. The collection supports studies of genetic and chemical diversity and facilitates targeted selection and breeding. This study presents a systematic approach to *H. italicum* genetic resources evaluation by integrating nuclear, chloroplast, and expressed sequence tag-derived simple sequence repeat (EST-SSR) markers. Genomic DNA and transcriptome were sequenced using the Ion Torrent platform in a shotgun approach, enabling the assembly of chloroplast genome, nuclear genome and transcriptome contigs. From these, 24 nuclear SSR, 16 chloroplast SSR and 23 EST-SSRs markers were identified as appropriate for genotype identification and population analysis. EST-SSRs showed clear polymorphisms predominantly in coding regions and all SSRs demonstrated successful cross-species transferability to related *Helichrysum* taxa (*Helichrysum litoreum* Guss. and *Helichrysum arenarium* (L.) Moench). The newly developed markers provide a robust tool for authentication, traceability, and marker-assisted selection, while addressing the challenges posed by its complex taxonomy and cross-pollination dynamics. Preliminary results indicate that genotype significantly influences the chemical diversity and variability of essential oil content, suggesting these markers can facilitate targeted exploitation of genotype–chemotype relationships.

Key Words: *H. italicum*, draft genome, transcriptome, microsatellite markers, genotype selection, authentication, traceability

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PHYTOCHEMICAL PROFILING AND ENZYME INHIBITORY POTENTIAL OF *LIMONIUM LILACINUM* VAR. *LAXIFLORUM*

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Soil salinity and drought represent two of the most detrimental abiotic stressors adversely affecting plant growth, development, and productivity. These environmental challenges induce the excessive generation and accumulation of reactive oxygen species (ROS), which are highly reactive molecules capable of causing significant oxidative damage to cellular macromolecules, including lipids, proteins, and nucleic acids. Halophytic plant species, characterized by their natural tolerance to high salinity conditions, have evolved highly efficient enzymatic and non-enzymatic antioxidant defense systems that enable them to mitigate the harmful effects of ROS. Notably, the biosynthesis and accumulation of polyphenolic compounds in halophytes are frequently upregulated in response to salt and drought stress. Polyphenols have emerged as key phytochemicals of interest due to their potent antioxidant properties and their broad spectrum of health-promoting effects. *Limonium* Mill., a genus within the family Plumbaginaceae, is represented in Türkiye by 27 taxa, of which 14 are endemic. Members of this genus are typically found growing naturally along seashores, sand dunes, and rocky coastal areas of the Aegean and Mediterranean regions, as well as in saline inland habitats in our country. In traditional medicine, various *Limonium* species have been employed for the treatment of ailments such as arthritis, fever, the common cold, neuralgia, hepatitis, bronchitis, diarrhea, menstrual irregularities, cramps, and genitourinary infections. Despite their ethnobotanical importance, a review of the existing literature reveals that phytochemical and pharmacological studies on *Limonium* species remain limited. In the present study, we aimed to investigate the phenolic profile and enzyme inhibitory potential of *Limonium lilacinum* (Boiss. et Bal.) Wagenitz var. *laxiflorum* Doğan et Akaydın. To this end, methanolic extract was prepared from the aerial parts of the plant, followed by successive fractionation using hexane, dichloromethane, ethyl acetate, and water. Enzyme inhibition assays were conducted to evaluate the inhibitory activities of the extract and fractions against a panel of clinically relevant enzymes, including acetylcholinesterase (AChE), butyrylcholinesterase (BChE), monoamine oxidase A (MAO-A), monoamine oxidase B (MAO-B), cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), and aromatase. The phenolic composition of the methanol extract was analyzed qualitatively and quantitatively using LC-MS/MS.

Key Words: *Limonium*, phenolics, LC-MS/MS, enzyme inhibiton, AChE, BChE, MAO-A, MAO-B, COX-1, COX-2

Acknowledgements: This study was supported by Anadolu University Scientific Research Project Commission under the grant number 1963.

ENHANCED ANTIOXIDANT POTENTIAL OF *BRASSICA OLERACEA* L. VAR. *ACEPHALA* (KALE) MICROSHOOTS THROUGH BIOFORTIFICATION WITH METAL NANOPARTICLES

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Brassica oleracea L. var. *acephala*, commonly known as green kale, is an increasingly popular leafy vegetable recognized for its potential as a superfood. It is an excellent source of essential vitamins, minerals, fiber, and bioactive phytochemicals that contribute to various health benefits. Among its key nutrients are vitamin K, vitamin C, B vitamins, potassium, calcium, and iron. Additionally, kale contains significant amounts of antioxidants, such as flavonoids and carotenoids, which help neutralize free radicals, support cardiovascular health, and exhibit anti-inflammatory properties. This study investigates the impact of biofortification with sub-5 nm metal nanoparticles (NPs) of gold (AuNPs), silver (AgNPs), copper (CuNPs), and platinum (PtNPs) on the antioxidant activity and total polyphenol content of kale microshoot extracts grown in an *in vitro* culture system. Microshoots were initiated from seeds and cultivated on Murashige-Skoog (MS) medium without plant growth regulators under continuous light. NPs were introduced into the liquid MS medium at concentrations of 0 (control), 5 mg L^{-1}, 10 mg L^{-1}, and 15 mg L^{-1}. The culture cycle lasted 14 days (three series, n=5). Methanolic extracts from the biomasses were analyzed for antioxidant activity using the CUPRAC and FRAP assays and for total polyphenol content *via* the Folin-Ciocalteu method. The presence of AuNPs at 10 and 15 mg L^{-1} significantly increased the total polyphenol content by 12.49% and 11.96%, respectively, compared to the control. Furthermore, AuNPs at 10 mg L^{-1} enhanced the antioxidant activity, as measured by CUPRAC, by 16.1% (from 42.8 to 49.7 nmol TE/mg d.w.), while the FRAP assay showed a smaller increase of 6.7%. These findings highlight the beneficial role of gold nanoparticle enrichment in enhancing polyphenol content and antioxidant potential in *in vitro* cultures of *B. oleracea* var. *acephala*.

Key Words: Nanoparticles, antioxidants, polyphenols, functional food, superfood

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ADAPTATION AND GROWTH DEVELOPMENT OF LAVENDER (LAVANDULA ANGUSTIFOLIA – SEVTAPOLIS) IN ŞANLIURFA CONDITIONS

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Since the 1970s, the cultivation of Lavandula spp. plants has been practiced in Türkiye. While not widely grown in the country, there has been an increasing demand for Lavandula angustifolia – SEVTAPOLIS in recent years. This study aimed to observe the adaptation and growth development of lavender varieties under local conditions. The study was conducted with lavender plants planted at a row spacing of 150 cm and an intra-row spacing of 40 cm between 2019 and 2021. The research was carried out in Şanlıurfa, as three repetative trial at the GAP Agricultural Research Institute Koruklu Talat Demirören Agricultural Research Station. During the experiment, all cultural practices such as fertilization, irrigation, weed control, spraying, and harvesting were applied. The harvest took place in May, during the flowering period. The parameters measured included plant height (cm), flowering branch count (number/plant), fresh herbage yield (kg/da), dry herbage yield (kg/da), essential oil yield (%), and essential oil components. The findings revealed that the lavender plants had an average plant height of 47.77–68.63 cm, a flowering branch count of 2.77–4.63 per plant, fresh herbage yield of 4.03–7.63 kg per plant, dry herbage yield of 1.81–3.16 g, an essential oil yield of 36.42–40.17%, and essential oil components ranging between 28.23–107.41 kg/da.

Key Words: Lavender, variety, yield, quality

THYMOQUINONE VALUES OF DIFFERENT BLACK CUMIN LINES

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Black cumin seed and its preparations have been widely used for many years, especially in food, spices, folk medicine, cosmetics and cultivation has been carried out accordingly. In the cultivation of black cumin, it is necessary to determine the active compounds in its structure as well as acquiring standard products in terms of yield and quality criteria required by the industry. When black cumin seeds are evaluated pharmacologically, it has been determined that the components contained in them show different therapeutic effects against various diseases. The active part of these compounds is thymoquinone and it has important antioxidant effects. Therefore, it has been determined that thymoquinone can show protective effects in heart, liver, kidney and stomach by reducing oxidative stress. Various researches are being carried out on black cumin and its components at different ecological conditions. Characterization and breeding researches examining the yield and quality criteria of black cumin seed in Tokat ecological conditions have been continuing for many years. In this paper, thymoquinone values of a total of 14 lines become prominent with breeding studies were analyzed. The experiment was established according to the Randomized Block Design with three replications and 30 cm row spacing and 1.5 kg/da seed was taken as basis. At the end of the research, thymoquinone values of 14 lines varied between 1.42-3.13% and the average was 2.43%. The highest thymoquinone proportion was obtained from the Tokat-59 line. This was followed by Tokat-61 line with 2.84. In addition to the need to evaluate the outstanding lines in terms of yield and quality criteria, they should also be standardized in terms of the active compounds in their structure. In addition, there is a need for various clinical and toxicological studies on thymoquinone.

Key Words: *Nigella sativa*, thymoquinone, quality

PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *BISCUTELLA LAEVIGATA* L. MICROSHOOT CULTURES AND PARENT PLANT EXTRACTS

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Biscutella laevigata L. (Brassicaceae) is a rare and protected in Poland plant species native to mountainous regions of Europe, ranging from the Iberian Peninsula to the Carpathians and the Balkans. Despite its ecological significance, its phytochemical composition and biological potential remain largely unexplored. This study aimed to characterize the phytochemical profile and antimicrobial activity of extracts derived from seeds, leaves, and *in vitro* microshoot cultures of *B. laevigata*. The parent plant material was sourced from the Botanical Garden in Kielce (Poland) in September 2021. Microshoot cultures were established on Murashige and Skoog (MS) medium supplemented with 1 mg/L 6-benzyladenine (BA) and 1 mg/L 1-naphthaleneacetic acid (NAA), cultivated under continuous LED lighting in 20-day cycles. Methanolic extracts were analyzed for glucosinolate content using UHPLC-DAD-MS/MS, and for polyphenolic compounds with HPLC-DAD. Total polyphenol content (TPC) was quantified by the Folin–Ciocalteu method. Antimicrobial activity was assessed against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Cutibacterium acnes*, and *Candida albicans*, using broth microdilution assays in accordance with EUCAST guidelines. Glucosinolate analysis revealed six compounds present exclusively in seed extracts, with glucohirsutin being the most abundant (15.06 mg/100 g DW). The highest TPC was also detected in seed extracts (2.11 mg GAE/mL), while leaf and microshoot extracts showed comparable TPC values (1.36 and 1.38 mg GAE/mL, respectively). Ferulic acid was the dominant phenolic acid in seed extracts, and rutin was the predominant flavonoid in leaf extracts (1609.21 mg/100 g DW). All extracts exhibited antimicrobial activity against tested bacterial and fungal strains. These findings highlight the significant phytochemical richness and comparable antimicrobial potential of both natural and *in vitro*-derived *B. laevigata* extracts, underscoring their potential for further pharmacological exploration.

Key Words: *Biscutella laevigata*, *in vitro* plant cultures, glucosinolates, polyphenols, antioxidant capacity, antimicrobial activity

LONG-CHAIN AND RARE AROMATIC GLUCOSINOLATES IN PLANTS OF *ARABIS* GENUS AND *PSEUDOTURRITIS TURRITA*

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The genus *Arabis* (Brassicaceae) comprises over 100 species distributed mainly in temperate and alpine regions. While not as extensively studied as other members of the Brassicaceae family, *Arabis* species are known to produce glucosinolates (GSLs), sulfur-containing secondary metabolites crucial for plant defense against herbivores and pathogens [1]. These compounds, upon enzymatic hydrolysis by myrosinase, generate bioactive products such as isothiocyanates, nitriles, and thiocyanates, which exhibit antimicrobial, antioxidant, and potential anticancer properties. The composition and concentration of GSLs in *Arabis* are influenced by genetic background, environmental conditions, and plant developmental stages.

In this study, GSLs in different plant parts from two *Arabis* species, *Arabis alpina* L. (alpine rockcress), *Arabis hirsuta* (L.) Scop. (hairy rockcress), as well as *Pseudoturritis turrita* (L.) Al-Shehbaz (syn. *Arabis turrita*, tower rockcress), were identified and quantified as their desulfo counterparts using LC-MS/MS. The detected compounds were classified into two major groups: methionine-derived aliphatic GSLs, and phenylalanine and/or tyrosine derived aromatic GSLs. Progoitrin (3.2–24.1 µmol/g DW) was the predominant GSL in *A. alpina*, alongside long-chain C7-C9 GSLs, with glucoarabin being the most abundant. In *P. turrita* seeds, long-chain C8-C10 aliphatic GSLs were also present, with the main being 9-(methylsulfonyl)nonyl GSL (60.4 µmol/g DW). In *A. hirsuta*, the dominant GSL was (*R*)-2-hydroxy-2-(4-hydroxyphenyl) ethyl GSL (8.6–57.2 µmol/g DW). These compounds are relatively uncommon within the GSL kingdom. Overall, the occurrence of such long-chain and rare aromatic GSLs suggests a potential evolutionary response to specific ecological pressures, contributing to enhanced chemical defenses against specialized herbivores. Their limited distribution within the Brassicaceae family may indicate lineage-specific selection or metabolic constraints. Furthermore, the presence of GSLs across multiple plant tissues underscores their role in stress adaptation and signaling processes. The distinct tissue-specific localization of these compounds provides further insights into their functional specialization and ecological significance in *Arabis* species.

Key Words: *Arabis* genus, glucosinolates, LC-MS/MS

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GLUCOSINOLATE PROFILING IN *BRASSICA* SPECIES: DIVERSITY, DISTRIBUTION, AND SIGNIFICANCE

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The *Brassica* genus (Brassicaceae) comprises a wide range of species with significant economic and nutritional value, largely attributed to their diverse glucosinolate (GSL) content [1]. These sulfur-rich secondary metabolites serve essential roles in plant defense mechanisms and are recognized for their potential health-promoting properties, primarily through their hydrolysis products, including isothiocyanates and nitriles. GSL profiles vary across *Brassica* species due to genetics, environment, and tissue-specific accumulation, drawing interest across biodiversity, biochemistry, biotechnology, and ethnobotany. This study presents a comprehensive analysis of GSLs in six *Brassica* species, identifying and quantifying their desulfo derivatives using LC-MS/MS across different plant tissues. The predominant GSLs were classified into aliphatic and indolic groups. Notably, *Brassica rapa* subsp. *chinensis* (var. white and purple pak choi) C3 and C4 aliphatic GSLs—progoitrin (0.87–1.67 µmol/g DW), gluconapin (up to 2.57 µmol/g DW), and gluconapoleiferin (up to 1.9 µmol/g DW)—were most abundant. Neoglucobrassicin was the main indolic GSL, especially in leaves (1.01–2.80 µmol/g DW). The seeds of *Brassica elongata* Ehrh. (elongated mustard) contained mainly progoitrin (1.60 µmol/g DW) and 4-hydroxyglucobrassicin (0.99 µmol/g DW), while *B. rapa* var. *niposinica* (mizuna) showed high levels of gluconapin (1.3 µmol/g DW) and glucobrassicanapin (2.78 µmol/g DW). Two Croatian endemics, *B. botteri* Vis and *B. cazzae* Ginzb. & Teyber, had remarkably high sinigrin and gluconapin (40.61 and 24.51 µmol/g DW) in *B. botteri* seeds, and significant progoitrin and gluconapin (28.45 and 30.63 µmol/g DW) in *B. cazzae*. The extensive diversity of GSLs among *Brassica* species highlights their pivotal role in plant defense strategies and their potential benefits in human nutrition. The presence of lineage-specific GSLs, particularly in seeds and flowers, indicates evolutionary adaptations for ecological interactions. A deeper understanding of their tissue-specific distribution and biological activity can pave the way for advancements in agricultural practices, functional food development, and biotechnological applications.

Key Words: *Brassica* genus, glucosinolates, LC-MS/MS

Acknowledgements: We are thankful for the scientific-research equipment financed by EU grant "Functional integration of the University of Split, PMF-ST, PFST and KTFST through the development of the scientific and research infrastructure" (KK.01.1.1.02.0018).

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IN VIVO EVALUATION OF THE ANTI-INFLAMMATORY EFFECT OF *SYZGIUM AROMATICUM* ESSENTIAL OIL

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Essential oils are widely studied for their therapeutic effects, and their anti-inflammatory activities are among the most explored properties. By reducing clinical signs such as edema and inhibiting inflammatory mediators at the cellular level, they can reduce arthritis or skin inflammation by modulating inflammatory mediators. Obtained by distillation of its flower buds, clove essential oil (*Syzygium aromaticum*) is widely used in aromatherapy due to its antiseptic, antibacterial, analgesic, antifungal, and antioxidant properties. It stimulates blood circulation and boosts the immune system. The objective of our study is an *in vivo* anti-inflammatory evaluation of several concentrations of clove essential oil by induction of topical inflammation on the ear of mice, followed by an estimation of the percentage of inhibition of edema, as well as a microscopic evaluation through histopathological examination. The results showed variability in the inhibitory effect of inflammation depending on the decrease in edema, vascular congestion, and the penetration of inflammatory elements, which varied with the different concentrations. Several *in vivo* studies on mouse models have shown that certain essential oils can have significant effects in reducing inflammation. Although these studies show promising results, the majority of research is preliminary and conducted on animal models. The effects in humans still require further investigation. Current research seeks to better understand the doses, specific mechanisms of action, and clinical efficacy of essential oils in the treatment of inflammation in humans.

Key Words: Essential oil, *Syzygium aromaticum*, *in vivo*, anti-inflammatory

EVALUATION OF BIOLOGICAL ACTIVITIES OF SOME *CITRUS* SP. ESSENTIAL OILS

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The genus *Citrus* of the Rutaceae includes approximately 17 species distributed in tropical regions. They are cultivated in the Mediterranean and Black Sea regions of Türkiye. *Citrus* sp., also known as “narenciye” in Türkiye, have significant commercial importance. The main species with such importance include citron (*C. medica* L.), lemon (*C. limon* L. Burm. f.), bitter orange or sour orange (*C. aurantium* L.), sweet orange (*C. sinensis* (L.) Osbeck), Mediterranean and Satsuma mandarins (*C. deliciosa* Ten. and *C. unshiu* Marc.), pomelo or shaddock (*C. grandis* (L.) Osbeck), grapefruit (*C. paradisi* McFad.), and lime (*C. aurantifolia* (Christm.) Swingle). *Citrus* sp., which have a significant share in the food industry, are also widely used in the cosmetic and perfumery industries. In addition, due to their antiseptic, anti-inflammatory effects, as well as positive effects on the immune system and central nervous system, they are used in the pharmaceutical industry and in aromatherapy applications. In this study, the chemical compositions of the essential oils of commercial *Citrus reticulata* Blanco, *C. sinensis* L. Osbeck, *C. limon* L., and *C. paradisi* Macfadyen obtained by cold pressing were primarily examined using gas chromatography (GC) and gas chromatography-mass spectrometry methods (GC-MS). Limonene (%71.8-96.6) was found as the main compound in all essential oils. Myrcene (%1.7-2.0), α-pinene (%0.5-1.7), sabinene (%0.2-1.7), β-phellandrene (%0.3), and linalool (%0.1-0.3) were detected as common major compounds in all the oils evaluated. *In vitro* antimicrobial activity of the essential oils was tested against Gram (+) and Gram (-) bacterial strains (*Streptococcus mutans* ATCC 25175, *Streptococcus pneumonia* ATCC 700699, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* NRRL B-3008, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* NRRL B-4378) and *Candida* sp. (*Candida albicans* ATCC 90028 and *C. krusei* ATCC 6258) pathogenic strains. Ciprofloxacin was chosen as the positive control for bacteria, and nystatin for fungi. Minimum inhibitory concentrations ranged from 20 to 0.62 mg/mL. The essential oils were combined with various antibiotics (ciprofloxacin, amphotericin B, chloramphenicol, ketoconazole, and nystatin), and their synergistic activities were evaluated using the Checkerboard method. 5 synergistic activities, 20 additive effects, and 22 independent effects were observed.

Key Words: Rutaceae, *Citrus* sp., volatiles, antimicrobial activity, synergistic activity

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LEMON MYRTLE (*BACKHOUSIA CITRIODORA*) — DELECTABLE AUSTRALIAN TRADITIONAL HERB RICH IN HEALTH-PROMOTING COMPOUNDS

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Lemon myrtle (*Backhousia citriodora*) is an evergreen tree native to subtropical rainforests of Queensland and northern New South Wales, Australia. The medium-height tree grows broad and glossy lanceolate leaves [1]. It belongs to the family *Myrtaceae* [1] to which many well-known native Australian plants including various eucalyptus trees, tea tree (*Melaleuca alternifoli*), lemon-scented tea tree (*Leptospermum citratum*) and anise myrtle (*Syzygium anisatum*) belong. Lemon myrtle is characterized by a strong citrus scent owning to high concentration of acyclic monoterpene citral in its leaves. The citral content of the essential oil frequently exceeds 90%, the highest among known citrus-scented plants [2]. While being caffeine-free, the leaf is also rich in polyphenols, including a range of flavonoids such as catechin, quercetin, myricetin and kaempferol, as well as phenolic acids, such as gallic acid and chlorogenic acid, and tannins. Studies have shown that the antioxidant properties of lemon myrtle tea were the highest among 27 herbal teas examined and comparable to several *Camellia sinensis* teas [3]. The polyphenol content of the tea extract was among the highest of 44 herbs analyzed [4]. Many of the key compounds in lemon myrtle are shown to offer various health effects. Citral is known to be strongly antimicrobial [5] but also exerts antinociceptive and potential anxiolytic effects by interacting with pain-sensing ion channels [6]. Many of the flavonoids in lemon myrtle are implicated in controlling body weight [7] and lifestyle diseases such as diabetes and non-alcoholic fatty liver disease [7–9]. Citral and flavonoids are also implicated in conditioning the gut microbiome [10,11]. Lemon myrtle extract was found to inhibit senescence of epidermal stem cells [12]. Casuarinin, a lemon myrtle-derived ellagitannin, was shown to enhance muscle growth in elderlies by activating muscle stem cells [13]. Lemon myrtle is not just a citrus herb with exquisite scent and flavor but also a botanical with many promising health and functional benefits.

Key Words: Lemon myrtle, Australian native, natural ingredients, citral, polyphenols, health benefits

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EXPLORING THE BIOLOGICAL ACTIVITIES OF *SIDERITIS ARGYREA* P.H.DAVIS

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Sideritis argyrea P.H.Davis (Boz eşekçayı) is a species belonging to the family *Lamiaceae* and is generally endemic to the Mediterranean region. They are generally distributed in rocky, mountainous and arid regions. Silver-gray leaves are characteristic of the genus *Sideritis*. It is traditionally preferred for colds, digestive problems and to support the immune system. It has important biological properties such as antimicrobial and antioxidant thanks to its phenolic compounds. This study aims to evaluate the biological activities of 70% ethanol and ethyl acetate extracts obtained from *S. argyrea*, focusing on their antioxidant and antimicrobial properties, total phenolic content (TPC). The TPC of both extracts was assessed, revealing comparable results. The ethyl acetate extract exhibited a TPC of 50.1 ± 0.03 mg GAE/g, while the 70% ethanol extract demonstrated a slightly higher TPC of 50.4 ± 0.026 mg GAE/g. Antioxidant activity was evaluated through the DPPH radical scavenging assay at concentrations of 0.125 mg/mL for the ethanol extract and 0.15 mg/mL for the ethyl acetate extract. At these concentrations, the 70% ethanol extract exhibited an inhibition of 80.09%, while the ethyl acetate extract showed an inhibition of 75.69%. Microdilution method was used to analyze the antimicrobial property and MIC value of the extracts against standard microorganisms was determined. According to the antimicrobial results, ethyl acetate extracts were effective than 70% ethanol extracts at concentrations of 51, 206 and 412 µg/mL, while ethanol extracts were effective at concentrations ranging from 825-1650 µg/mL against *Bacillus subtilis* NRRL B478, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 90028 and *Candida krusei* ATCC 6258 cultures.

Key Words: *Sideritis argyrea, Lamiaceae,* total phenolic content, antioxidant, antimicrobial

α-GLUCOSIDASE AND LIPASE INHIBITORY ACTIVITY OF SOUR CHERRY FRUIT

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Prunus cerasus L. (sour cherry) is widely utilized in the production of various processed food products, including fruit juice, jam, marmalade, and sauces, and is therefore regarded as an industrially significant fruit. The objective of the present study was to evaluate the in vitro inhibitory effects of sour cherry fruit extracts on α-glucosidase and pancreatic lipase enzymes. For this purpose, an 80% ethanol extract was prepared from fruits harvested in the Çubuk district of Ankara, followed by successive fractionation using *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. The resulting crude extract and its corresponding solvent fractions were subjected to enzyme inhibition assays. Notably, both the 80% ethanol extract and the ethyl acetate fraction demonstrated significant inhibitory activity against α-glucosidase, with IC_{50} values of 235.3 ± 0.8 µg/mL and 110.8 ± 1.1 µg/mL, respectively, and against pancreatic lipase, with IC_{50} values of 78.1 ± 1.5 µg/mL and 56.7 ± 0.5 µg/mL, respectively. These findings suggest that *P. cerasus* exhibits potential as a source of bioactive compounds with dual enzyme inhibitory activity. Further investigations, including bioactivity-guided fractionation, molecular docking studies, and in vivo evaluations, are warranted to elucidate the active constituents and their mechanisms of action.

Key Words: *Prunus cerasus*, glucosidase, lipase, sour cherry

COMPARATIVE PHYTOCHEMICAL ANALYSIS OF DIFFERENT PARTS OF *ARUM RUPICOLA* VAR. *RUPICOLA* BOISS.

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Arum rupicola var. *rupicola* Boiss., is a member of the Araceae family, a perennial rhizomatous species found in the flora of Turkey. It grows across rocky slopes, maquis, and open forest margins in South-Eastern Anatolia and extends to regions including the Eastern Mediterranean, Iran, Iraq, and the Caucasus. Commonly referred to as "yılan yastığı" by local folk, the plant has been traditionally used both as a food source and in folk medicine. In this study, three different extracts were obtained from fruits, aerial parts and rhizomes of *A. rupicola* var. *rupicola* by ultrasonic extraction method. The extracts were analysed and compared using GC-MS and HPLC techniques. GC-MS results show that the fruit and rhizome extracts contained higher levels of volatile compounds, while the aerial parts were rich in long-chain aliphatic alcohols. Specific compounds identified included 6.63% isoamyl laurate (fruit), 1.44% phytane, 1.17% phytol, 1.02% squalene, 0.93% benzofulvene (rhizome), and 0.49% phytane (aerial parts), all of which are known for their bioactive potential. HPLC analysis of phenolic compound profiles revealed notable differences among the plant parts. The fruit extract showed high levels of coumaric acid (34.03%), syringic acid (12.67%), and rutin (10.92%). The aerial part was especially rich in coumaric acid (63.13%) and rutin (30.49%), while the rhizome was characterised by chlorogenic acid (26.11%) and gallic acid (14.71%). These phytochemical differences may reflect part-specific biochemical responses to environmental factors. The results show that *A. rupicola* var. *rupicola* is a chemically rich species with notable phenolic content, supporting its pharmacological potential.

Keywords: *Arum rupicola* var. *rupicola*, GC-MS, HPLC, secondary metabolites

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EVALUATING THE EFFECT OF *NIGELLA* SATIVA IN INFLAMMATION: TRADITIONAL APPLICATIONS, EXPERIMENTAL STUDIES, AND CLINICAL PERSPECTIVES IN THE GASTROINTESTINAL INFLAMMATION

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Herbal products are widely used for their recognized health benefits in treating diseases. Among these, *Nigella sativa* seeds, commonly referred to "black seed," are members of the Ranunculaceae family and are indigenous to regions such as the European and Southwest Asia. These seeds are cultivated across various countries, including Egypt, Iran, Greece, Syria. The Nigella L. genus is represented by 12 species in Türkiye. The seeds contain an array of pharmacologically active compounds, with "*Thymoquinone*" being of particular interest. Numerous studies have demonstrated that its antiviral, anti-inflammatory, antihypertensive, and anticancer properties. While there is an increasing body of research investigating its potential effects on gastrointestinal health, some studies have reported mild adverse effects, such as stomache, diarrhea, and nausea, as well as more serious adverse events, including weight loss. Some animal studies have shown that treating DSS-induced mice with various doses of Thymoquinone (TQ) improves colitis damage, suggesting its potential therapeutic application in colonic inflammation management. Research indicates that the anti-inflammatory effects of *Nigella sativa* are attained through several key mechanisms, such as the modulation of inflammatory pathways and the regulation of cytokine production. It has been noted, it might reduce the expression of molecules associated with inflammatory responses, especially TNF-α, interleukins, and others, through the inhibition of NF-κB, which plays a role in regulating the response to inflammation. Furthermore, *Nigella sativa* may inhibit another signaling pathway, the MAPK pathway, which could potentially decrease the production of pro-inflammatory cytokines and mitigate the inflammatory process. Even though the primary therapeutic strategy cannot replace advanced treatments, it relies on its anti-inflammatory and antioxidant properties, providing at least complementary option to conventional therapies. Further research is needed to clarify the effects of *Nigella sativa*, particularly Thymoquinone, on intestinal inflammation and other conditions, to explore the underlying mechanisms, and to assess its overall impact.

Key Words: *Nigella sativa*, Thymoquinone, intestinal inflammation

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NEW ETHNOBOTANICAL DATA ON ALGERIAN MEDICINAL FLORA AND TAXONOMIC REMARKS.

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Abstract: Medicinal plants have been widely used by the local population for millennia in North Africa; this practice, which is part of popular heritage, remains underexploited in pharmacology. Therefore, this work is based on an analysis of ethnobotanical surveys conducted between 2018 and 2024 in Algeria with the objective of determining the plants used in traditional herbalism mostly in the context of cancerous diseases. The inventory and characterization of more than fifty species was performed. Remarks, recommendations and statistical data have been carried out around the problem of toxicity caused by the lack of knowledge, or approximate control of their bioactive effects, by users. In addition, a taxonomic determination and nomenclatural update are conducted for the sampled plants, as part of our project to renew the Algerian medicinal flora.

Key Words: Medicinal plants, Algeria, taxonomy, evolution, ethnobotany.

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FULL PAPERS

MORPHOLOGICAL, MICROMORPHOLOGICAL AND KARYOMORPHOLOGICAL FEATURES OF MEDICINAL *SILYBUM MARIANUM* (ASTERACEAE) IN TÜRKIYE

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Abstract

Silybum marianum is a well-known medicinal plant traditionally used for its hepatoprotective properties. It has been employed in the treatment of various liver conditions, including those involving functional impairment or degenerative necrosis. The plant's hepatoprotective effects are multifaceted, acting through antioxidant and anti-inflammatory mechanisms, regulating cell permeability, stabilizing membranes, promoting liver regeneration, and inhibiting collagen deposition, which could potentially lead to cirrhosis. While much of the research on *S. marianum* has focused on liver diseases, recent studies have also highlighted its potential benefits for a range of other conditions. These include renal protection, hypolipidemic and anti-atherosclerotic effects, cardiovascular health, prevention of insulin resistance (especially in cirrhosis patients), and potential roles in cancer and Alzheimer's disease prevention. Additionally, *S. marianum* is also utilized as a food remedy. After being collected in 2023 from the Gaziantep province of Türkiye, *S. marianum* was studied in detail in terms of its morphological, karyomorphological, and micromorphological characteristics. This study presents the first detailed report on various characteristics of *S. marianum*, including stem diameter, measurements of upper stem leaves, bract series, shapes and dimensions of phyllaries, and floret traits. Additionally, the chromosome number was determined to be 2n=34, and the seed surface pattern was identified as reticulate.

Key Words: chromosome, Compositae, milk thistle, plant

1. Introduction

Silybum marianum L. Gaertn (milk thistle), belonging to the Asteraceae family, has been used for nearly 2000 years in the treatment of liver and gallbladder diseases, toxin and fungal poisoning, snake bites, and insect stings. Its seeds contain a high amount of silymarin. Chemically, silymarin is composed of isomers including silibinin, isosilibinin, silicristin, silidianin, and dehydrosilibinin (Ding et al., 2001). In this way, silymarin has a complex structure formed from a mixture of multiple flavonolignan isomers. This compound has low solubility and oral bioavailability, which is why nanoemulsion formulations have been developed to enhance its therapeutic activity (Parveen et al., 2011). Silibinin, the main component of silymarin, makes up approximately 60% of it (Demartini and Esposti, 2002).Since silymarin is not water-soluble, it cannot be consumed as an herbal tea. A standard capsule plant extract is used instead. Silymarin possesses estrogenic activity, regulates drug-carrying glycoproteins, and affects DNA expression by inhibiting nuclear factor kappa B (NF-κB) (Kren and Walterova, 2005).

Silymarin inhibits the carcinogenic effects of certain chemicals. In human prostate carcinoma, silymarin has been observed to suppress mitogenic signaling pathways and cellular cycle regulators. Additionally, silymarin inhibits tumors dependent on steroid hormones and shows anti-angiogenic effects (Kren and Walterova, 2005; Zi and Agarwal, 1999; Kang et al., 2004). The main mechanisms of silymarin's hepatoprotective effects include antioxidant action, prevention of lipid peroxidation, strong detoxification, and protection against GSH depletion. Studies have also shown that it inhibits lipoxygenase, prevents leukotriene formation in the liver, enhances protein synthesis in hepatocytes, reduces the activity of tumor precursors, stabilizes mast cells, and modulates immune functions (Miguez et al., 1994).

Due to its antioxidant properties, silymarin has been considered beneficial in the treatment of certain neurodegenerative diseases (Nencini et al., 2007). In hyperlipidemic mice, plasma cholesterol and LDL (Low-Density Lipoprotein) levels were reduced with the effect of silymarin (Skottova and Kreeman, 1998). The powerful antioxidant property of silymarin has been proven in viral hepatitis, toxic hepatitis, fatty liver disease, cirrhosis, ischemic damage, and radiation toxicity (Fraschini et al., 2002). Silymarin is also known to stimulate the production of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase (Dvorák et al., 2003). Silymarin has protective effects against apoptotic cell death caused by tumor necrosis factor-alpha (TNF-α) as well (Srivastava et al., 1994). The ultrastructural patterns of seed coats, as observed through scanning electron microscopy (SEM), have been recognized as a valuable method for assessing phylogenetic relationships and addressing taxonomic challenges (Heywood, 1971; Bouman, 1975; Koul et al., 2000). Chromosome number, size, and asymmetry are key traits that contribute to understanding species phylogenetic relationships (Eroğlu et al., 2013). Karyological studies have proven valuable for the systematic classification of various Asteraceae genera, demonstrating connections between karyological, morphological, and molecular characteristics (Hellwig, 2004).

This study aims to elucidate the karyomorphological, micro- and macromorphological characteristics of *S. marianum*.

2. Material and methods

The locality of *S. marianum* is as follows: Gaziantep: Şahinbey, park edge, 675 m, 20 v 2023, 07 vi 2023. The morphology of the *S. marianum* has been thoroughly examined, with detailed measurements taken of the stem, leaves, involucre, appendages, achenes, and pappus. Floral and involucral traits were sampled from the terminal capitula. Measurements were conducted on dried herbarium specimens, with size data based on capitula that were not distorted by pressing. The appendage margin was included in measurements, though the involucral bracts were excluded. For chromosomal analysis, mature achenes were selected and germinated at regular intervals. Chromosome counts were performed at somatic metaphase using the squash technique, with primary root meristems providing the metaphase plates. Samples were pretreated with 0.002 M 8-hydroxyquinoline for 8 hours at 4 °C, followed by fixation in Carnoy's solution for 24 hours at 4 °C. The material was hydrolyzed in 5 N HCl for 30 minutes at room temperature and stained with 1% aceto-orcein. Permanent slides were prepared following the method of Bowen (1956). For each taxon, a minimum of 10 metaphase stages were analyzed, and the best metaphase images were captured at 100× magnification using an Olympus DP-72 digital camera mounted on an Olympus BX53 microscope.

For SEM analysis, at least ten achenes per species were dehydrated through a graded alcohol series (70%, 80%, 96%, and 100%) to ensure cleanliness. The achenes were then gold-coated in high-vacuum mode using a ZEISS EVO LS-10 SEM and examined at magnifications of 30X, 1000X, and 2000X to observe surface details. Achene characteristics were described according to the terminology established by Stearn (1992) and Koul et al. (2000).

3. Results and discussion

Plants 10–110 cm. Stem erect, sparcely villous, usually branched from base, 7–9 mm diameter at base. Leaves heteromorphic, glabrous; basal leaves obovate, petiolate, strongly spiny–dentate, 8–22 × 5–13 mm; upper stem leaves simple ovate–lanceolate, auriculate–amplexicaul, 9.5–22 × 4–18 mm. Involucre ovoid–subglobose, 2.4–4.2 × 2–4.1 mm (excl. spreading part of phyllaries). Bracts usually with 3–4 series. Phyllaries with adpressed spiny ovate base widening abruptly to ovate–subulate appendage, outer phyllaries ovate-triangular, 15–17 × 10–12 mm; median phyllaries ovate-oblong and obtuse, 19–21 × 8–10 mm; inner phyllaries linear-lanceolate, 23–25 × 4–6 mm. Florets purple, marginal, 29–31 mm, without staminodes, with 5 linear-filiform, acute lobes 9–11 mm; central florets 27–29 mm, with 5 lobes 8–10 mm; anther tubes violet. Achene obovoid, brownish streaked with black, glabrous, ornamentation reticulate, boundaries straight, thick, grooved, cell shape elongated, centres raised,

Figure 1. Flower of *S. marianum*

grooved, length 5.549-6.445 mm, width 2.696-3.036 mm, perimeter 15.417-16.492 mm, surface area 13.048-15.875 mm^2. Pappus barbellate, whitish, 10–15 mm (Figures 1–2).

Karyological data and asymmetry values are as follows: 2n=34, x=17, PL=2x, HCL=16.79, TF%=45.15, AsK%=54.85, S%=51.35, KF=17m, AI=1.07, A_1=0.17, A_2=0.21, X_{ca}=9.45, X_{ci}=0.45, CV_{cl}=21.19, CV_{ci}=5.07, Stebbins=4A (Figure 3).

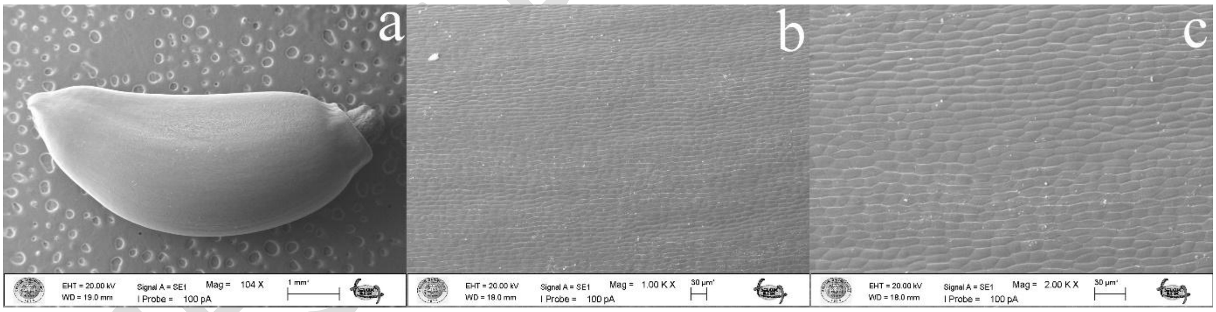

Figure 2. Achene mikrographs of *S. marianum*. General view (a), 1000× magnification (b), 2000× magnification (c)

Kupicha (1975) studied the morphological characteristics of the *Silybum marianum*. With this study, stem diameter, upper stem leaves measurements, bract series, phyllaries measurements and shapes, florets characters of *S. marianum* were reported for the first time. Ozcan and Demir (2021) stated the achene and pappus characteristics of the *S. marianum* species as follows: Achene (as cypsela) 79.5–180.4 × 2.69–3.34 mm, ornamentation (as cellular pattern) scalariform, pappus 14.5–22.33. In this study, the same characters were interpreted as follows: Achene 5.549-6.445 × 2.696-3.036, ornamentation reticulate, pappus 10–15 mm. Also, achene perimeter and surface area were studied for the first time for *S. marianum*.

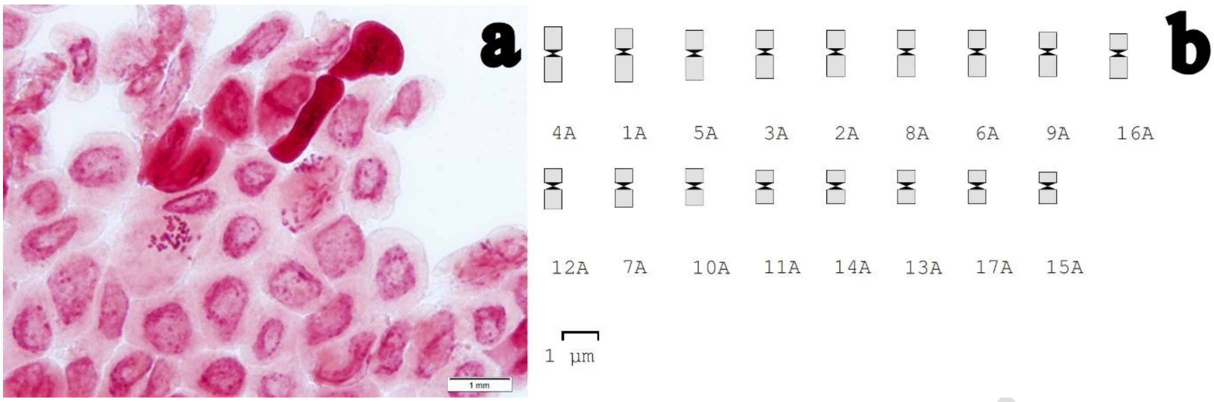

Figure 3. Metaphase (a) and idiogram (b) of *S. marianum*

The chromosome number of *S. marianum* was counted as 2n=34, which is consistent with previous studies (Asghari-Zakaria et al., 2008; Abrahampour et al., 2012). Asghari-Zakaria et al. (2008) investigated the karyomorphology of different populations of *S. marianum* and found S as 3.45-4.34% and TF as 33.87-38.36. In this study, S was found as 51.35% and TF as 45.15. Abrahampour et al. (2012) found the A_1 value to be 0.36-0.41 and the A_2 value to be 0.25-0.31 in their study examining *S. marianum* populations in Iranian regions. In this study, the A_1 value was found to be 0.17 and the A_2 value was found to be 0.21.

4. Conclusion

In conclusion, the morphological, micromorphological, and karyomorphological characteristics of *S. marianum*, a medicinally significant plant, have been thoroughly examined and documented.

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MEDICINAL AND AROMATIC PLANTS AND TERRITORIAL DEVELOPMENT MODELS

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Abstract

In Basilicata, the cultivation of Medicinal and Aromatic Plants (MAPs) remains a niche sector, although in certain areas, structured production systems have developed within micro-supply chains. Despite the presence of successful examples of cooperation among enterprises in the sector, the local MAP supply chain remains poorly structured and highly fragmented (De Luca, Ricciardi, 2022). The main challenges identified include the dispersion of the production sector, the limited capacity for aggregation among small enterprises, and difficulties in implementing integrated and efficient organizational models. Within this context, the transnational cooperation project ME.PLA.SU.S. (MEdicinal PLAnts in a SUstainable Supply chain. Experience of land-use practices) has been launched, promoted by the Council for Agricultural Research and Economics – Research Center for Policies and Bioeconomy (CREA-PB). The aim of this study is to present how the project has analyzed, within specific work packages, the sector's dynamics in relation to aggregation processes, highlighting how cooperative models and integrated strategies can contribute to improving the overall production framework, strengthening economies of scale, and enabling the adoption of more sustainable and innovative organizational models (D'Oronzio, Costantini, 2021). The research activity on the MAP sector in Basilicata has revealed, from the early stages, a significant fragmentation of data, making it necessary to adopt an integrated methodological approach. This approach combined quantitative and qualitative analyses, structuring the investigation process into multiple phases to ensure a multidimensional assessment of the sector. Specifically, an initial desk analysis of the sector was conducted, followed by the administration of a structured questionnaire to a representative sample of enterprises. Subsequently, three in-depth Focus Groups were carried out, allowing for the identification of key critical issues, business needs, and barriers to the establishment of collaborative networks. The results obtained contributed to the identification of a **development model** aimed at strengthening supply chain dynamics in line with the needs of local producers.

In the productive context of MAPs in Basilicata - characterized by the coexistence of well-established relationships and areas where connections between operators remain weak - the **Network Contract** could represent a strategic solution to enhance competitiveness, foster innovation, and promote the integration of local resources. The creation of a structured network could facilitate access to new development opportunities, expand international market outlets, promote know-how exchange, and optimize the shared use of equipment and infrastructure. Moreover, a network-based organization would ease access to funding provided by regional and national Rural Development Programs, thereby enhancing the sector's sustainability and competitiveness.

Key Words: Sustainable supply chain, competitiveness, medicinal and aromatic plants.

1. Introduction

The evolution of consumer lifestyles has sparked renewed interest in the valorization of traditional knowledge, ancestral practices, and flavors, including those associated with Medicinal and Aromatic Plants (MAPs). This trend is also evident in Basilicata, a region in southern Italy characterized by rich plant biodiversity, encompassing over 400 native medicinal plant species, both cultivated and wild (Sansanelli et al., 2017). MAPs comprise a vast and diverse range of species, each distinguished by unique botanical traits, biological characteristics, habitat preferences, and practical applications. A key commonality among these plants is the presence of bioactive compounds, which, despite their diversity, exhibit significant efficacy across multiple fields of application (Primavera, 2021). These compounds can be utilized directly or undergo processing and extraction. According to Assoerbe, 45% of MAPs produced in Italy are destined for pharmaceutical use, 40% for food applications, and 15% for the cosmetics industry (Martinasso, 2022).

In Italy, the MAP sector demonstrates significant dynamism and increasing entrepreneurial activity, though it remains a niche industry compared to both large-scale crops and so-called minor crops. In Basilicata, the MAP sector has followed the national growth trend, with steady development over the years. In various areas of the region, MAPs constitute an important productive resource, with established micro-supply chains that have been active for over a decade (Tab.1). These supply chains cater to an increasingly demanding market, which continuously seeks products with high nutritional standards and medicinal properties (Sica et al., 2021; D'Oronzio et al., 2023).

Table 1. Lucanian's micro supply chains of MPs

PRODUCER	NOTE	PROCESSING COMPANY	PRODUCT
Sud-Officinale Coop. in Irsina (MT)	Over 16 hectares of biological agriculture	**Bioplanta Company**	ESSENZIAL OILS
F.L.E.O. partnership (n. 53 partners, 3 public)	Produces 17 MPs for "Amaro Lucano"	**Amaro Lucano** in Pisticci (MT)	LIQUOR
	Plants for small and medium distribution	**SpeSì (brand)**	SEASONING PLANTS
Lucana Officinali Society Cooperative in Lauria (PZ)	Over 70 hectares	**EVRA Italia srl** in Lauria (PZ)	INTEGRATORI
Lucanian Regional Association of MPs and Saffron Producers in Chiaromonte (PZ)	N. 40 producers		SAFFRON
ORTI LUCANI (O.L.P.O.) Piante officinali	Business network		

Source: CREA PB

The entrepreneurial fabric of Basilicata related to the cultivation and primary processing of medicinal plants began to take shape in the 1970s, driven by the initiative of young agricultural entrepreneurs who organized themselves into associations. Over time, some of these associations have taken on a central role in the development of local certified medicinal plant value chains. Among the main reference players are the Lucana Officinali micro-supply chain, led by the secondary processing company EVRA (Extracts of Vegetal Research and Applications), and the Filiera Lucana Erbe Officinali (F.L.E.O.), coordinated by Lucano 1894 S.R.L. These stakeholders play a strategic role in enhancing the sector by promoting innovation in production processes and strengthening integrated supply chain models. In particular, EVRA is a specialized processing company, recognized for the production of high-quality botanical extracts, partly derived from cultivated or wild species within the Pollino National Park area. In 2010, with support from the Lucanian Agency for Agricultural Development and Innovation (ALSIA), EVRA launched the pilot project *"Italian Micro Supply Chain of Medicinal Plants"*, involving around twenty organic farms operating within the Pollino National Park.

Another key player on the international scene in the medicinal plant sector is Lucano 1894 S.R.L., producer of *Amaro Lucano*, a bittersweet herbal liqueur characterized by citrus and floral notes. Today, it stands as one of the flagship products of Made in Italy, officially recognized in 2021 as a "Historic Brand of National Interest." In recent years, a significant contribution to stakeholder aggregation within the sector has come from the Rural Development Programs (RDPs) of the Basilicata Region, which have activated innovative processes aimed at enhancing the competitiveness and sustainability of agri-food value chains, increasing production volumes, and placing greater emphasis on the processing and marketing of Lucanian agri-food products, in order to improve their valorization and strengthen their bargaining power. In addition to traditional value chains, attention has also been directed toward so-called "minor supply chains", which can equally contribute to the socio-economic development of specific areas within the region. Despite the presence of good practices and virtuous examples of cooperation among some primary producers, the local medicinal plant supply chain still faces several structural challenges.

The most critical issues include a high level of fragmentation within the sector, a limited capacity for aggregation among small enterprises, and the difficulty in implementing integrated organizational models. These factors hinder the optimization of economies of scale, the standardization of product quality, and access to broader markets, thereby limiting the sector's competitive potential. To support the strengthening of the supply chain, the transnational cooperation project MEdicinal PLAnts in a SUstainable Supply chain – ME.PLA.SU.S. has been launched. The project is promoted by the Council for Agricultural Research and Economics – Research Centre for Agricultural Policies and Bioeconomy (CREA-PB). It involves several Italian research institutions (CREA-PB, DiS-UNIBAS, ALSIA) and international partners such as the Hellenic Agricultural Organisation DEMETER – Research and Experimental Centre of Athens, and the Faculty of Agriculture at the University of Belgrade, along with numerous stakeholders from the sector. One of the key objectives of the project is to strengthen the medicinal plant supply chain in Mediterranean areas by fostering cooperative processes among agricultural producers, processing companies, research institutions, and advisory services. The initiative has established a structured network that brings together the expertise and knowledge of all actors involved in various project activities, organized into work packages. Through this collaborative framework, the project aims to define best practices and operational guidelines to promote new aggregation processes within the sector, enhance the technological innovations used in production, and maximize the sustainability of the supply chain through the recovery and valorization of by-products derived from the processing of medicinal plants.

The goal of the present work is to illustrate how the project has analyzed the sector's dynamics in relation to aggregation processes, highlighting how cooperative models and integrated strategies can improve the overall production framework, reinforce economies of scale, and enable the adoption of more sustainable and innovative organizational models (D'Oronzio, Costantini, 2021).

2. Material and Methods

The analysis of the medicinal plant sector in the Basilicata region revealed, from the very beginning, a significant fragmentation of available data, which made it necessary to adopt an integrated approach. This approach combined both quantitative and qualitative analyses, structuring the research process into multiple phases in order to ensure a multidimensional assessment of the sector.

- The first phase involved a desk analysis of the sector, aimed at identifying the cultivated species and mapping the farms engaged in the cultivation of medicinal plants (MP) across the regional territory. This activity was carried out through the consultation of official sources such as the Chamber of Commerce, Industry, Agriculture and Crafts (C.C.I.A.A.), the National Agricultural Information System (SIAN), the Farm Accountancy Data Network (RICA), the Basilicata Region, and the Lucanian Agency for Agricultural Development and Innovation (ALSIA), as well as through direct contact with local stakeholders.
  The integration of this information enabled the development of a detailed and structured database on the businesses operating in the sector and their main production characteristics (Verrascina et al., 2023).

- The second phase involved two different types of qualitative investigations as part of the "Filiera in Tour" initiative. This activity was structured into two key components:
  On one hand, a series of local stakeholder meetings were held to administer a structured questionnaire to agricultural entrepreneurs.

On the other, dedicated focus groups were organized to explore, through a participatory approach, key issues that emerged from the questionnaire analysis.

The direct engagement with operators represented a crucial element in understanding the production dynamics, identifying critical issues and recognizing opportunities within the sector. This participatory process provided valuable insights for the formulation of future value chain development strategies.

From the initial database, a sample of 12 farms was selected based on specific criteria designed to ensure an adequate representation of the regional production landscape. The selection criteria included: **type of cultivation, geographic distribution, level of integration across different stages of the production process, and the presence of women and young farmers**.

The interviews focused on the following key aspects:

- **Production processes**: covering all stages from cultivation, harvesting, and primary processing (such as drying, essential oil extraction, etc.) to packaging and marketing of the final products.
- **Target markets**: identification of the main sales channels and marketing strategies adopted by the farms.
- **Type of production**: classification of the various product categories, including dried products, processed goods such as essential oils, and by-products obtained through bioeconomy approaches (e.g., valorization of processing residues).
- **Multifunctionality**: identification of complementary activities that add value to the farming business, such as experiential tourism or the use of medicinal plants in cosmetic and therapeutic applications.
- **Business relationships and value chain dynamics**: analysis of existing collaborations and networks among enterprises in terms of production, commercial partnerships, and socio-cultural linkages.

To better understand value chain dynamics, three Focus Groups were organized involving primary producers and processing farms, institutional representatives (Regional Government), and stakeholders from the Lucanian agri-food sector, including producer organizations, the Agri-Food District, Local Action Groups (GALs), and the Regional Federation of Chefs.

- The first Focus Group aimed to identify the structural weaknesses of the sector and to define the needs of the stakeholders.
- The second Focus Group focused on identifying the main barriers to the establishment of networks and local value chains.
- The third Focus Group concentrated on the analysis of organizational models capable of addressing the specific needs of Lucanian producers, assessing their effectiveness in relation to the territorial context and market dynamics.

The outcomes of the desk analysis, interviews, and participatory activities enabled the identification of strategies and actionable measures to support the development of a regional medicinal plant value chain in Basilicata, grounded in principles of sustainability and innovation.

3. Results and Discussion

The sources consulted during the **desk analysis** outlined a growing sector, consisting of approximately 100 farms operating over a total area of about 360 hectares, with an average farm size of 5.8 hectares.The farms involved in the study cultivate a diverse range of medicinal and aromatic plant species found in the Basilicata region, ranging from widely grown crops such as coriander and fennel, to more niche species like thyme, rosemary, lavender, and lemon balm (Melissa officinalis).

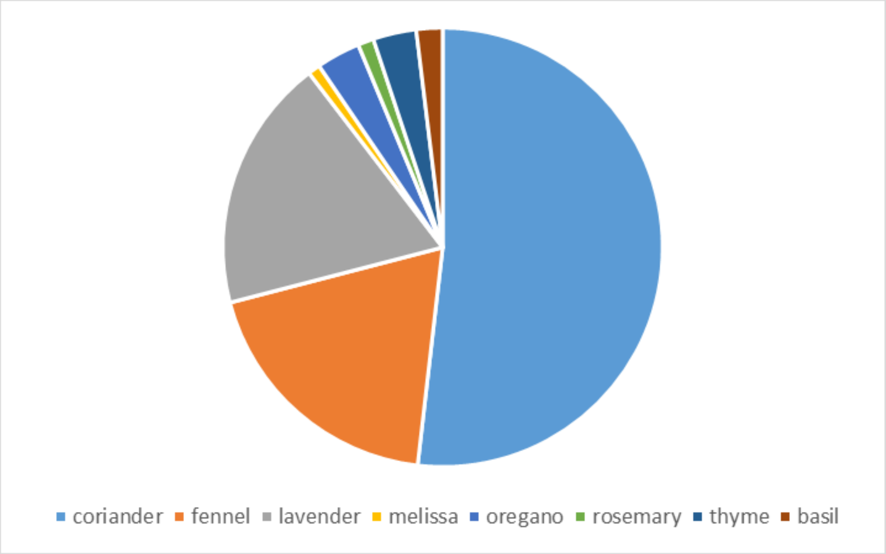

Figure 1. Main crops cultivated by Lucanian farms Source: CREA PB

The analysis of the Lucanian production sector revealed a partially consolidated organizational structure, with a predominance of small-scale, family-run farms. These characteristics were confirmed during the **second phase of the investigation**, which was based on direct interviews. The sample analyzed - composed primarily of farms led by young people and women - shows the following characteristics: 57% of farm managers are between 30 and 45 years old; 36% of the farms are managed by women; 64% of respondents hold a university degree, making it the most common level of education within the sample. The interviews also revealed that:

- Most of the farms carry out post-harvest processing of medicinal and aromatic plants. Only a small portion of the product is marketed fresh and in bulk, mainly referring to culinary herbs such as basil, rosemary, thyme, etc.
- After harvest, the main operations include selection, cleaning, and preparation for drying. In the case of certain species such as lavender, rosemary, and rosehip, part of the crop is selected for essential oil extraction.
- Generally, the interviewed farms supply fresh material to local distillers, due to the high perishability of the product. However, two of the farms have invested in their own distillation units, becoming reference points for other producers in the area. Overall, the majority of the harvested product is destined for drying.
- All the farms are equipped with manual or controlled dryers. The drying process can be carried out outdoors, leveraging solar exposure, or indoors in dedicated facilities, typically using self-built dryers, or through a combination of both methods.
- A portion of the dried product is entrusted to local professionals for packaging and labeling, while another portion undergoes further processing, enabling a wide diversification of output. Value-added products include food items (honey, candies, chocolate, flavorings), baked goods (panettone, pastries, pasta, and saffron-based cookies), liqueurs, herbal teas, craft beers, cosmetics, dietary supplements, animal feed, and household hygiene products.
- Each interviewed farm manages its own sales channels independently. Direct sales on-farm are widespread, as well as distribution to retail points both within and outside the region, especially for farms producing branded herbal teas, supplements, food products, liqueurs, and beers. Only a small number of farms (five) rely on intermediaries (buyers, wholesalers, distributors) to access out-of-region markets. In most cases, farmers actively seek out new clients, particularly in the hospitality and restaurant sectors. A noteworthy aspect is the increasing effectiveness of online sales via dedicated websites and e-commerce platforms.

- Some farms have adopted multifunctionality strategies to enhance their productive assets, developing agritourism, cultural, and recreational activities directly in the field. A significant example is represented by lavender farms, which, in addition to focusing on niche products such as essential oils and derivatives, have invested in immersive tourism experiences, capitalizing on the environmental and scenic value of their local landscapes.
- In terms of business relationships and cooperation, several barriers to the creation of structured networks, collaborations, and supply chains have emerged. These limitations negatively impact the valorization of production and the bargaining power of individual farms.
- However, the analysis also identified success stories where small farms located in distinctive areas (e.g., within natural parks) have joined organized supply chains, reorganizing their activities around collaborative models. This has allowed them to access new markets and strike a better balance between profitability, experimentation, and innovation.

Despite the presence of micro-supply chains in the regional territory, as highlighted in Table 1, the medicinal plants sector remains poorly structured (De Luca, Ricciardi, 2022). This condition can be attributed to persistent challenges that hinder the establishment of an integrated supply chain and limit aggregation processes, as evidenced by the results of the Focus Groups, reclassified in Table 2 based on the main thematic areas that emerged from the analysis.

Table 2. Results of the Focus Groups

1st FOCUS **Critical Issues**	2nd FOCUS **Obstacles to Aggregation**	3rd FOCUS **Organizational Model**
Poor accessibility of the road network	Aging of agricultural entrepreneurs	Flexibility in defining objectives, action boundaries, and the level of partner involvement
Lack of collective storage and processing facilities	Lack of information on the benefits of association	Preserving business autonomy
Absence of irrigation systems specifically for MAPs	Low interest in cooperation	Sharing experiences
Low level of mechanization	Limited awareness MAPs culture in Basilicata	Economies of scale
Shortage of labor	Shortage of specialized training	Encouraging continuous training
Poor alignment of crops with market demands	Absence of consulting services	Non-binding from a legal and economic standpoint
Lack of a brand and/or quality certification	Small-sized enterprises with limited bargaining power	Exchange of industrial, commercial, technical, or technological services
Insufficient promotion of the product	Restricted generational turnover	Common goals of innovation, promotion, and marketing

Source: CREA PB

The main challenges highlighted by agricultural entrepreneurs primarily concern deficiencies in infrastructure and structural aspects, as well as limitations related to marketing and promotion activities, particularly in the restaurant sector. They also face high production costs and a lack of specialized consultants in the agricultural sector. At the same time, obstacles to the creation of networks and various forms of collaboration have mostly been attributed to factors related to human and social capital. In the third Focus Group, the key characteristics of a potentially suitable and coherent aggregation model that meets the needs expressed by local businesses were identified. The analysis of these characteristics revealed that the Network Contract is the most appropriate tool to facilitate cooperation among enterprises. This agreement allows companies to collaborate in order to enhance their innovative capacity and competitiveness in the market by pooling certain activities while maintaining their managerial autonomy (Licciardo, Tarangioli, 2021).

4. Conclusion

The medicinal plant sector in Basilicata is characterized by a heterogeneous production context, where established relationships coexist, as seen in the case of the regional supply chains Lucana Officinali and F.L.E.O., alongside situations where connections among operators remain weak. However, thanks to the engagement and involvement activities promoted by the ME.PLA.SU.S. project, there is a gradual emergence of renewed interest in cooperative dynamics. In this scenario, the Network Contract stands out as a potentially effective solution to address the needs for competitiveness, innovation, and integration of local resources. This is because it represents an agile organizational model that allows for the effective integration of technology, processes, and strategies, responding promptly to the demands of the local production reality. The medicinal plant sector, while representing a niche market, is experiencing significant expansion due to the increasing demand for quality products and Made in Italy goods. The multifunctional value and broad diversification of production have enhanced the sector's potential, supported by consumers' need for natural substances for therapeutic, cosmetic, health, and food purposes, which is met by the spontaneous collection of known species that have now become a collective interest everywhere (D’Oronzio et al., 2023).

The establishment of a business network would enable access to new development opportunities, promoting the expansion of markets, including at the international level, and facilitating the exchange of know-how among companies. Furthermore, this organizational model would improve planning and investment management capabilities, easing access to funding within the framework of Rural Development Programs (PSR) at both regional and national levels. Structuring the supply chain through Network Contract would also enhance the competitiveness of participating companies, encouraging the creation of new products and optimizing the quality of existing ones. Simultaneously, the network would allow for the development of synergies in strategic areas such as marketing, training, research, technological innovation, and experiential tourism, thereby strengthening the sustainability and resilience of the sector. A key element for the success of this model will be the involvement of the research sector, which is essential for stimulating collective project initiatives, fostering the aggregation of operators, and providing specialized consultancy.

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Conflict of Interest

No conflict of interest between authors.

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THE POTENTIAL OF SOME WILD PLANTS COMMONLY CONSUMED IN THE EASTERN BLACK SEA REGION FOR MIXED CULTIVATION IN HAZELNUT ORCHARDS

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Abstract

A soil survey was conducted in the Eastern Black Sea region of Turkey Ordu province Gölköy district) to evaluate soil characteristics relevant to intercropping wild edible plants sakarca (*Ornithogalum umbellatum* L.), melocan (*Smilax excelsa* L.), and galdirik (*Tra*chystemon *orientalis* L.) within hazelnut orchards. Twenty-two soil samples were collected from Gölköy (Ordu), and their coordinates were recorded. Soil texture, pH, electrical conductivity (EC), lime content, and organic matter (O.M) were analyzed. The results showed a range of soil textures including clay loam, clay, sandy loam, and loamy sand. Soil pH was moderately acidic to near-neutral. EC values were generally low, indicating a lack of salinity. Lime content was low across all samples. O.M varied, suggesting differing levels of fertility. Based on these soil properties, the suitability for intercropping sakarca, melocan, and galdrik varies. The moderate acidic pH is generally favorable for these plants, which often thrive in slightly acidic conditions. Low lime content is also beneficial, as high lime levels can inhibit nutrient availability. While specific requirements for each plant need to be considered, the range of soil textures observed suggests that with appropriate management, including organic matter amendments where necessary, intercropping these wild plants could be viable in the studied hazelnut orchards.

Keywords: Aromatic plants, Hazelnut orchards, Soil Analysis, Soil Fertility

1. Introduction

Hazelnut cultivation in Gölköy district of Ordu province in the Black Sea Region is greatly affected by soil properties such as organic matter content, pH level, soil depth and calcium carbonate content. These factors affect nutrient availability, water retention and root development in hazelnut orchards. Understanding these properties is crucial for optimizing agricultural practices, improving soil health, increasing hazelnut productivity and the development of some widely consumed wild plants. In the Ordu region, particularly in Gölköy, wild plants like Galdirik, Melocan, and Sakarca are cultivated in diverse soil conditions. These plants thrive in areas with mild climates and humid environments, often found in hazelnut orchards and forests. Galdirik grows on north-facing slopes at elevations of 50-400 meters, while Melocan is abundant in forests and hazelnut gardens up to 800 meters (Gündüz & Çiçek, 2023). Sakarca is commonly found in hazelnut fields across Ordu, including Gölköy, where it grows at various elevations. The growth of wild plants like Galdirik, Melocan, and Sakarca in the Ordu region, particularly in Gölköy, is influenced by several soil factors including organic matter, pH, and lime content. Generally, wild plants can thrive in diverse conditions, often indicating soil characteristics such as pH levels and nutrient availability (Lambers et al., 2011). For instance, plants growing in acidic soils (low pH) might suggest a need for lime to adjust the pH for other crops, while those in alkaline soils (high pH) could indicate suitability for plants like Hindiba (Atabey et al., 2020). Organic matter content also plays a crucial role, as it affects soil fertility and moisture retention, which can influence plant growth (Lal, 2020). The integration of wild plants into hazelnut orchards through mixed cropping offers several advantages. This practice can effectively control weeds, reducing the need for herbicides and promoting a more sustainable agricultural system. Additionally, mixed cropping enhances soil fertility by increasing organic matter content, which supports beneficial microbial activities and improves nutrient cycling. It also provides an opportunity for producing quality forage, helping to address feed shortages in livestock farming. Furthermore, by maintaining a diverse soil cover, mixed cropping helps mitigate soil erosion, protecting water quality and ensuring long-term soil health. Overall, this approach supports a more resilient and biodiverse agricultural ecosystem, contributing to sustainable and productive hazelnut cultivation.

2. Material and Methods

The study's material includes 22 soil samples taken from Ordu provinces Gölköy district. The soil samples constituting the research material were taken from hazelnut gardens from 0-30 cm soil depth and the location points of the soil samples taken with GPS and X and Y coordinates are given in Table 1.

Table 1. GPS Coordinates of Locations Where Soil Samples Were Taken

		Coordinate Values				Coordinate Values	
Sample No	**Altitude**	**X-GPS**	**Y-GPS**	**Sample No**	**Altitude**	**X-GPS**	**Y-GPS**
1	1088	383538	4508538	12	952	383364	4506161
2	872	382961	4503902	13	838	382499	4504737
3	904	382254	4503897	14	1140	382698	4499357
4	965	381645	4505235	15	995	383603	4507112
5	1005	379315	4505154	16	1034	384178	4507186
6	841	376527	4504450	17	1137	384994	4506448
7	947	374394	4505291	18	978	385092	4520973
8	1091	372174	4507050	19	1176	382790	4514574
9	834	389501	4514713	20	1101	383430	4512820
10	922	387673	4512862	21	1116	379554	4517587
11	900	385850	4511707	22	1162	378733	4517452

The soil samples taken from the hazelnut gardens were brought to the laboratory and were broken up, taking care not to crush the clods. Then, they were dried by spreading them in nylon bags to make them air-dried soil. After drying, the air-dried soil sample was sieved through a 2 mm sieve and made suitable for analysis.

Analyses of Soil Samples

Soil Texture: The % clay, % sand, % silt amounts of the soil samples were determined by the hydrometer method and the texture classes of the soil samples were determined by using the texture triangle (Bouyoucos, 1951).

Soil pH: The soil samples were made suitable for analysis and the soil pHs were determined with a glass electrode pH-meter in a 1:2.5 soil:water mixture as reported by Grewelling and Peech, (1960).

EC Analysis: Total salt was measured with an EC meter in a 1:2.5 soil/water ratio suspension as reported by Richards (1954).

Lime Determination: It was determined with a Scheibler calcimeter as reported by Çağlar (1949).

Organic Matter: It was made according to the modified Walkley-Black wet combustion method as reported by Jackson (1958).

3. Results and Discussion

Of the 22 soil samples taken from Gölköy, 3 were determined as clayey, 7 as clayey-loam, 3 as sandy clayey loam, 6 as sandy loam, 1 as loamy sand and 2 as loamy soil (Table 2).

Table 2. Texture Status of Soil Samples

Sample No	Soil Textured	Sample No	Soil Textured
1	clay loam	12	clay
2	clay	13	clay loam
3	sandy loam	14	loam
4	clay	15	sandy clay loam
5	sandy loam	16	clay loam
6	Sandy clay loam	17	loam
7	clay loam	18	sandy loam
8	clay loam	19	sandy loam
9	clay loam	20	sandy loam
10	sandy clay loam	21	sandy loam
11	clay loam	22	loamy sand

The soil analysis results obtained from the Gölköy region offer significant insights for hazelnut cultivation and mixed cropping systems. The generally slightly acidic nature of the soils (pH 4.77-7.49) indicates a suitable range for nutrient uptake by hazelnut plants. Hazelnut prefers slightly acidic soils, and this pH range increases the solubility of the nutrients required by the plant, facilitating their uptake (Figure 1). The low electrical conductivity (EC) values (35.5-339 µS/cm) indicate the absence of salinity issues, providing an environment where hazelnut plants, as well as other plant species, can easily grow. Salinity can hinder water uptake by plant roots, negatively affecting growth; therefore, low EC values are a positive factor Figure 1.

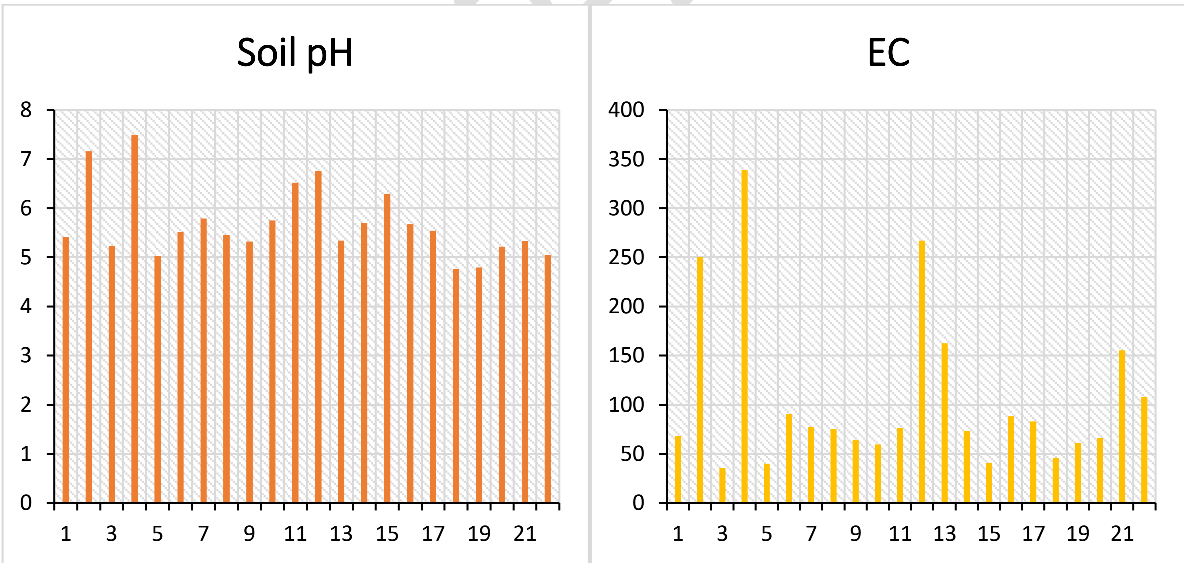

Figure 1. pH and EC Values of Soil Samples

The low lime content (0.74-2.36%) suggests a low risk of soil calcification and considering the sensitivity of hazelnut plants to calcareous soils, this situation provides an advantage for hazelnut cultivation. The variability in organic matter (O.M) content (1.05-8.21%) indicates significant differences in soil fertility. Soils with high organic matter content are more advantageous in terms of water retention capacity, nutrient content, and soil structure. This can increase hazelnut productivity and positively affect the development of other plant species grown in mixed cropping systems (Figure 2).

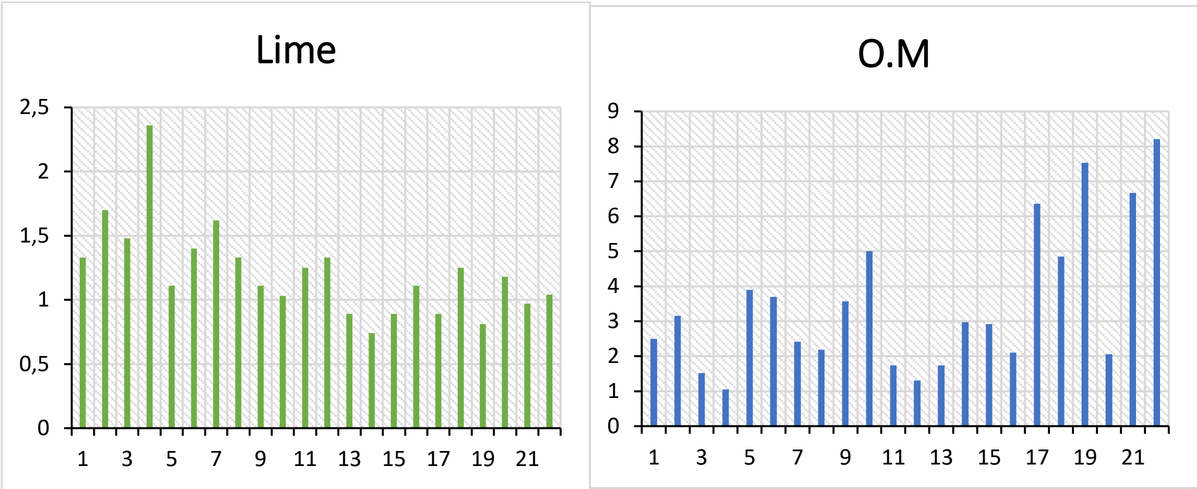

Figure 2. Lime and Organic Matter Values of Soil Samples

The impact of soil properties on hazelnut orchards directly affects the plant's growth and productivity (Dugan et al., 2022). The slightly acidic structure of the soils in the Gölköy region provides a suitable environment for hazelnut cultivation, while low salinity and lime content support the healthy development of the plant. However, differences in organic matter content necessitate the development of management strategies to enhance soil fertility (Magdoff & Weil, 2004). In areas with low organic matter content, soil fertility can be increased through practices such as organic fertilization or green manure application. Mixed cropping systems have the potential to increase biodiversity in hazelnut orchards, control harmful organisms, and improve soil health (Van Sambeek, 2017).. In these systems, different plant species are grown alongside hazelnut plants, ensuring more efficient use of soil nutrients and suppression of harmful organisms. The general characteristics of the soils in the Gölköy region provide a suitable environment for mixed cropping systems. In particular, low salinity and lime content allow the co-cultivation of different plant species, while differences in organic matter content provide clues as to which plant species may grow better in certain areas.

The presence of wild plants is also closely related to soil properties. Some wild plant species that grow in acidic soils can further lower the soil pH, while others can increase the soil's organic matter content (Bueno et al., 2013). Therefore, wild plant management is important for maintaining and improving soil properties. In mixed cropping systems, controlling wild plants and supporting beneficial species can yield positive results in terms of soil health and plant productivity.

4. **Conclusion**

The soil properties in the Gölköy region exhibit a favorable potential for both hazelnut cultivation and the implementation of mixed cropping systems. The slightly acidic nature, low salinity, and low lime content of the soils collectively contribute to an environment conducive to plant growth. However, it is imperative to address the variability in organic matter content through targeted soil management practices to optimize soil fertility. Additionally, effective management of wild plants is crucial to ensure the sustainability of agricultural practices in the area. Regularly assessing soil analysis results and implementing tailored management strategies will be essential for maximizing agricultural production and maintaining the ecological balance in the Gölköy region.

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THERAPEUTIC POTENTIAL AND BIOACTIVE PROPERTIES OF POMEGRANATE PEEL EXTRACT FROM ISFAHAN VARIETIES IN VETERINARY MEDICINE

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Abstract

Pomegranate peel extract, especially from the Isfahan variety, has garnered significant attention in veterinary medicine due to its rich bioactive composition and numerous therapeutic potentials. This study explores the phytochemical profile of pomegranate peel and evaluates its therapeutic effects, particularly its antioxidative, antimicrobial, and anti-inflammatory properties. Four different samples of pomegranate peel were analyzed, and the results revealed varying levels of bioactive compounds. The total phenolic content (TPC) ranged from 12.8 to 23.3 mg GAE/ml, while the total flavonoid content (TFC) varied between 27.6 to 61.9 mg RU/ml. Notably, the antioxidant activity exhibited strong inhibition values ranging from 90.4% to 95.7%, indicating the extract's powerful potential in countering oxidative stress. These findings suggest that pomegranate peel extract could be effectively used in veterinary applications to support animal health, particularly in oral hygiene products and as a hepatoprotective agent. However, further research is required to optimize extraction techniques and evaluate the long-term safety and efficacy of the extract in animal models. If this research progresses, pomegranate peel could become a valuable and sustainable alternative in veterinary medicine.

Key words: Pomegranate peel extract, Antioxidant activity, Phenolic content, Flavonoid content, Veterinary medicine

1.Introduction

Pomegranate (*Punica granatum* L.) is a widely cultivated fruit known for its rich nutritional and medicinal value. Among the different parts of the pomegranate, the peel is an abundant by-product with significant bioactive compounds such as polyphenols, flavonoids, tannins, and essential oils. These compounds possess strong antioxidant, antimicrobial, and anti-inflammatory properties, which have garnered increasing attention in both human and veterinary medicine (Akuru et al., 2022). The Isfahan variety of pomegranate, grown in the central regions of Iran, is particularly known for its unique phytochemical composition. Research indicates that environmental factors, including soil composition and climate conditions in Isfahan, enhance the accumulation of bioactive compounds in the peel. This makes the Isfahan pomegranate peel a valuable natural resource for therapeutic applications (Noor et al., 2024). Oxidative stress is a common underlying factor in many animal diseases, leading to tissue damage and inflammation. The antioxidants present in pomegranate peel can mitigate these effects by scavenging free radicals and reducing oxidative damage. Moreover, the antimicrobial activity of pomegranate peel extract has shown promising results against various pathogens, making it a potential natural alternative to conventional antibiotics in veterinary practice (Silva et al., 2021).

The use of natural plant-based products in veterinary medicine aligns with the increasing demand for safer and more sustainable treatments. Pomegranate peel extract has been incorporated into various veterinary formulations, including oral hygiene products and hepatoprotective treatments. Despite these promising results, further research is required to standardize extraction methods, evaluate long-term safety, and optimize the efficacy of pomegranate peel-derived therapies in veterinary medicine (Abd

Elzaher et al., 2024). This study aims to investigate the bioactive properties of pomegranate peel extract from Isfahan varieties and evaluate its therapeutic potential through a comprehensive analysis of phenolic content, flavonoid content, and antioxidant activity. The findings of this study will contribute to the development of innovative and sustainable veterinary treatments.

2. Material and method

2.1. Sample Collection and Preparation

Four pomegranate varieties (Black peel, red peel, white peel and wild white peel) were collected from different regions of Isfahan, Iran. The peels were separated, washed, and air-dried at room temperature for 10 days. Dried peels were then ground into a fine powder and stored in airtight containers until extraction.

Number	Variety	Collection Location
1	Black Peel	Isfahan Agricultural and Natural Resources Research and Education Center
2	Red Peel	Shahreza
3	Wild White Peel	Shahrekord
4	White Peel	Isfahan Agricultural and Natural Resources Research and Education Center

2.2. Extraction Procedure

The extraction of bioactive compounds was performed using the maceration method. For each sample, 5 g of pomegranate peel powder was soaked in 30 mL of 70% ethanol and kept at room temperature for 4 hours with occasional stirring. The extract was obtained, and the filtrate was concentrated by incubating at 40°C. The concentrated extracts were stored at 4°C for further analysis.

2.3. Phytochemical Analysis

Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method according to Spanos and Wrolstad (1990) method. Gallic acid was used as the standard, and results were expressed as mg of gallic acid equivalent per gram of dry extract (mg GAE/ml). Total Flavonoid Content (TFC) was measured using the aluminum chloride colorimetric method according to Quettier-Deleu et al., (2000), with Rutin the standard, and results were reported as mg Rutin equivalent per gram of dry extract (mg RU/ml). Antioxidant activity was evaluated using the DPPH radical-scavenging assay using by Pyrzynska and Pękal (2013) method. The percentage of DPPH inhibition was calculated to assess the antioxidant capacity of the extracts.

3.Result and Discussion

Phenolic, Flavonoid, and Antioxidant Analysis

Total Phenolic Content (TPC) indicates the concentration of phenolic compounds, which are powerful antioxidants. Wild White Peel Pomegranate has the highest TPC at 23.3 mg GAE/ml, suggesting it is the most phenolic-rich sample. Black Peel Pomegranate and Red Peel Pomegranate show similar TPC values (14.7 mg and 14.3 mg GAE/ml, respectively). White Peel Pomegranate exhibits the lowest TPC at 12.8 mg GAE/ml. Flavonoids, a subclass of polyphenols, are known for their anti-inflammatory and antioxidant properties. Wild White Peel Pomegranate again leads with the highest TFC at 61.9 mg RU/ml, almost twice as much as other samples. Black Peel Pomegranate follows with 39.5 mg RU /ml. Red Peel Pomegranate (32.2 mg RU /ml) and White Peel Pomegranate (27.6 mg RU /ml) show lower flavonoid levels. Antioxidant Activity (DPPH % Inhibition) reflects the ability of the pomegranate peel extract to scavenge free radicals, thereby reducing oxidative stress. Wild White Peel Pomegranate exhibits the highest antioxidant activity at 95.7% inhibition. White Peel Pomegranate is similarly effective, with 95.4% inhibition. Black Peel Pomegranate (91.2%) and Red Peel Pomegranate (90.4%) show slightly lower but still strong antioxidant capacities. To place these findings in context, research on various pomegranate varieties shows a wide range of TPC, TFC, and antioxidant activities. A comparative study of 46pomegranate peel cultivars reported the average of TPC between 121.38 and 297.70 mg GAE/g DW of dry weight (Sweidan et al., 2023). Wild White Peel Pomegranate in the present dataset (23.3 mg GAE/ml) falls near this range, suggesting it is phenol-rich. Black, Red, and White Peels fall slightly below the reported range, indicating moderate phenolic content.

Total Flavonoid Content of other studies show that pomegranate peels typically contain between 58.81 and 116.08 mg RU /ml, depending on extraction methods and geographical origin. Wild White Peel Pomegranate (61.9 mg RU /ml) approaches the upper limit, highlighting its flavonoid richness (Sweidan et al., 2023). Explore the influence of cultivar, climatic conditions, and soil composition on polyphenol and flavonoid content. Other samples exhibit moderate levels but remain within the expected range. Published data on pomegranate peel extracts typically report antioxidant activities between 70% and 85%. All four samples in this dataset exceed 90% inhibition, indicating exceptional antioxidant capacity. Notably, Wild White Peel (95.7%) and White Peel (95.4%) demonstrate superior antioxidant performance, surpassing values reported in prior literature. Wild White Peel Pomegranate emerges as the most bioactive sample, having the highest levels of TPC, TFC, and antioxidant activity. White Peel Pomegranate shows strong antioxidant activity despite having the lowest TPC and TFC, suggesting the presence of other potent antioxidants.

Table 2. The phytochemical composition of the four pomegranate peel samples

No	Sample	TPC (mg GAE/ml)	TFC (mg RU/ml)	Antioxidant Activity (DPPH % inhibition)
1	Black Peel Pomegranate	14.7	39.5	91.2%
2	Red Peel Pomegranate	14.3	32.2	90.4%
3	Wild White Peel Pomegranate	23.3	61.9	95.7%
4	White Peel Pomegranate	12.8	27.6	95.4%

The antioxidant activity in all four samples surpasses that found in previous studies, suggesting that these specific pomegranate varieties or extraction methods yield particularly potent bioactive compounds. Differences across studies can be attributed to genetic variability, geographic conditions, and extraction protocols. For example, ethanolic or methanolic extraction often yields higher polyphenol and flavonoid concentrations than aqueous methods. Standardize extraction protocols for consistent bioactive compound measurements. Investigate the synergistic effects of different bioactive compounds contributing to enhanced antioxidant activity. (Silva et al., 2021). In contrast, while pomegranate peel exhibits significant antioxidant and therapeutic potential, the extraction methods and conditions can greatly influence the yield and efficacy of these bioactive compounds. This variability suggests that further research is needed to standardize extraction processes for optimal health benefits.

Therapeutic Implications in Veterinary Medicine

Pomegranate peel extract (PPE) has been widely studied for its beneficial effects in animal models, displaying remarkable antioxidant, anti-inflammatory, and protective properties. These therapeutic effects are primarily attributed to the extract's high polyphenol content, which plays a crucial role in modulating oxidative stress and inflammatory responses across various biological systems. The following sections highlight key findings from recent animal studies on PPE. Pomegranate peel extract exhibits potent antimicrobial effects, particularly against Gram-positive bacteria and fungal species. In a comparative analysis, Pomegranate peel extract of organic sample demonstrated the most robust antimicrobial activity, which correlated with its elevated polyphenol content. These findings suggest that PPE holds promise as a natural antimicrobial agent in veterinary medicine (Alnees et al., 2023). Moreover, research indicates that PPE is effective against common vaginal bacteria, further supporting its application in animal health (Ziya Motalebipour & Pirestani, 2022). The antioxidative properties of PPE have practical implications in veterinary care. In studies involving irradiated rats, PPE demonstrated radioprotective effects by enhancing antioxidant defenses and reducing oxidative damage. This was evidenced by decreased malondialdehyde (MDA) levels and improved red blood cell membrane integrity (Hamieda et al., 2024). Additionally, antioxidant-rich formulations of PPE have been shown to mitigate oxidative damage and support hepatic function, contributing to overall animal health (Abd Elzaher et al., 2024). Bioactive compounds present in PPE may alleviate inflammation-associated conditions, improving animal well-being. These compounds have shown potential in reducing inflammatory markers and supporting immune system function (Noor et al., 2024). By modulating inflammatory responses, PPE can play a pivotal role in managing chronic inflammatory diseases in animals.

PPE has also demonstrated promising nematicidal properties against plant-parasitic nematodes. In particular, the ethanolic extract of pomegranate peel was found to be more effective than aqueous extracts, achieving a high mortality rate in nematodes and inhibiting egg hatching. These findings suggest that PPE could be employed as a sustainable agricultural solution for controlling parasitic nematodes (AbdelRazek, 2024). Despite the promising findings regarding the therapeutic potential of pomegranate peel extract, further research is required to solidify its role in veterinary and agricultural applications. Key areas for future exploration include: Comprehensive studies are needed to assess the prolonged use of PPE in various animal models to confirm its safety and sustained therapeutic effects. Developing standardized extraction techniques is crucial to ensure the consistent yield of bioactive compounds, improving the reliability of PPE-based treatments. There is a need to create targeted PPE-based veterinary products addressing specific animal health concerns, optimizing dosage, and enhancing bioavailability. Further investigation in these areas will enhance our understanding of PPE’s full potential and facilitate its integration into mainstream veterinary practice and sustainable agricultural solutions.

4. Conclusion

Pomegranate peel extract from Isfahan varieties offers significant therapeutic potential in veterinary medicine due to its rich phytochemical profile. Its antioxidative, antimicrobial, and anti-inflammatory properties make it a valuable natural resource for treating various animal health conditions.

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Conflict of Interest

The authors declare no conflicts of interest.

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INVESTIGATION OF GENES AND KEY FACTORS ASSOCIATED WITH DROUGHT RESISTANCE IN MEDICINAL PLANTS: INSIGHTS INTO GENETIC AND ENVIRONMENTAL INTERACTION

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Abstract

Drought stress is one of the most significant abiotic factors limiting plant growth and productivity. Understanding the genetic and biochemical mechanisms underlying drought resistance in medicinal plants is crucial for enhancing their tolerance and ensuring sustainable agriculture. Recent advances in molecular genetics have led to the identification of key genes involved in drought response, including those associated with polyphenol biosynthesis, flavonoid metabolism, antioxidant enzyme activity, and glycyrrhizin synthesis. This study investigates the genetic and environmental interactions influencing drought resistance in medicinal plants. Research findings indicate that certain genes play a fundamental role in plant adaptation to drought stress and can serve as potential targets for genetic engineering and breeding programs. For example, flavonoid biosynthesis, glycyrrhizin production and antioxidant activity plays a key role in mitigating oxidative stress. The findings of this research suggest that increasing polyphenol and flavonoid production, activating antioxidant defense systems, and optimizing secondary metabolite biosynthesis can enhance drought tolerance in medicinal plants. These insights provide valuable strategies for plant breeding, genetic modification, and sustainable cultivation of drought-resistant medicinal plants.

Key Words: Drought Resistance, Medicinal Plants, Gene Expression, Antioxidant Enzymes, Polyphenols and Flavonoids

1.Introduction

Drought is a critical environmental stress that significantly affects plant metabolism, growth, and survival. Medicinal plants, which serve as valuable sources of pharmaceutical compounds, are also vulnerable to drought stress (Hu et al., 2014). Understanding the molecular mechanisms behind drought tolerance can help enhance their resilience and sustain the production of bioactive compounds. Advances in genetic research have led to the identification of genes that regulate plant responses to drought conditions (Baghery et al., 2022). These genes are involved in key biochemical pathways such as polyphenol and flavonoid biosynthesis, antioxidant enzyme activity, and glycyrrhizin production. Literature reviews and experimental studies were used to examine the involvement of key drought-responsive genes. The genes of interest include CHS (chalcone synthase), CHI (chalcone isomerase), and F3H (flavanone 3-hydroxylase) in *Achillea millefolium* , which are responsible for flavonoid biosynthesis. F3H catalyzes the hydroxylation of flavanones, leading to the formation of dihydroflavonols, which are precursors to various flavonoids(Yin et al., 2019). Also, CHI is a rate-limiting enzyme in flavonoid biosynthesis, facilitating the intramolecular cyclization of chalcones into flavanones(Yin et al., 2019). In *Glycyrrhiza glabra*, genes such as SQS1, SQS2, BAS, CYP88D6, CYP72A154, and UGT73 play essential roles in glycyrrhizin biosynthesis and antioxidant enzyme activity. Squalene Synthase (SQS1 and SQS2): These genes are involved in the initial steps of triterpenoid biosynthesis, catalyzing the conversion of farnesyl pyrophosphate to squalene, a precursor for glycyrrhizin production(Khakpour et al., 2019). Although not directly mentioned in the provided contexts, genes like CYP72A154 are typically involved in the hydroxylation processes within the triterpenoid biosynthesis pathway(Chiyo et al., 2023). hense properties are attributed to the modulation of various signaling pathways, which are involved in cellular defense mechanisms against oxidative stress Additionally, RcGPX5 in *Salvia miltiorrhiza* has been identified as a key factor in oxidative stress regulation. Overexpression of RcGPX5 in Salvia miltiorrhiza leads to increased tolerance against oxidative stress, as evidenced by reduced malondialdehyde (MDA) levels and enhanced activities of antioxidant enzymes like glutathione reductase and ascorbate peroxidase(Zhang et al., 2019). Transgenic plants with RcGPX5 exhibit improved drought tolerance, wilting later than wild types and recovering more quickly after re-watering(Zhang et al., 2019). This indicates a broader role in stress response beyond just oxidative stress. We will evaluate the effects of drought stress on these plants based on previous experimental data, which analyzed changes in metabolite accumulation and gene expression levels. In this study, we will investigate the impact of drought stress on medicinal plants by examining the expression of key drought-responsive genes and their role in stress mitigation. Additionally, based on the genes studied in prior research, we aim to explore the relationship between these genes and identify a suitable pathway for enhancing drought resistance in medicinal plant.

2. Material and method

This study focused on three medicinal plant species: *Achillea millefolium*, *Glycyrrhiza glabra*, and *Salvia miltiorrhiza*. The study focused on genes associated with drought tolerance in medicinal plants. The genes analyzed included CHS, CHI, and F3H in *Achillea millefolium* for flavonoid biosynthesis, SQS1, SQS2, BAS, CYP88D6, CYP72A154, and UGT73 in *Glycyrrhiza glabra* for glycyrrhizin biosynthesis, and RcGPX5 in *Salvia miltiorrhiza* for oxidative stress regulation. Data from previous studies were collected from peer-reviewed literature and used for comparative analysis. This comparative approach enabled a more comprehensive understanding of the molecular mechanisms underlying drought tolerance in the selected medicinal plants (Table 1).

Table 1. Genes Associated with Drought Tolerance in Medicinal Plants

Medicinal Plant	**Genes**	**Gene Function**
Achillea millefolium	CHS, CHI, F3H	Flavonoid biosynthesis
Glycyrrhiza glabra	SQS1, SQS2, BAS, CYP88D6, CYP72A154, UGT73	Glycyrrhizin biosynthesis
Salvia miltiorrhiza	RcGPX5	Regulation of oxidative

3.Result and discussion

Medicinal plants exhibit a range of adaptive responses to drought stress, which influence their physiological functions, genetic regulation, and interactions with environmental factors. Understanding these responses is crucial for optimizing both plant survival and the production of pharmacologically valuable compounds. This section explores key physiological mechanisms, genetic adaptations, and environmental interactions that contribute to drought tolerance in medicinal plants. Additionally, the impact of drought stress on the biosynthesis of medicinal compounds is examined, highlighting the balance between stress resistance and secondary metabolite production. By analyzing the role of drought-responsive genes such as CHS, CHI, F3H in Achillea millefolium, SQS1, SQS2, BAS, CYP88D6, CYP72A154, UGT73 in Glycyrrhiza glabra, and RcGPX5 in Salvia miltiorrhiza, this discussion provides insights into the molecular and biochemical pathways underlying drought resilience. Furthermore, the implications of these findings for genetic engineering, selective breeding, and conservation strategies are considered, emphasizing the need for an integrated approach to improving the drought tolerance of medicinal plants without compromising their medicinal value.

Physiological Mechanisms

Drought stress induces a range of physiological responses in medicinal plants, primarily through the accumulation of osmoprotectants such as proline and changes in antioxidant enzyme activity. These mechanisms help stabilize cellular structures and reduce oxidative stress, thereby enhancing drought tolerance (Liu et al., 2023). At the molecular level, flavonoid biosynthesis genes such as CHS (chalcone synthase), CHI (chalcone isomerase), and F3H (flavanone 3-hydroxylase) in *Achillea millefolium* play a crucial role in plant adaptation to drought. These genes regulate the production of polyphenols and flavonoids, which function as antioxidants and stress protectors. Specifically, F3H catalyzes the hydroxylation of flavanones, leading to the formation of dihydroflavonols, essential precursors to various flavonoids (Yin et al., 2019). Meanwhile, CHI accelerates the cyclization of chalcones into flavanones, which are key intermediates in flavonoid biosynthesis (Yin et al., 2019). Drought stress has been shown to increase the production of polyphenolic and flavonoid compounds, likely as a protective mechanism against oxidative damage. For instance, prolonged drought exposure in *Achillea millefolium* resulted in a significant accumulation of these metabolites, reinforcing their role in stress adaptation. Similarly, in *Glycyrrhiza glabra*, drought-induced stress triggered an increase in glycyrrhizin biosynthesis, which was linked to the activation of antioxidant enzymes. These findings indicate that drought not only enhances plant survival but may also increase the medicinal value of these plants by stimulating the production of bioactive compounds.

Environmental Interactions

Environmental factors, particularly soil microbiomes, significantly influence plant metabolism, genetic expression, and drought responses. For example, beneficial rhizosphere microorganisms such as mycorrhizal fungi and plant-growth-promoting bacteria (PGPB) can enhance water uptake efficiency, facilitate nutrient acquisition, and regulate stress-responsive genes, improving overall drought resilience (Liu et al., 2024). In *Glycyrrhiza glabra*, genes involved in glycyrrhizin biosynthesis—such as SQS1, SQS2, BAS, CYP88D6, CYP72A154, and UGT73—exhibit environmentally influenced expression patterns. For instance, SQS1 and SQS2 encode squalene synthases, which catalyze the conversion of farnesyl pyrophosphate to squalene, a crucial precursor in glycyrrhizin biosynthesis (Khakpour et al., 2019). Additionally, CYP72A154 is involved in hydroxylation processes within triterpenoid biosynthesis, highlighting its role in modulating glycyrrhizin levels in response to drought stress (Chiyo et al., 2023). While genetic and physiological adaptations are essential for drought resistance, excessive reliance on specific genetic traits may limit the broader adaptability of medicinal plants to rapidly changing environments. Therefore, a holistic conservation strategy should consider both genetic and ecological factors, ensuring the sustainability of these valuable medicinal species.

Impact of Drought on Medicinal Compounds

Drought stress leads to the generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), which can cause oxidative damage (Yang et al., 2022). However, plants mitigate this damage through the activation of antioxidant enzymes, which help neutralize ROS and maintain cellular integrity. In *Salvia miltiorrhiza*, the RcGPX5 gene plays a pivotal role in oxidative stress regulation by enhancing antioxidant enzyme activities, such as glutathione reductase and ascorbate peroxidase (Zhang et al., 2019). This genetic mechanism contributes to improved drought tolerance, as transgenic plants overexpressing RcGPX5 exhibit reduced oxidative damage, delayed wilting, and faster recovery after re-watering. However, overexpression of RcGPX5 has been linked to a decrease in the accumulation of secondary metabolites, highlighting a potential trade-off between stress resistance and medicinal compound production. These findings emphasize the importance of genetic regulation in plant adaptation to drought and the need to balance stress tolerance with the maintenance of pharmacologically valuable metabolites. Optimizing the expression of stress-related genes could help maintain both plant survival and medicinal compound yield.

Genetic Regulation and Adaptation

The increased production of polyphenolic and flavonoid compounds under drought conditions underscores their function as protective agents against oxidative stress. The enhanced glycyrrhizin biosynthesis in *Glycyrrhiza glabra* exemplifies an adaptive metabolic shift, where antioxidant enzymes help mitigate stress effects while promoting the synthesis of medicinal compounds. Similarly, in *Salvia miltiorrhiza*, the RcGPX5 gene contributes to drought tolerance through oxidative stress regulation, reinforcing its role in stress adaptation (Haghpanah et al., 2024; Zhang et al., 2019). Although genetic responses enhance drought resistance, they also introduce trade-offs between stress adaptation and secondary metabolite production. Excessive stress resistance could lead to reduced biosynthesis of bioactive compounds, potentially compromising the medicinal value of these plants. Therefore, it is essential to strategically regulate the expression of key drought-responsive genes to achieve an optimal balance between survival and metabolite production. By applying genetic engineering and selective breeding techniques, future studies can enhance drought tolerance in medicinal plants while preserving their pharmacological potential. Identifying and modulating key genes such as CHS, CHI, F3H, SQS1, SQS2, CYP88D6, UGT73, and RcGPX5 will be essential for developing climate-resilient medicinal plant varieties that thrive in changing environmental conditions while maintaining their therapeutic efficacy.

4.Conclusion

Drought stress profoundly impacts the growth and metabolism of medicinal plants, requiring adaptive mechanisms to ensure survival. The importance of key drought-responsive genes in regulating biochemical and physiological processes under water-deficient conditions is evident in many species. A thorough understanding of genetic and environmental interactions that influence drought resistance is essential for developing stress-tolerant medicinal plants. Future research should focus on integrating genetic modification and advanced breeding techniques to enhance drought tolerance while preserving the medicinal properties of these plants, ensuring their sustainable use in both conservation and commercial applications.

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STRATEGIES FOR INCREASING SECONDARY METABOLITE YIELD IN MEDICINAL AND AROMATIC PLANTS

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Abstract

The enhancement of secondary metabolite production in medicinal and aromatic plants is an important research topic to increase the biological activity and commercial value of these plants. Secondary metabolites play a critical role in protecting plants against environmental stressors and in carrying out various biological functions. To increase the production of these compounds, various methods such as plant growth regulators, tissue culture techniques, polyploidy, and mutation are used. Plant growth regulators (PGRs) are chemical substances that control plant growth and development and stimulate secondary metabolite production. In particular, the use of plant growth regulators at correct concentrations and timings facilitates the synthesis of higher amounts of secondary metabolites. Tissue culture techniques are another significant method used to optimize the biological and chemical properties of plants, as they enable the controlled production of secondary metabolites under in vivo conditions. Polyploidy is the presence of more than two sets of chromosomes in somatic cells and is achieved by increasing the chromosome number in plants. This condition enhances the metabolic capacity of plants, thereby promoting the production of greater quantities of secondary metabolites. Therefore, polyploidy is an effective method used to enhance secondary metabolite production in plants. Mutation, which involves sudden and permanent changes in the genetic structure of plants, can also be employed to produce new and desired metabolites. Mutations in plants can be induced by chemical or physical mutagens, leading to an increase in both the diversity and quantity of secondary metabolites. These methods collectively contribute to the enhancement of secondary metabolite production in medicinal and aromatic plants, thereby ensuring more efficient and effective extraction of these compounds for use in pharmaceutical and cosmetic industries. This review aims to summarize the approaches used to enhance secondary metabolite production in plants.

Key Words: Medicinal and aromatic plants, secondary metabolites, plant growth regulator, tissue culture, polyploidy

1.Introduction

Secondary metabolites are defined as organic compounds that are produced by plants, which do not play a direct role in their growth, development, or reproduction. However, they are vital for their defence mechanisms and interaction with the environment. These compounds, which include alkaloids, flavonoids, terpenoids and phenolics, possess significant pharmacological properties and are widely used in the pharmaceutical and cosmetic industries. These metabolites are secreted by plants to provide protection against pathogenic attacks or injuries (Özyiğit et al., 2023). Secondary metabolites have been demonstrated to play a significant role in the adaptation of organisms to stressful conditions (Chandana et al., 2018; Erb & Kliebenstein, 2020; Özyiğit et al., 2023). The induction of secondary metabolite biosynthesis in plants begins with treatment by any elicitor, which is any stress factor that triggers defense responses in plants (Naik & Al Khayri, 2016; Khanam et al., 2022; Özyiğit et al., 2023). It is imperative to enhance the production of secondary metabolites in medicinal and aromatic plants to ensure optimal biological efficacy and economic value. The present review has been prepared for the purpose of summarising some of the approaches that have been used to enhance secondary metabolite production in plants.

Approaches for Enhancing the Yield of Secondary Metabolites

1. Polyploidy

Ploidy is defined as changes in the number of chromosomes in living organisms. The classification of plants according to their ploidy levels can be categorised into seven distinct categories: monoploid (haploid), diploid, triploid, tetraploid, pentaploid, hexaploid, and octaploid. Polyploidy refers to a chromosomal condition in which somatic cells contain more than two complete sets of chromosomes (Şehirali & Özgen, 2013). In the field of botany, polyploidy can be either naturally occurring or artificially induced, through the use of antimitotic agents which disrupt the process of mitotic division. As demonstrated by Goluch et al. (2021), colchicine, oryzalin and trifluralin are widely utilised antimitotic agents in the induction of polyploidy in plants. Furthermore, chemical substances such as chloral hydrate, ether, chloroform, and phenylurea, as well as heat shock treatments, are among the methods employed to induce polyploidy (Şehirali & Özgen, 2013). Despite the fact that the polyploidy of plants can be estimated based on morphological characteristics, the accurate identification of polyploid plants can be achieved through a variety of methods. These include chromosome counting under a microscope, the quantification of chloroplasts in stomatal cells, the measurement of pollen grain size, and analysis using flow cytometry.

It is widely acknowledged that artificial polyploidization represents one of the safest and most effective modern breeding methodologies, employed both in vitro and in vivo to induce polyploidy in organisms (Gupta et al., 2024). A well-known method for enhancing secondary metabolite content in plants is the use of chemical mutagenesis. The application of polyploidy-inducing agents or chemicals has been demonstrated to function as a mutagenic mechanism in the breeding of polyploid plants. Polyploid plants have been observed to exhibit changes in organ size, including increased leaf size, plant height, and overall biomass. Variations in the yield of secondary metabolites have also been documented, particularly in medicinal plants (Ahmadi et al., 2013; Azizan et al., 2020). Polyploidy has been demonstrated to exert considerable influence on diverse plant organs, including seeds, fruits, and leaves, and even on the entire plant. This influence can be characterised by an increase in organ size, a deeper intensity and darker pigmentation, and an elevated chemical content. Polyploidization is regarded as a robust and modern approach that generates new genotypes and induces overexpression of genes involved in the biosynthesis of important metabolites. The enhanced production of natural metabolites has been demonstrated to improve the bioactivity of plant extracts, thereby increasing tolerance to environmental stresses and ensuring uniformity. This enhancement in the potency and amount of plant secondary metabolites has the potential to substantially advance the medicinal value of plants (Gupta et al., 2024).

Considering the increasing demand for valuable secondary metabolites derived from plants, artificial polyploidization has emerged as a notable breeding strategy that may improve the phytochemical potential and efficacy of medicinal and aromatic species (Niazian & Nalousi, 2020; Gupta et al., 2024). The process of direct chromosome doubling, otherwise referred to as allopolyploidization, has been highlighted as a potentially efficacious strategy for enhancing the yield of phytopharmaceuticals (Lavania, 2005). This approach is consistent with the increasing consumer preference for natural products. Nevertheless, the general evidence indicates that inducing polyploidy artificially may represent a viable and long-term strategy for advancing medicinal and aromatic plant species, particularly through the enhancement of metabolite synthesis and associated biological properties (Gupta et al., 2024). Table 1 presents a summary of studies related to polyploidy and secondary metabolites.

Table 1. Some Polyploid Studies on Secondary Metabolites

Species	Polyploidy Agent	Applied Dose	Application Duration	Application Method	Result	References
Cannabis sativa L.	Colchicine	0,0 0,1 0,2 (%)	24 48 (hour)	Dropping	Tetraploid hemp plants have been reported to be unsuitable for fiber production and medicinal use, as they exhibit reduced fiber and cannabinoid contents compared to mixoploid plants.	Mansouri & Bagheri, (2017)
Chamaemelum nobile L.	Colchicine	0 0,05 0,10 0,50 (%)	12 (hour)	Addition to the culture medium	An analysis was conducted on the essential oils of diploid and tetraploid plants subjected to a regeneration process. The results of this analysis indicated that there was no significant difference in total oil production per unit weight between the two groups. Furthermore, no compositional changes were observed, indicating that there was no alteration in aroma in tetraploid plants. However, tetraploid plants produced 1237.3 ± 87.9 mg of essential oil per spike, compared to 663.5 ± 137.0 mg produced by diploid plants. These results suggest that cell size enhances essential oil production, but this increase applies to a specific organ rather than total weight.	Tsuro et al., (2016)
Cuminum cyminum L.	Colchicine	0,025 0,050 0,100 0,200 (%)	30 (3x) (minute)	Dropping	It was reported that tetraploid genotypes exhibited a 30% to 100% enhance in essential oil yield, and that this increase was influenced by colchicine treatment. It was also observed that tetraploid plants demonstrated a increased level of cuminaldehyde content.	Sanaei-Hoveida et al., (2024)
Melissa officinalis L.	Oryzalin	20 40 60 (mM)	24 48 (hour)	Explant exposure technique	While the citronellal level remained stable in both diploid and tetraploid plants, an increase in geranial and neral was observed in the tetraploid form. It has been reported that polyploidization can result in a significant increase in essential oil yield and composition in *Melissa officinalis*.	Bharati et al., (2023)
Mentha piperita *Mentha villosa* *Mentha suaveolens*	Colchicine	0 0,0125 0,0250	3 6 9	Immersion	It has been reported that in the hexaploid form of mojito mint, the essential oil yield, as well as the levels of 1,8-sineol and pulegone, were increased, and phytochemicals such as α-pinene, sabinene, limonene, mentofuran, and borneol were synthesized. Additionally, it has been stated that the secondary metabolites contained in the triploid and hexaploid forms of *M.* × *villosa* differ.	Moetamedipoor et al., (2022)

		0,050 (%)	(hour)			
Mentha spicata L.	Oryzalin	20 40 60 (µM)	24 48 (hour)	Addition to the culture medium	An increase in essential oil yield, particularly in carvone and limonene content, has been reported in hexaploid Mentha spicata	Bharati et al., (2023)
Nigella sativa L.	Colchicine	0,025 0,050 0,100 (%)	4 6 8 (hour)	Immersion	An increase in total phenolic compounds was observed in tetraploid plants compared to the controls. Additionally, lines with 46% more thymoquinone in their seed oil were developed.	Dixit et al., (2015)
Salvia officinalis L.	Colchicine	0 0,05 0,10 0,25 0,50 (%)	12 24 48 (hour)	Immersion	The application of 0.25% colchicine for 48 hours and 0.50% colchicine for 24 hours have been highlighted as the most effective treatments for tetraploid induction. In tetraploid plants, increases were observed in various morphological traits, certain enzymes, and secondary metabolites such as total phenolics and flavonoids.	Hassanzadeh et al., (2020)
Thymus persicus	Colchicine	0 0,05 0,10 0,30 0,50 (%)	12 48 (hour)	Immersion	Compared to diploid plants, tetraploid plants exhibited shorter stature, reduced root length, thicker stems, and darker leaves; while stomatal size increased, stomatal density decreased. Furthermore, triterpenoid production was elevated in both tetraploid and mixoploid plants. In tetraploid plants, significant increases in various secondary metabolites, such as betulinic acid, oleanolic acid, and ursolic acid, were observed compared to their diploid forms. These results indicate that phytochemical characteristics are strongly influenced by the ploidy level of the plant.	Tavan et al., (2015)

2. Plant Growth Regulators (PGRs)

Plant Growth Regulators (PGRs) are naturally occurring or synthetic substances that regulate various physiological functions in plants. Plant Growth Regulators (PGRs) play a crucial role in influencing the biosynthesis of secondary metabolites in plants, which are vital for their medicinal and economic value. These regulators, including auxins, cytokinins, gibberellins, abscisic acid, ethylene, brassinosteroids, and methyl jasmonate, modulate various biosynthetic pathways and signal transduction mechanisms to enhance the production of secondary metabolites such as terpenoids, alkaloids, and phenylpropanoids. When applied at precise concentrations and developmental stages, PGRs can markedly enhance the yield and quality of these compounds. The interaction between PGRs and secondary metabolite biosynthesis is complex and involves multiple pathways and regulatory networks.

Mechanisms of PGR Influence

Perception and Signal Transduction:

PGRs activate specific signal transduction pathways that lead to the expression of genes involved in secondary metabolite biosynthesis. PGR signaling begins with the perception of the hormone by specific receptors. The molecular mechanisms of hormone action have been elucidated for most PGRs, with the discovery of their receptors being a major milestone. For example, methyl jasmonate is known to upregulate genes responsible for the production of terpenoids and phenolic compounds (Chauhan et al., 2024). The identification of the SCF (Skip/Cullin/Fbox) (COI1) complex in jasmonate signaling revealed that JAZ proteins (jasmonate ZIM-domain) are direct targets of this E3 ubiquitin ligase, leading to their proteasome degradation and the activation of jasmonate responses (Chini et al., 2007). Similarly, the perception of auxins involves F-box proteins that target transcriptional repressors for degradation, allowing auxin response factors to activate gene expression (Smith et al., 2017).

Gene Expression Modulation: PGRs can modulate the expression of key enzymes in the biosynthetic pathways of secondary metabolites. This regulation is often species-specific and can vary with the plant's developmental stage (Li et al, 2020).

Enhancement of Biomass: By promoting plant growth and biomass accumulation, PGRs indirectly increase the substrate availability for secondary metabolite production. This is particularly evident in tissue culture systems where PGRs are used to optimize growth conditions (Chauhan et al., 2024).

Environmental Interaction: PGRs mediate plant responses to environmental stimuli, which can trigger the production of secondary metabolites as a defense mechanism. This interaction is crucial for plants in the Lamiaceae family, where essential oil production is influenced by both genetic and environmental factors (Sharafzadeh & Zare, 2011).

Crosstalk Among PGRs

PGRs do not function in isolation; instead, they interact in complex networks to regulate plant growth and development. Crosstalk occurs at multiple levels, including hormone biosynthesis, signaling, and gene expression. For example, auxins and cytokinins interact antagonistically to regulate cell division and differentiation, while gibberellins and abscisic acid exhibit both synergistic and antagonistic interactions depending on the developmental context (Ohri et al., 2015) (Wang & Irving, 2011).

Antagonistic and Synergistic Interactions

Antagonistic interactions often involve hormones with opposing functions. For instance, salicylic acid and jasmonic acid exhibit antagonistic crosstalk during plant defense responses, with salicylic acid promoting resistance to biotrophic pathogens and jasmonic acid regulating defense against necrotrophic pathogens (Robert-Seilaniantz et al., 2011). In contrast, synergistic interactions, such as those between brassinosteroids and gibberellins, enhance growth-promoting effects (Xiong et al., 2009).

Applications of PGR Signaling

Agricultural Applications

Understanding PGR signaling has practical applications in agriculture, including the development of transgenic plants with improved stress tolerance and yield. For example, manipulating the biosynthesis or signaling of abscisic acid can enhance drought tolerance in crops (Kulaeva & Prokoptseva, 2004) (Cutler et al., 2010). Similarly, modulating jasmonic acid signaling can improve resistance to insect pests (Chini et al., 2007).

Hormone Crosstalk and Breeding

The complex interactions between PGRs provide opportunities for breeding crops with desirable traits. For instance, the antagonistic interaction between gibberellins and abscisic acid can be exploited to develop crops with improved seed dormancy and germination properties (Verma et al., 2016). Additionally, the crosstalk between auxins and cytokinins can be manipulated to regulate plant architecture and yield (Ohri et al., 2015)

Table 2. Key PGRs and Their Functions

Hormone	Key Functions	Signaling Mechanism
Auxins	Promote cell elongation and root development	F-box proteins target Aux/IAA repressors for degradation (Smith et al., 2017) (Kulaeva & Prokoptseva, 2004)
Jasmonik Acid	Regulate defense responses and stress tolerance	JAZ repressors are degraded via the SCF(COI1) complex (Chini et al., 2007) (Verma et al., 2016)
Abcisic Acid	Mediate drought tolerance and stomatal closure	PYR/PYL/RCAR receptors regulate PP2C phosphatases (Cutler et al., 2010) (Waadt et al., 2022)
Ethylene	Promote senescence and fruit ripening	Membrane-anchored receptors activate protein phosphorylation cascades (Smith et al., 2017) (Bari & Jones, 2009)
Salicylic Acid	Regulate defense against biotrophic pathogens	Interacts with jasmonic acid signaling (Robert-Seilaniantz et al., 2011) (Bari & Jones, 2009)

Jasmonic acid (JA) and methyl jasmonate (MeJA) are lipid-derived phytohormones synthesized from linolenic acid, a compound prevalent in the plant kingdom. These endogenous phytohormones are integral to the processes of plant growth and serve as powerful stimulators or signaling molecules that modulate a plethora of biochemical and physiological phenomena (Howe 2010; Koca and Karaman, 2015). Among the diverse array of stimulatory agents, MeJA and salicylic acid (SA) have been extensively documented as significant phytohormonal stimulants (Jeyasri et al., 2023). The exogenous administration of JA and MeJA has been shown to provoke the accumulation of secondary metabolites, thereby inducing defensive responses (Thiem & Krawczyk 2010). Ashrafi et al. (2012) demonstrated that the foliar application of JA at a concentration of 100 µL resulted in an enhancement of thymol and carvacrol levels within the essential oil extracted from the aerial portions of Thymus daenensis in comparison to both the control and other experimental treatments. Ghasemi Pirbalouti et al. (2014) indicated that varying concentrations of JA and SA exerted significant influences on the chemical constituents of Satureja hortensis L. essential oils, while having no notable impact on the overall oil yield and dry biomass of the herb. The foliar application of 50 µL of SA was found to elevate the levels of monoterpene hydrocarbons in S. hortensis oil, whereas it concurrently caused a decrease in oxygenated monoterpenes and sesquiterpenes. It was highlighted that JA at a concentration of 50 µL could be effectively employed as a foliar treatment on S. hortensis to enhance the content of monoterpene hydrocarbons in the oil.

3. Tissue Culture Techniques

Plant tissue culture refers to the cultivation of various plant components, such as cells, tissues, and organs, in a synthetic medium under aseptic and regulated environmental conditions. This process can be performed in various forms, including solid, semi-solid, or liquid, with the objective of producing plant products. The tissue culture method is based on the totipotency of plants. Totipotency is a property that enables the regeneration of an entire plant from a single plant part (Finer & Dhillon, 2016; Özyiğit et al., 2023). Plant tissue culture methods have numerous applications, including the production of valuable compounds such as plant-derived secondary metabolites and recombinant proteins that are utilized as biopharmaceuticals. They also include the propagation of plants through micropropagation, the conservation of endangered plant species, the production of disease- and pest-free plants, and the development of hybrid species through interspecific hybridisation. The generation of plants with novel traits via somaclonal variation has been achieved. The production of haploid plants through anther and microspore culture has been successful, as has cross-pollination and embryo formation between distantly related species. Chromosome doubling has been accomplished, as have creation of new hybrids through protoplast fusion and regeneration, preservation and storage of genetic resources, the use of cell cultures in in vitro selection studies, and the generation of transgenic plants (Özyiğit, 2012; Mukta ve ark., 2017; Dağüştü, 2018; Huyop ve ark., 2019; Phillips ve Garda, 2019; Seçkin ve Okumuş, 2022; Özyiğit vd. 2023). To summarise, in vitro propagation has a significant role in plant breeding and in the enhancement of secondary metabolite production.

Table 3. Tissue Culture Techniques and Their Common Applications

Technique	Description and Applications
Embryo Culture	This term refers to the transfer and development of embryos on nutrient media. The use of both mature and immature embryos is a possibility (Kocaçalışkan, 2017). Its applications include the germination of non-viable seeds (Uysal et al., 2006; Kayın & Turan, 2023), the development of embryos that are unable to grow due to incompatibility, the production of haploid plants, and the study of embryo development (Uysal ve ark., 2006; Akpınar, 2006; Ellialtıoğlu ve Taşkın, 2021; Kayın ve Turan 2023).
Meristem Culture	This is an in vitro technique in which the apical part of the meristem, otherwise known as the plant's growth point, is cultured in a aseptic condition on an artificial medium with the objective of regenerating virus-free plants. Its applications include virus elimination, disease control, the regeneration of plant material, and the enhancement of plant production (Yücel, 2022).
Callus Culture	The cultivation and proliferation of undifferentiated plant cells (callus) formed in response to wounding or hormonal stimuli under laboratory conditions (Aktaş & Çölgeçen, 2017; Yücel, 2022). It is utilized for indirect organogenesis or somatic embryogenesis, as starting material for suspension cultures, physiological and cytogenetic studies, protoplast isolation, gene transfer, graft incompatibility studies, stress analyses, and particularly secondary metabolite production (Erdemel & Aygün, 2016).
Cell Suspension Culture	This term refers to the liquid culture of single cells or small groups of cells (Vuran & Türker, 2021). These alternative methods of production represent a significant development, as they allow for the generation of secondary metabolites without the necessity of using whole plants (Topcu & Çölgeçen, 2015).
Anther (Microspore) and Ovary Culture	In androgenesis, male reproductive organs (anther or microspore) and in gynogenesis, female reproductive organs (ovule or ovary) are cultured in artificial media (Yücel, 2022). These techniques are effectively utilised in breeding programmes to obtain 100% homozygous plants.
Protoplast Culture	Protoplasts are defined as plant cells in which the cell walls have been removed. Protoplast culture is defined as culturing of cells on nutrient media for the purpose of plant regeneration. Its primary applications are in somatic hybridisation and gene transfer (Kocaçalışkan, 2017).

The tissue culture technique offers numerous advantages, including the ability to produce a large number of plants or products from a small amount of plant material, the phenotypic uniformity of the resulting plants, high production yield per unit area, independence from climatic conditions due to cultivation under controlled environments—allowing for year-round production—and, most importantly, the generation of clean plants and products thanks to sterile growth conditions. Nevertheless, the technique is not without its drawbacks. These include the considerable expenses incurred during the investment and production phases, the requirement for skilled personnel and advanced technology, and the difficulties encountered during the sterilisation process.Tissue culture offers a controlled environment for the cultivation of plant cells, tissues, or organs, enabling the consistent and scalable production of secondary metabolites. Techniques such as callus culture, cell suspension culture, hairy root culture, and organ culture are commonly employed. These in vitro methods allow researchers to manipulate growth conditions, nutrient media, and elicitors to optimize metabolite production. Moreover, tissue culture facilitates the conservation of rare and endangered medicinal plants while ensuring a sustainable supply of bioactive compounds.

The successful production of significant compounds, including secondary metabolites, has also been achieved through plant tissue culture (Özyiğit et al., 2023). In vitro plant cultures have emerged as a prominent technological approach for the production of valuable secondary metabolites (Topcu & Çölgeçen, 2015). The employment of diverse in vitro culture techniques as an alternative to whole plants provides a promising avenue for the synthesis of high-value secondary metabolites. In comparison with conventional production techniques, this method offers a number of benefits, including independence from climatic and environmental factors, the capacity for uninterrupted production with consistent quality and quantity, the identification of novel compounds not typically present in the original plant, resistance to fluctuations in agricultural policy, and the capability to facilitate expedited production (Ramachandra & Ravishankar, 2002). Praveen and Murthy (2012) conducted a study where hairy root cultures of Withania somnifera were optimized in flask scale experiments. Maximum biomass was obtained using 3% sucrose at pH 5.8 while the highest withanolide A production was obtained with 4% sucrose at pH 6.0. These findings support large scale cultivation for withanolide A production. In another case, cell suspension cultures of Panax ginseng have been successfully used to enhance ginsenoside content under the influence of sucrose, and the ratio of NO3+/NH4− (Lian et al., 2002).

5.Conclusion

While the use of PGRs shows great potential for enhancing secondary metabolite production, challenges such as low productivity and the need for further research into large-scale applications remain. Addressing these issues is essential for the sustainable exploitation of MAPs in the pharmaceutical industry. The enhancement of secondary metabolite production in medicinal and aromatic plants is essential for meeting the growing demand in health-related industries. Techniques involving plant growth regulators, tissue culture, polyploidy, and mutation breeding offer promising avenues for improving both the quantity and quality of these valuable compounds. By leveraging these biotechnological and genetic approaches, researchers can ensure a more efficient and sustainable production of secondary metabolites, thereby supporting advancements in pharmaceuticals, nutraceuticals, and cosmetics.

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THE ROLE OF SECONDARY METABOLITES IN MEDICINAL AND AROMATIC PLANTS

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Abstract

Secondary metabolites are natural compounds other than primary metabolites such as proteins, carbohydrates, and fats that are not essential for plant life. Unlike primary metabolites, which are essential for plant growth and development, secondary metabolites are not directly involved in these processes but serve more specialized functions. These natural compounds synthesized by plants as a defense against abiotic and biotic stress factors are also known as efficient or active substances in plants. They exhibit various bioactive properties such as antimicrobial, anti-inflammatory, antioxidant, and anticancer activities. Secondary metabolites can be classified into three main groups: alkaloids, terpenoids, and phenols. Generally, alkaloids play a significant role in the development of pharmaceuticals, while flavonoids contribute to tranquilizers and anti-inflammatory effects. Terpenoids are known for their sedative properties. Plants with a high content of secondary metabolites are typically classified as medicinal and aromatic plants. These plants contain varying amounts of secondary metabolites in different organs. Medicinal and aromatic plants are categorized according to their active compounds, which determine their uses, such as perfume plants, spice plants, dye plants, and medicinal plants. For example, jasmine (Jasminum) is classified as a perfume plant due to its benzyl acetate content, mint (Mentha spicata) is classified as a spice plant due to its menthol content, madder (Rubia tinctorum) is classified as a dye plant due to its alizarin content, and poppy (Papaver somniferum L.) is categorized as a medicinal plant due to its morphine content. These compounds generally play a defense role in plants, protecting them from herbivores, pathogens, and environmental stressors. The diverse roles of secondary metabolites make them essential for plant survival and offer significant potential for developing natural drugs and products in the pharmaceutical and cosmetic industries. Consequently, secondary metabolites in medicinal and aromatic plants not only contribute to their ecological success but also form the basis for many herbal medicines and commercial products. In this review, it is aimed to summarize the functions of secondary metabolites secreted by plants and the uses of some plants according to their active ingredients.

Key Words: Medicinal and aromatic plants, secondary metabolites, alkaloids, terpenoids, phenol compounds.

1.Introduction

Plants synthesize a wide range of compounds, classified as either primary or secondary metabolites, which directly or indirectly influence their growth and development. Among these, substances such as nucleic acids, proteins, carbohydrates, fats and lipids that are directly involved in plant life and essential for development are referred to as primary metabolites (Bakır, 2020). Secondary metabolites, on the other hand, are compounds that, unlike primary metabolites such as proteins, carbohydrates and fats, are not essential for plant life. These compounds are generally produced in lower quantities and have different chemical structures. Secondary metabolites are synthesized through specific biosynthetic pathways mediated by specialized enzymes and are stored in plant organs (Alaca and Arslan, 2012). Plant secondary metabolites are known to serve various functions, including the provision of protection against abiotic and biotic stress factors, the enhancement of the plant's competitive ability, and the attraction of organisms that are essential for pollination. Secondary metabolites are present in different amounts in various organs of plants, and they are utilized for diverse purposes depending on the types of metabolites they contain. Secondary metabolites have been demonstrated to play a crucial role in plant growth and development, due to their contribution to resistance against abiotic and biotic stressors, and their allelopathic effects (Seigler, 1998; Ramakrishna and Ravishankar, 2011; Tiring et al., 2020). Furthermore, secondary metabolites are currently employed as raw materials in a variety of industrial sectors, including dyes, fibers, oils, adhesives, pharmaceuticals, flavors, and perfumery. Moreover, they have a variety of applications in fields as diverse as agriculture, food production, and flavor enhancement (Çalışkan et al., 2019). Secondary metabolites have been demonstrated to play a role in the development of properties such as colour, taste, and aroma in plants. For instance, indigo found in the leaves of woad is responsible for colour formation, glycyrrhizin in the rhizomes of licorice contributes to taste, and menthol in peppermint oil is responsible for aroma (Baydar, 2016).

The medicinal uses of many plants have been scientifically validated due to the secondary metabolites they contain. Examples of such plants include Ginkgo biloba, which has been shown to enhance memory, Hypericum perforatum, which has been shown to have antidepressant effects, and Echinacea purpurea, which has been shown to boost the immune system. Consequently, secondary metabolites derived from plants are industrially converted into pharmaceuticals (Baydar, 2016). A multitude of plants, including Allium sativum, Salvia officinalis, Rosmarinus officinalis, Ocimum basilicum, Melissa officinalis, Mentha piperita, and Foeniculum vulgare, have been shown to possess antibiotic properties, with the secondary metabolites found in these species being regarded as natural antibiotics (Baydar, 2016). Secondary metabolites can also be utilized through the consumption of herbal teas prepared by infusion or decoction of plants (Baydar, 2016). It has been established that certain plant species, including Chrysanthemum cinerariaefolium, Azadirachta indica, and Acorus calamus, are characterized by insecticidal properties. These properties are attributed to the presence of secondary metabolites within the plants. In a similar manner, plants such as Pimpinella anisum, Salvia officinalis, Rosmarinus officinalis, Anethum graveolens, Mentha piperita, and Cuminum cyminum have been observed to exhibit fumigant effects as a consequence of their secondary metabolites. Furthermore, the secondary metabolites present in various plant species contribute significantly to pollination and fertilization processes by imparting distinctive features such as color and aroma (Baydar, 2016).

The present review aims to provide significant insights into the pharmaceutical potential of medicinal plants by presenting selected medicinal and aromatic plants and their uses, clarifying the classification and biological activities of secondary metabolites, and highlighting recent studies conducted on secondary metabolites in light of current research.

Certain Important Medicinal and Aromatic Plants

Table 1. The Utilized Plant Parts, Active Compounds, Essential Oil Contents, and Purposes of Using Certain Medicinal and Aromatic Plants

Family	Plant Species	Commonly Used Plant Part	Utilized Active Compounds	Essential Oil Content and Constituents	Common Uses
Apiaceae	*Pimpinella anisum* L.	Fruit	Terpenoids	Contains 1,50–6,00% essential oil, with anethole being the main constituent.	Used as a spice and for medicinal purposes. It is also commonly used in the production of alcoholic beverages.
Apiaceae	*Anethum graveolens* L.	Leaf	-	Contains 2,00–5,00% essential oil, with limonene as the primary component.	Used as a spice.
Apiaceae	*Cuminum cyminum* L.	Fruit	Terpenoids Phenolic Compounds	Contains 2,00–5,00% essential oil, where the main constituent is cuminaldehyde.	Used as a spice.
Apiaceae	*Coriandrum sativum* L.	Fruit	Terpenoids Phenolic Compounds	Contains 0,30–1,50% essential oil, with linalool as the major component.	Used as a spice.
Apiaceae	*Foeniculum vulgare* Mill.	Fruit	Terpenoids Phenolic Compounds	Contains 1,00–8,50% essential oil, and the main constituents are anethole and fenchone.	Used as a spice and for medicinal purposes.
Asteraceae	*Achillea milledolium* L.	Leaf Flower	Terpenoids Phenolic Compounds	Contains 0,60–0,85% essential oil.	Used for medicinal purposes.
Iridaceae	*Crocus sativus* L.	Stigma	Terpenoids	Contains 0,40–1,50% essential oil.	Used as a dye, spice, and for medicinal purposes.
Lamiaceae	*Salvia fruticosa* Mill.	Leaf	Terpenoids Phenolic Compounds	Contains 0,80–2,60% essential oil, with cineole being the major compound.	Used for tea and medicinal purposes.
Lamiaceae	*Rosmarinus officinalis* L.	Leaf	Terpenoids Phenolic Compounds	Contains 1,00–2,00% essential oil, with camphor, α-pinene, 1,8-cineole, and limonene as the main constituents.	Used as a spice, in cosmetics, and for medicinal purposes.

Lamiaceae	*Ocimum basilicum* L.	Leaf	Terpenoids Phenolic Compounds	Contains 0,30–1,00% essential oil, with the main constituents being linalool, methyl chavicol, eugenol, and estragole.	Used in the spice, cosmetics, and perfumery industries.
Lamiaceae	*Origanum spp.* *Thymus spp.* *Satureja spp.* *Thymbra spp.* *Coridothymus sp.*	Leaf	Terpenoids Phenolic Compounds	Contains 0,50–10,00% essential oil, with the main constituents being carvacrol and thymol.	Used as a spice, in tea, and for medicinal purposes.
Lamiaceae	*Melissa officinalis* L.	Leaf	Terpenoids Phenolic Compounds	Contains 0,01–0,50% essential oil, with the main constituent being nerol.	Used as a spice, in tea, and in the cosmetics and perfumery industries.
Lamiaceae	*Mentha x piperita* L.	Leaf	Terpenoids Phenolic Compounds	Contains 1,00–3,00% essential oil, with the main constituent being menthol.	Used as a spice, in tea, in cosmetics, and for medicinal purposes.
Laureceae	*Laurus nobilis* L.	Leaf Fruit	Terpenoids Phenolic Compounds	Contains 0,50–5,00% essential oil, with the main constituent being 1,8-cineole.	Used for medicinal purposes.
Papaveraceae	*Papaver somniferum* L.	Fruit Seed	Alkaloids	Contains 40,00–55,00% oil, with the main fatty acids being linoleic acid and oleic acid.	Used as a spice and for medicinal purposes.
Ranunculaceae	*Nigella sativa* L.	Seed	Terpenoids	Contains 0,10–0,50% essential oil, with the main constituent being thymoquinone.	Used as a spice and for medicinal purposes.
Rubiaceae	*Rubia tinctorum* L.	Rhizome	Phenolic Compounds	The active compound is alizarin, and it does not contain essential oil.	Used as a dye.

(Arslan et al., 2015, Baydar 2016)

Classification of Secondary Metabolites

There are more than 50,000 secondary metabolites, which can be classified into alkaloids, essential oils, phenols, glycosides, heterosides, steroids, saponins, flavonoids, tannins, colorants, and resins (Baydar, 2013; Çalışkan et al., 2019). These secondary metabolites, which have different chemical structures, are generally classified into three main groups: alkaloids, terpenoids, and phenolic compounds (Figure-1).

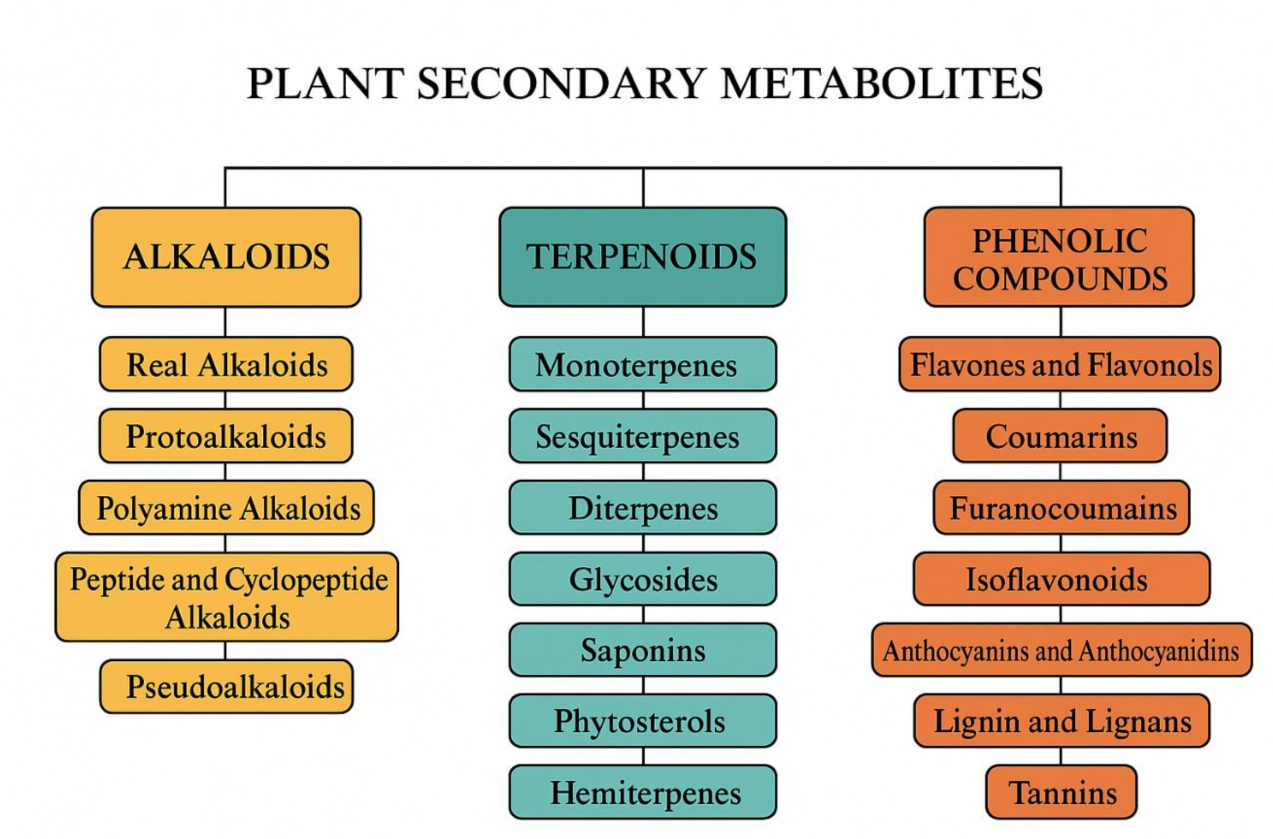

Figure 1. Classification of Secondary Metabolites

1. Alkaloids

Alkaloids are basic compounds that are typically derived from plants and contain one or more nitrogen atoms. They exhibit strong physiological and pharmacological activity (Alaca and Arslan, 2012). Plants that are rich in alkaloids and are cultivated for the purpose of extracting alkaloids are referred to as alkaloid plants. For instance, the opium poppy (Papaver somniferum), which contains the alkaloid morphine, and tobacco (Nicotiana tabacum), which contains the alkaloid nicotine, are pertinent examples of alkaloid-producing plants. These substances characteristically possess a bitter taste. In the present day, some of the most widely studied alkaloids include atropine, ephedrine, ergotamine, berberine, ellipticine, hyoscyamine, caffeine, quinine, colchicine, reserpine, morphine, cocaine, camptothecin, scopolamine, vinblastine, and vincristine (Baydar, 2016). Alkaloids have been demonstrated to possess both stimulatory and sedative effects, and therefore, these substances, whether natural or synthetic, are used as active pharmaceutical ingredients. When used in high doses, they can exhibit toxic effects.

Alkaloids are classified into 11 groups based on the active compounds they contain: pyridine alkaloids, piperidine alkaloids, tropane alkaloids, quinoline alkaloids, isoquinoline alkaloids, phenylethylamine alkaloids, indole alkaloids, purine alkaloids, terpenoid alkaloids, betaine alkaloids, and pyrazole alkaloids (Baydar, 2016).

2. Terpenoids

Terpenoids are a structurally diverse group of oxygen-containing organic compounds synthesized from terpenes. In other words, secondary metabolites with a hydrocarbon structure are referred to as terpenoids. Over 22,000 compounds belong to the terpenoid class (Connolly and Hill, 1991; Tiring et al., 2020). Terpenoids have numerous functions, such as attracting beneficial insects for pollination, repelling pests, inhibiting the germination of other plant seeds, preventing the growth of surrounding weeds, and repelling pathogenic microorganisms (Baydar, 2016). Additionally, terpenoids play a role in hormones, photosynthetic pigments, and the structural components of membranes (Tiring et al., 2020). Terpenoids are classified into five groups based on isoprene units: monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes.

3. Phenolic Compounds

Chemical substances that contain one or more hydroxyl groups attached to an aromatic ring are referred to as phenols (Shahidi and Naczk, 1995; Tiring et al., 2020). Phenolic compounds fulfil a variety of functions in plants, including protecting genetic material from UV radiation, deterring herbivores, preventing infections, and ensuring timely seed dispersal (Alaca and Arslan, 2012). Furthermore, they have been demonstrated to play a role in the processes of plant growth and development, fertilization, and defense mechanisms (Tiring et al., 2020). Specifically, a high concentration of phenolic compounds has been observed in red fruits and vegetables (Baydar, 2016).

A Brief Overview of Recent Scientific Research on Secondary Metabolites

In a research project conducted by Acar et al. (2019) on Bituminaria bituminosa, commonly known as Arabian pea or pitch clover, the researchers investigated the secondary metabolites found in the leaves of 12 different genotypes at various developmental stages, as well as their allelopathic and antimicrobial effects. They reported that leaf extracts obtained from the plant exhibited a positive allelopathic effect on wheat during germination and early growth stages. The study identified the presence of secondary metabolites such as psoralen, angelicin, daidzein, genistein, daidzin, and genistin, with variations observed across different growth stages. Furthermore, the genotypes examined were found to be effective in terms of their secondary metabolite content. However, the researchers noted that the quantities of secondary metabolites identified in their study did not exhibit any significant effect on antimicrobial activity. Çakır and Akyüz (2020) purified three metabolites from the chloroform extract of Pistacia vera L. stems using different methods and characterized their chemical structures. They introduced the molecule pistachionic acid to the scientific literature. Additionally, they reported that inulobiose was isolated from this species for the first time.

Göktürk Baydar (2020) aimed to develop an appropriate in vitro protocol for the production of secondary metabolites in Hyoscyamus niger. For this purpose, L-phenylalanine (FA) was applied to root cultures, and different harvest periods were evaluated. The levels of hyoscyamine, scopolamine, and phenolic compounds in both the roots and culture media were determined using HPLC analysis. As a result of the study, the highest amounts of scopolamine and hyoscyamine were obtained in the cultures harvested on the 3rd day following the application of 0.5 mM FA. Additionally, the highest levels of phenolic compounds were found in the cultures harvested on the 1st and 3rd days after the application of 0.5 and 1.0 mM FA. It was thus concluded that the levels of hyoscyamine, scopolamine, and phenolic compounds in Hyoscyamus niger root cultures can be enhanced by optimizing FA application and harvest timing. Yaman et al. (2020) conducted a study with the objective of determining the total alkannin/shikonin, total phenolic and flavonoid contents, and radical scavenging activity in Alkanna taxa. In their study, seeds from two Alkanna taxa were collected from nature and cultivated in MS media with different compositions. Consequently, the highest content of total alkannin/shikonin was obtained from the roots of Alkanna sieheana under in vivo conditions. The root extract of Alkanna orientalis was found to contain the highest total phenolic content in in vivo conditions. For flavonoid content, the highest amounts were obtained from the extracts of both taxa under in vivo conditions. With regard to radical scavenging activity, the highest activity was reported in the roots of Alkanna orientalis under in vivo conditions.

Yıldırım et al. (2021) aimed to determine the total flavonoid, phenolic compounds, radical scavenging activity, and condensed tannin content in Melilotus alba. For this purpose, they used 17 Melilotus alba genotypes collected from nature. As a result of their study, the average flavonoid content was determined to be 17.962 mg QE g^{-1}, the average phenolic compound content was found to be 3.901 mg GA g^{-1}, the average radical scavenging activity was 26.523%, and the average condensed tannin content was 0.611%. They reported that there were differences among Melilotus alba genotypes in terms of these secondary metabolites. Çetiz and Memon (2021), in their review study on plant secondary metabolites based on plant-microbe interactions, reported that plant-microbe interactions can alter the amount of secondary metabolites. Ayhan and Yıldırım (2021), in their study, aimed to determine the effects of autumn and spring sowings on yield and yield criteria in Papaver somniferum. They used the "Ofis 3" variety in their study. Various characteristics, including plant height, capsule length, capsule width, number of capsules per plant, capsule yield, seed yield, and morphine content, were examined. As a result of their study, the highest observations were obtained in all characteristics except for morphine content in autumn sowings. In spring sowings, while morphine content was higher, it was reported that the other characteristics were significantly negatively affected. They reported that morphine content ranged from 1.07-1.17% in autumn sowings and from 1.29-1.46% in spring sowings.

Turfan (2022) investigated the changes of certain chemical compounds, including pigments, nitrogenous compounds, secondary metabolites, reducing sugars, lipid peroxidation, pyruvic acid, and hydrogen peroxide, in fresh leaves and cloves of Allium sativum under field and greenhouse conditions. It was reported that the environment in which the subjects were cultivated was of crucial importance for all of the characteristics that were investigated. With regard to secondary metabolite content, it was stated that higher levels were found in fresh leaves of the OF-TD and GH-TD samples, and in cloves, the highest levels were found in the OF-KUC and GH-KUC samples. Gedik et al. (2022) determined the essential oil components of two different varieties of Mentha longifolia subsp. typhoides. The essential oils were obtained from the flowers and leaves of M. longifolia subsp. typhoides var. typhoides, with 16 and 19 different essential oil components identified, respectively. The essential oil components of the flower were determined as 56.87% piperitenone oxide, 15.89% cis-piperitone oxide, and 11.30% eucarvone, while the leaf essential oil components were identified as 35.69% cis-piperitone oxide and 35.20% piperitone oxide. In the M. longifolia subsp. typhoides var. calliantha, essential oil was extracted from the herb, and the essential oil components were reported to be 47.72% pulegone, 17.19% menthone, 7.89% trans-dihydrocarvone, 7.55% eucarvone, and 5.17% piperitone. Kuru et al. (2022) aimed to determine the total phenolic compounds, flavonoids, and antioxidant capacities in Pelargonium quercetorum Agnew grown under in vivo and in vitro conditions. According to the results of their study, the highest amounts of phenolic compounds and flavonoids were found in the in vitro shoot extract. A linear relationship between total phenolic compounds, flavonoids, and antioxidant activity was reported.

5.Conclusion

Secondary metabolites are of significant chemical importance to plants due to their roles in defense mechanisms, pollination and fertilization processes, and therapeutic properties. Due to their multifaceted biological activities, including but not limited to antimicrobial, anti-inflammatory, antioxidant, and anticancer properties, they hold significant potential for the treatment of various diseases. Consequently, there is a necessity to increase the production of these compounds, and numerous studies have been conducted with this objective in mind. In this context, a detailed investigation of the structural and functional characteristics of secondary metabolites will contribute both to the development of novel therapeutic approaches in modern medicine and to the more sustainable use of natural resources.

Conflict of Interest

There is no conflict of interest among the authors.

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POTENTIAL AROMATIC PLANT IN TÜRKİYE: *Iris germanica* L.

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Abstract

Iris germanica L., a perennial rhizomatous plant of the family Iridaceae, is an economically valuable species with high medicinal and aromatic value. Essential oil, absolute and tea obtained from the rhizomes of the species are used in the treatment of cough, diarrhea, anti-inflammatory, arthritis, rheumatism and sinus infections, mental problems such as distress, anxiety, fear and mental fatigue. It also has antioxidant properties. The triterpenoid iridals in iris essential oils are biosynthetic precursors of iron and are widely used in the perfume and cosmetics industries for their pleasant violet-like aroma and their ability to fix other fragrances. Iris species contain more than 250 chemical constituents, including flavonoids, isoflavonoids and their glycosides, as well as various secondary metabolites such as benzoquinones, triterpenoids and stilbene glycosides. α, β, ɣ-irones, which are the main constituents of *Iris germanica* essential oil and determine the quality of the essential oil, are formed by the gradual oxidation of structures called irides during the aging process of the rhizomes. 3-year-old rhizomes are dried after harvesting and extraction processes are carried out after the dry rhizomes are aged (2-5 years). In Turkey, *Iris germanica* is cultivated only in a limited area of 250 da in Isparta/Kuyucak. With this review, scientific researches on the botanical characteristics, uses, contents and cultivation of *Iris germanica* were examined and it was aimed to provide a source for future studies.

Key Words: *Iris germanica*, Orris Butter, Essantial Oil, Süsen, iron, Absolute

1.Introduction

Iris germanica is a perennial, rhizomatous plant belonging to the Iridaceae family (Ceylan, 1997). Although there are more than 300 iris species globally, 56 naturally occurring iris taxa are found in the flora of Turkey (Erken et al., 2016). The species does not have a specific climate requirement and has high adaptability. Although it grows well in well-aerated soils (Ceylan, 1997), it can also grow in soils with low organic matter content and no particular preference for lime (Erken et al., 2013). It can be propagated both by seed and rhizome, but requires 2–3 years before harvesting (Baydar, 2022). Harvest time affects the yield and content of the rhizomes (Kara & Gürbüzer, 2019).

The compounds in the rhizomes of *Iris germanica* have a pleasant violet-like fragrance, making them a valuable raw material in the perfume industry (Kara & Baydar, 2024). Irises are rich in secondary metabolites, with over 250 identified components (Sevim, 2018). Additionally, compounds found in *iris spp.* have known antineoplastic, antioxidant, anti-inflammatory, antiulcer, antiplasmodial, antituberculosis (Yousefsani et al., 2021), antimicrobial, and antifungal effects (Öztaş et al., 2024; Başgedik, 2013). In folk medicine, they are used for skin conditions, as antispasmodics, to regulate menstruation, relieve cough and diarrhea, as diuretics, anti-inflammatories, and expectorants (Asghar et al., 2011; Demirezer et al., 2022; Demirezer et al., 2024).

Taxonomy and Botanical Characteristics

Plants of the Iridaceae family are perennial herbaceous species with underground bulbs, corms, or rhizomes (Ceylan, 1997). The family, which is distributed throughout the Northern Hemisphere and thrives in tropical and subtropical regions, comprises approximately 70 genera and 1800 species (Gülgün et al., 2009). In Turkey, it is represented by six genera—Iris, Gynandriris, Romulea, Gladiolus, Hermodactylus, and Crocus—with a total of 91 species (Karacan et al., 2019). The genus

Iris, which lends its name to the family, is the largest in the group, with over 300 species globally. In Turkey, 56 natural Iris taxa have been recorded, 24 of which are endemic. Due to the significant morphological differences among species found in Turkey, the genus has been divided into five subgenera: Limniris, Oncocyclus, Scorpiris, Iris, and Hermodactyloides. Some subgenera are rhizomatous, while others are bulbous. Irises have a wide ecological tolerance, growing in aquatic environments, rocky terrains, arid, and sunny habitats (Erken et al., 2016). The name “Iris” comes from the Greek word for “rainbow,” referencing the flower's diverse colors (Öztaş & Öztaş, 2024). In Turkey, Iris species are known by various local names such as nevruz flower, sultan navruz, mavruz, süsen, yılan zambağı, yayla zambağı, and many others (Erken et al., 2013). Irises are ornamental plants with showy, fragrant flowers and are often grown in parks and gardens. The rhizomes of species such as Iris florentina, Iris pallida, and Iris germanica are valued in the perfume industry for their violet-like scent (Kara & Baydar, 2024).

Iris germanica is a species in the Iris genus of the Iridaceae family. While its exact origin is not definitively determined, it is believed to be native to southern Europe and Eurasia (Yousefsani et al., 2021). Genetic studies have shown close relationships with I. variegata and I. pallida (Li et al., 2020), and some sources suggest that these two species may be hybrids (Anonymous, a). Today, it is widely cultivated in many countries, including China, Pakistan, Egypt, Turkey, Morocco, and Iran (Yousefsani et al., 2021). In Turkey, industrial production is carried out on a 250-decare area in Kuyucak village, Isparta province (Baydar, 2022). Common and English names for Iris germanica include Bearded Iris, Common Orrisroot, Flag, Florentine Orris, Florentine Iris, Garden Iris, German Iris, German Orrisroot, Iris, Orris, Orrisroot, Purple Flag, Queen Elizabeth Root, Tall Bearded German Iris, and Tall Bearded Iris. In Turkish, it is known as mor süsen, Türk süseni, and navruzi (Lim, 2016).

Iris germanica is a flowering, perennial plant with thick underground stems (rhizomes). The plant can grow to a height of 30–100 cm and produces fragrant flowers that consist of four to five blossoms on long stems. The flowers lack pedicels and are few in number but have a pleasant fragrance. They undergo artificial fertilization (Ceylan, 1997). The sepals are spread out, hanging down or reflexed, and show color transitions resembling shadows on their surfaces. The sepals are in spreading, drooping, or reflexed (curved backward) positions and come in a variety of colors, including blue-purple, yellow, brown, or white. They are decorated with a dark blue-purple pattern. A beard (a white or yellow structure) runs along the middle vein of the claw section and beneath the lobe (Lim, 2016). The seeds are angular, measuring 7–10 mm by 4–5 mm (Ceylan, 1997). The seed capsule is approximately 5 cm long, with brown seeds arranged in sections like rolls of paper. The leaves are wide, sword-shaped, typically curly, and gray-green in color, with a width ranging from 2.5 to 4.5 cm. The medically used parts of the plant are its thick, short rhizomes and roots (Yousefsani et al., 2021). While some sources report a chromosome number of 2n=20 (Azimi et al., 2018), cytogenetic analyses in a research project (Erken et al., 2009) revealed that the chromosome number in the yellow-flowered *Iris germanica* variety is 2n = 48, while in the *Iris germanica biliottii* subspecies, it is 2n = 44.

Cultivation

Climate and Soil

Due to their high adaptability, *iris spp.* do not require a specific climate. They need strong light, but areas that receive morning sun or indirect light are preferred over direct sunlight. Although not selective in terms of soil, they prefer well-aerated soils (Ceylan, 1997; Lim, 2016). In a study conducted in Isparta (Kara & Baydar, 2024), the trial plots were reported to have low salinity, sandy-loam texture, pH: 8.19, lime content of 28.14%, and organic matter content of 1.68%. In another study conducted in Yalova, natural growing soils of three different Iris germanica ecotypes were analyzed and found to have pH levels of 7.71, 7.54, and 7.9; lime contents of 18.79%, 3.62%, and 19.14%; and organic matter contents of 6.71%, 20.42%, and 2.54% respectively (Erken et al., 2009). In a trial conducted in Diyarbakır, soil properties were defined as pH 7.45, salt content 0.16%, clay-loam texture, and poor in phosphorus and organic matter (1.16%) (Pala, 2006). In a study on salt stress, four different Iris germanica genotypes were exposed to NaCl levels of 4, 6, 8, and 12 dS/m (Azimi et al., 2025). Another study tested salinity tolerance at 70, 105, 140, and 175 nM, and found that different varieties responded differently to salt stress (Zhao, Li et al., 2021). As part of a project on Iris populations in Turkey, samples were collected from provinces including Adıyaman, Bursa, Gaziantep, Hatay, İzmir, Kocaeli, Kahramanmaraş, Mardin, Muğla, Sinop, Trabzon, Karaman, and Yalova. In the same study, adaptation abilities of 45 Iris populations were evaluated, and Iris germanica was found to have the highest adaptability among 11 different Iris species (Erken et al., 2013).

Propagation

Iris germanica can be propagated both by seeds and rhizomes; however, for seed propagation, artificial pollination of the plant is required (Ceylan, 1997). Seed propagation is used in large-scale production and breeding studies. Additionally, there is a necessity for the germination of hybrid seeds obtained from breeding hybridization studies (Erken et al., 2009). Seed propagation requires a longer period for the plants to flower, which is why propagation by rhizomes is more common (İpek et al., 2013). Iris seeds can germinate within 2 to 3 years. It is stated that a germination rate of 50% or higher in seeds sown in spring is considered normal for Iris seeds. However, in a study where pre-treatments and different sowing times (July, August, September, October) were applied, seeds sown in August had a germination rate of 58.7% in the first year and 10.7% in the second year, with the highest germination rate (69.3%) found. Furthermore, it was found that cold stratification at +5°C and moist stratification at +5°C were ineffective, and the highest germination rate (79%) was in the control seeds without any treatment (Erken et al., 2016). In another study where seeds were sown in September, a germination rate of 55.7% was found. In the same study, subsequent trials in the following year involved pre-treatments such as moist stratification in +5°C perlite (25.40%), dry stratification at +5°C (17.14%), and storage at room temperature (33.85%). However, pre-treatment procedures were also unsuccessful in this study (Erken et al., 2009).

Iris plants propagate from rhizomes by producing new shoots from each node. The dug-up rhizomes are cut into pieces with one or two leaves and roots, and these pieces are planted between September and March (İpek et al., 2013). The spacing should be 20–25 cm between rows and 20 cm within the rows (Ceylan, 1997). Rhizomes for seed production can survive in a soilless environment for a maximum of 2 weeks and should be planted immediately if possible. Planting in the same direction allows the rhizomes to grow comfortably without overcrowding (Anonymous b, 2025).

Another vegetative propagation method includes studies on in vitro propagation of *Iris germanica*, which has shown positive results in micropropagation and greenhouse transfer (Xu & Huang, 2015).

Soil Preparation

Leguminous plants, such as those from the legume family, and root crops can be used as suitable preceding crops for *Iris germanica* (Ceylan, 1997). Soil should be prepared 1-2 weeks before planting, and organic matter can be used to increase yield. For poor soils, a 5-10-5 compound fertilizer can be added. In heavy soils, drainage should be improved with organic matter and compost. Weed control should be carried out, and care should be taken to avoid damaging the rhizomes during post-planting operations. In cold climates, rhizomes can be mulched with straw or grass during the first season to protect them from frost damage (Graper, 1991).

Irrigation and Fertilization

While Iris species are drought-resistant, for rhizome-producing varieties, irrigation should be avoided during the resting period between July and September (Yılmaz, 2020). Irrigation may only be done during the first year of field establishment (Gürbüzer, 2018). Excessive irrigation can increase fungal diseases (Erken et al., 2009). The species is sensitive to root rot caused by excessive moisture and irrigation. Fertilization can be done based on soil analysis, using the appropriate type and amount of fertilizer (Anonymous b, 2025). Fertilizer can be applied at a rate of 5 kg/da for nitrogen and 5 kg/da for phosphorus (Gürbüzer, 2018). It has been determined that increased nitrogen applications (5-10-15 mM) in *Iris germanica* enhance rhizome size and increase the uptake of N, P, Mg, Mn, Zn, and B (Zhao et al., 2021).

Diseases and Pests

A sunny, spacious field with good drainage prevents disease outbreaks. Major diseases and pests include bacterial soft rot, fungus rots, iris leaf spot, rust and bacterial leaf spot, mosaic, iris borer, aphids, verbena bud moth, iris thrips, and nematode infection (Graper, 1991). In a project conducted in Yalova, where different Iris species were cultivated, fungal diseases were frequent, especially due to humid air and irrigation, with the most common pathogens being Rhizoctonia solani and Fusarium oxysporum, which cause rhizome rot. Additionally, Botrytis cinerea was also detected. Among pests, the larvae of Eumerus amoenus (Diptera: Syrphidae) feed on the rhizomes, causing them to rot and stunting the plant, while the wounds they create serve as entry points for secondary infections. Pests like Heliothis sp. (green caterpillar), Otiorhynchus sp. (vine weevil), Epicometis hirta (flower beetle), and Agrotis spp. (cutworm) cause damage to leaves, buds, and flowers. Snails and slugs have also been identified as pests and controlled (Erken et al., 2009).

Harvest and Yield

For essential oil production, rhizomes are harvested in the third year during the summer months. Small pieces are used for seed production, while larger pieces are cut to prevent sprouting and to dry quickly, then stored in the shade at room temperature (Kara & Baydar, 2024). Pieces are cut to a thickness of 3 cm and a length of 5–10 cm. The older the plantation, the higher the yield. Fresh yield can range from 400 to 1200 kg/da (Ceylan, 1997). One kilogram of dry rhizome is obtained from approximately 4 kg of fresh rhizomes (Baydar, 2022). In a study conducted in a 3-year-old trial field, it was found that *Iris germanica* has 3.27-6.47 rhizomes per plant, with weights ranging from 85.55 to 186.52 g. The fresh rhizome yield per decare was 972.8-1651.2 kg, and the dry rhizome yield was 212.33-457.50 kg (Kara & Gürbüzer, 2019). The duration rhizomes stay underground and the harvest time affect the iron content of iris rhizomes. A study to determine the optimum rhizome harvest time found that in Isparta conditions, the highest essential oil yield occurs in August, the highest resinoid yield occurs in July, and the highest quality resinoid is obtained in August. Kara and Gürbüzer (2019) also noted that September is the most suitable time for both fresh and dry rhizome yield.

Fresh rhizomes are not directly suitable for extraction since the aromatic compounds known as iridals have not yet formed. Iridals are formed during the storage of rhizomes, as precursor triterpenoid compounds called iridals undergo oxidative transformation over time. Particularly in young rhizomes, free and monosyclic iridals are abundant, but with age, these compounds turn into cyclic structures. For this reason, dried rhizomes are typically stored for 2 to 5 years. As the storage period increases, the amount of iridals also increases, which directly affects the odor quality and economic value of the essential oils (Baydar, 2022; Kara & Baydar, 2024). A study examining the proportion of iridals in fresh and dry rhizomes suggested that rhizomes should be stored for at least 24 months after harvest (Gürbüzer, 2018).

Secondary Metabolites

Irises are notable for their rich secondary metabolite content. Phytochemical studies conducted on species of this genus have identified over 250 compounds. These compounds mainly include flavonoids, isoflavonoids and their glycosides, benzokinones, triterpenoids, and stilbene glycosides. The leaves of the plant contain ascorbic acid and various vitamins, while the rhizome contains terpenoid derivatives, organic acids, and iridin glycosides (Sevim, 2018). In *Iris germanica*, the iron compounds, which are the main components of the essential oil, determine its value in perfumery. As rhizomes age, the iron content increases. Although the essential oil yield from rhizomes ranges from 0.25% to 0.50%, it can vary depending on the month in which the rhizomes are harvested. The most suitable harvesting month has been found to be August in Isparta conditions (Baydar, 2022; Kara &

Baydar, 2024). Iris essential oil is a thick, viscous oil with a color ranging from pale yellow to cream and a woody, violet-like aroma. It is widely used as a fixative in perfumery and is also found in the production of scented candles, room sprays, and incense. In skincare, it is used for its moisturizing properties and in the treatment of certain dermatological conditions. The essential oil can be diluted with carrier oils for application. Iris absolute is a concentrated product extracted from the rhizomes using a hydrocarbon solvent and then processed with ethanol. This absolute has a violet-like, woody aroma. In medical use, it is highlighted for its expectorant, diuretic, anti-inflammatory, and soothing effects. It is commonly used in aromatherapy, especially in bath water applications. However, internal use of the essential oil is not recommended. Instead, an infusion made from dried rhizomes is used for its expectorant and diuretic properties (Demirezer et al., 2022; Demirezer et al., 2024).

There are many extraction methods developed to isolate the active compounds in medicinal plants. Standards have been established for the identification of natural raw materials in industrial production. According to the ISO 9235:2021 standard, published in Turkey under the title "Aromatik Doğal Hammaddeler – Sözlük", Iris products are defined under categories such as essential oil, rectified essential oil, non-acid essential oil, concrete essential oil, resinoid, and absolute. In the U.S. (CAS, FEMA), EU (ECHA), and China (INCI) systems, these products are named differently, and it has been pointed out by researchers that some definitions may lead to confusion. A clear classification in line with standards is of great importance for the perfumery and cosmetics industries (Bicchi & Joulain, 2024). Iris essential oil is usually obtained from rhizomes by steam distillation. However, to volatilize the high-boiling iron compounds in the content, distillation must be conducted under low pressure. For this purpose, a reflux column filled with Raschig rings is used. The system operates without cooling, allowing not only the irons but also long-chain hydrocarbons to be carried directly to the top of the column. At approximately 98°C, the oil and water phases are separated and removed from the Florentine flask. The resulting product is semi-solid at room temperature and is known in the perfume industry as orris essential oil or orris butter. Since no solvents are used in this process, the term "orris concrete" used in some sources is technically incorrect (Başer & Buchbauer, 2009). From *Iris germanica* rhizomes, resinoid can be obtained with yields approximately ten times higher than essential oils by using organic solvents such as n-hexane and ethyl acetate. In this process, dried rhizomes aged for two years are ground and subjected to three extraction cycles with a mixture of n-hexane and ethyl acetate (70:30). The resulting extracts are filtered and the solvents are removed under low pressure to obtain the resinoid in semi-solid form. The resinoid obtained differs from the essential oil in terms of chemical composition and aroma profile (Roger et al., 2012).

In a study conducted in Pakistan (Asghar et al., 2011), the extract obtained from the entire plant using petroleum ether was analyzed using gas chromatography-mass spectrometry (GC-MS). The analysis revealed compounds such as 9-hexadecanoic acid methyl ester, 9-octadecenoic acid methyl ester, 8-octadecenoic acid methyl ester, 11-octadecenoic acid methyl ester, 10-octadecenoic acid methyl ester, 13-octadecenoic acid methyl ester, 16-octadecenoic acid methyl ester, 1,2-benzenedicarboxylic acid diisooctyl ester, bis(2-ethylhexyl) phthalate, methyl 6-methyl heptanoate, nonanoic acid, and 9-oxo-methyl ester. In Iran, a study (Ghasemi et al., 2023) conducted on Iris rhizomes obtained essential oil by hydro-distillation and analyzed it using GC-FID and GC-MS methods. Compounds such as α-pinene, γ-terpinene, decanoic acid ethyl ester, tetradecane, α-ionone, α-irone, β-irone, dodecanoic acid, ethyl dodecanoate, 1-hexadecene, hexadecane, trans-farnesol, and β-acoradienol were detected. In Turkey, two separate studies analyzed essential oils obtained from rhizomes using steam distillation and GC-MS. In the study (Kara & Baydar, 2024), compounds such as α-irone, γ-irone, and 6-methyl-ionone were identified. In a study conducted by Kara and Gürbüzer, 2-pentanone, 4-hydroxy-4-methyl, α-irone, γ-irone, and myristic acid were detected. In a study on the hydro-distillation of *Iris germanica* flowers and flower buds (Dulgheru & Burzo, 2011), GC-MS analysis of the volatile oils revealed that the buds contained 8 main components from 26 compounds: tricosane, heptadecadiene, heptacosane, heneicosane, methyl tricosane, and nonadecane. The differences in the compounds and their ratios can be explained by the influence of genetic, ontogenetic, morphogenetic, and environmental factors that can alter the content of secondary metabolites in the biosynthesis of these compounds (Verma & Shukla, 2015).

Medicinal and Aromatic Uses

Iris species attract attention due to the various phytochemical compounds they contain, such as flavonoids and their glycosides, triterpenoids, benzoquinones, and stilbene glycosides, which exhibit remarkable pharmacological effects. These compounds have antineoplastic (anti-tumor), anti-inflammatory, antiulcer, antiplasmodial (anti-malaria), antituberculosis (Yousefsani et al., 2021), and antioxidant (Ullah et al., 2016) properties. Historically, the peeled and dried rhizomes (orris root) of species such as *Iris germanica* and *Iris pallida* have been used for various medicinal purposes. In folk medicine, they are used for skin disorders, as antispasmodics, menstruation stimulants, antitussives, antidiarrheals, diuretics, anti-inflammatories, and expectorants (Asghar et al., 2011).

Extracts obtained from these species have been shown in many studies to possess antimicrobial, antifungal, and anti-inflammatory properties. Furthermore, isoflavones isolated from *Iris germanica* rhizomes have been reported to exhibit inhibitory effects in cancer treatment (Öztaş et al., 2024; Başgedik, 2013). A study conducted on mice with Alzheimer's disease reported that it could improve spatial memory (Borhani et al., 2017). Ethanolic extracts of Iris germanica rhizomes and leaves were reported to have molluscicidal activity on Biomphalaria alexandrina (snail) (Singab et al., 2006). Apart from its medicinal uses, it has been reported that the roots are used as spices, as flavoring agents in ice cream, candy, cakes, gin, and syrups, and that its starch is used as bread flour (Lim, 2016).

Perfume

The history of perfume is as old as humanity and was first discovered with natural scents and then through the burning of resinous woods. In Ancient Egypt, aromatic oils and incense were used, and over time the perfume culture spread to various civilizations and was brought to Europe with the Crusades. The perfume industry developed especially in Italy and France, and in 1370, the first alcohol-based perfume “Hungary Water” was produced. From the 16th century onwards, with the abundance of raw materials and the discovery of synthetic fragrances in the 19th century that made perfume accessible rather than luxurious, the modern perfumery industry was born (Demir et al., 2020). In 1893, Ferdinand Tiemann, in a study investigating the oil of the violet flower, accidentally isolated the fragrance components of iris root and identified the irone compounds. Despite the long time since their identification, commercial compounds do not meet the demand of the perfume industry from a sensory perspective, and natural iris oil continues to be used in perfumes (Brenna et al., 2003). Today, more than 200 perfumes contain iris in their formulation (Anonymous e, 2025). Some commercial products containing iris include Cartier Iris Ganach, Extravagance d’Amarige, and Chanel 19 (Öztaş et al., 2024). The price of iris essential oil is reported in sources to be 120,000 USD (Başer & Buchbauer, 2010) and 15,000 euros (Roger et al., 2012). Although prices vary, it was observed that in April 2025, when this research was conducted, the retail price of 1 gram of orris butter was between 35 USD (with 15% irone, from I. pallida) and 44 euros (with 13% irone, from I. germanica) (Anonymous c, 2025; Anonymous d, 2025).

Conclusion

With this review, the botanical characteristics, cultivation, usage, and content of the Iris germanica species, which belongs to the Iridaceae family, and its potential in Türkiye have been addressed. Türkiye, with its rich biological diversity and various climatic conditions, has significant potential. This species, which can be grown in different regions of Türkiye, stands out as a valuable raw material in the perfumery sector. Its cultivation is carried out in a very limited area in Türkiye. However, its adaptability and yield potential indicate that its cultivation can be tried in all regions. It is seen that studies on the cultivation of Iris germanica are insufficient. For rhizome cultivation, detailed research should be conducted on appropriate cultural practices for each region, considering regional differences, and on issues affecting quality and yield. In the initial stage of production, the method of propagation by seed, which is economical and easy, should also be emphasized. New studies to be conducted with different pre-treatment techniques, germination environments, and hormone applications on the seeds, which have a germination period extending up to almost 3 years, can increase the quality of the seed material and reduce the initial installation cost. The irones found in the essential oils extracted from iris rhizomes are important as fixatives in the perfume industry. Its characteristic violet-like scent is obtained after the rhizomes are dried and stored for a long time. The fact that 3 years must pass for the harvest of the rhizomes and that a long storage period of 2–5 years is required after harvesting for the irones to reach sufficient levels in the essential oil that gives the desired fragrance quality may reduce the producer's willingness to invest in this product. Iris cultivation, with its resistance to drought, salt stress, and lime, can be tried in agricultural lands with low productivity and economic return. Its high commercial potential can support rural development and provide a source of income for local producers. With practices such as offering purchase guarantees and providing seed material support, supporting local producers through public-private partnerships, providing necessary training to producers, training qualified labor at all stages, and investing in technology infrastructure, raw materials that comply with standards can be produced in a sustainable production model, contributing to the increase of Türkiye's export potential.

Although there are many scientific studies in the literature on the medicinal, cosmetic, and perfumery uses of *Iris germanica*, clinical studies are lacking. Verifying its effects on human health is important for the widespread recognition of its potential. It will also increase its reliability as a natural product with medicinal use. In conclusion, the cultivation of *Iris germanica* in Türkiye can create new potential in terms of economic, social, and cultural aspects.

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IN SILICO ANALYSIS OF SAFETY AND EFFICACY OF HERBAL SKIN-LIGHTENING AGENTS: A TOXICOLOGICAL PERSPECTIVE

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Abstract

Skin-lightening products represent a multi-billion-dollar global industry driven by complex sociocultural factors and beauty standards. While synthetic agents like hydroquinone, mercury compounds, and topical corticosteroids dominate this market, their documented toxicity profiles have raised significant safety concerns. This study critically evaluates the historical context, usage patterns, and toxicological implications of conventional skin-lightening agents, with a focused analysis of plant-derived alternatives. Natural compounds, including glabridin (*Glycyrrhiza glabra* L.), aloesin (*Aloe vera* (L.) Burm.f.), mulberroside F (*Morus alba* L.), and ellagic acid (various plant sources), demonstrate tyrosinase inhibition with potentially improved safety profiles compared to synthetic alternatives. However, a comprehensive assessment of their pharmacokinetic parameters and toxicity profiles remains inadequate. This research addresses this gap through comprehensive in silico analysis examining the drug-likeness of selected herbal compounds using computational models to predict Log Kp (skin permeation) values, passive intestinal absorption, and potential brain penetration as functions of lipophilicity and apparent polarity. In addition to assessing bioavailability, the active efflux by P-glycoprotein is evaluated. Beyond established mechanisms, ligand-based virtual screening by similarity and target prediction algorithms are employed to identify both therapeutic targets and potential off-target interactions responsible for toxicity. This multifaceted computational approach provides critical insights into the safety and efficacy of herbal skin-lightening agents, offering a foundation for developing safer alternatives to conventional products with established toxicity concerns. The findings contribute to evidence-based selection of herbal compounds for skin-lightening applications and establish methodological frameworks for safety assessment of cosmeceutical ingredients derived from medicinal and aromatic plants. The computational methodology we use is applicable to all classes of herbal bioactives across various therapeutic domains, delivering crucial drug-likeness parameters, comprehensive ADME profiles, and toxicological risk assessments as demonstrated in our extensive work with diverse medicinal plants, including *Reynoutria japonica* Houtt.

Key Words: Skin-lightening, herbal compounds, tyrosinase inhibitors, in silico toxicology, pharmacokinetic prediction, medicinal plants.

1. Introduction to In Silico Approaches for Cosmetic Ingredient Safety Assessment

Computational toxicology and in silico prediction methods have emerged as valuable tools for preliminary safety assessment of cosmetic ingredients, particularly in the post-animal testing regulatory environment (Cronin et al., 2017). When the data are interpreted by a toxicologist and pharmacognost, one such computational platform may provide critical physicochemical and pharmacokinetic parameters relevant to the toxicological evaluation of potential cosmetic ingredients. Understanding these parameters offers significant insights into both efficacy and safety profiles of natural compounds intended for cosmetic applications.

Key Parameters for Toxicological Assessment in Cosmetics

The toxicological evaluation of cosmetic ingredients necessitates careful consideration of several physicochemical and pharmacokinetic parameters that significantly influence both safety and efficacy. Parameters such as **molecular weight**, **lipophilicity (LogP)**, **topological polar surface area (TPSA)**, **molecular flexibility (rotatable bonds)**, as well as absorption and distribution-related characteristics including **gastrointestinal absorption**, **blood-brain barrier permeability**, **P-glycoprotein substrate status**, **cytochrome P450 enzyme inhibition**, and **skin permeation potential (Log Kp)**, play a crucial role in risk assessment. These factors, when misinterpreted or disregarded, can lead to substantial errors in safety conclusions, particularly in estimating systemic exposure and local tolerability (Bos & Meinardi, 2000; Naegel et al., 2013; Ertl et al., 2000; Veber et al., 2002; Pajouhesh & Lenz, 2005; Schinkel & Jonker, 2003; Zhou et al., 2005; Potts & Guy, 1992).

In addition, the presence of specific **structural alerts**—such as PAINS (Pan-Assay Interference Compounds) and toxicophoric substructures identified by **Brenk alerts**—can indicate the potential for undesirable effects, including skin irritation or sensitization, even at low exposure levels (Baell & Holloway, 2010; Brenk et al., 2008). Moreover, while originally designed for pharmaceuticals, established **druglikeness filters** (Lipinski et al., 2001) offer valuable insights into the general biocompatibility and "chemical reasonableness" of cosmetic compounds. It is therefore essential that these parameters are not only identified but also interpreted appropriately in context. Oversimplified or incorrect assessments may compromise the reliability of toxicological evaluations and ultimately the safety of cosmetic products. The integration of these parameters enables a comprehensive preliminary assessment of cosmetic ingredient safety, identifying potential areas of concern for further experimental validation while reducing reliance on animal testing methodologies, in line with current regulatory trends in cosmetic safety evaluation (Buyukyildirim et al., 2025).

2. Toxicological Assessment of Herbal Skin-Lightening Agents

Comparative Analysis of Natural Tyrosinase Inhibitors

The four evaluated herbal skin-lightening compounds—glabridin, aloesin, mulberroside F, and ellagic acid—demonstrate distinct physicochemical and pharmacokinetic profiles with significant implications for their safety and efficacy in cosmetic applications.

Glabridin (*Glycyrrhiza glabra* L.)

Glabridin exhibits optimal physicochemical properties for dermal application, with a moderate molecular weight (328.32 g/mol) and balanced lipophilicity (consensus LogP 2.78). The moderate TPSA value (89.13 Å²) and limited number of rotatable bonds (3) contribute to its favorable predicted skin permeation coefficient (Log Kp -5.94 cm/s), suggesting efficient delivery to melanocytes in the basal layer of the epidermis (Nerya et al., 2003). The absence of PAINS or Brenk structural alerts indicates minimal potential for non-specific reactivity or toxicity. However, the predicted inhibition of multiple CYP enzymes (CYP1A2, CYP2C9, CYP2D6, and CYP3A4) represents a potential safety concern if significant systemic absorption occurs. Current evidence suggests that percutaneous absorption of glabridin through intact skin remains limited, but application to damaged skin or excessive use could potentially lead to systemic effects and drug interactions (Yokota et al., 1998). Glabridin satisfies all druglikeness criteria, reflecting its favorable overall molecular behaviour. The moderate synthetic accessibility score (3.53) suggests feasibility for commercial-scale production, an important consideration for cosmetic formulation.

Aloesin (*Aloe vera* (L.) Burm.f.)

Aloesin demonstrates a favorable molecular weight (280.23 g/mol) for skin permeation but exhibits significantly higher hydrophilicity (consensus LogP 0.16) compared to glabridin. This property, combined with an elevated TPSA (124.29 Å^2), contributes to its reduced predicted skin permeation (Log Kp -7.82 cm/s), potentially limiting efficacy but also reducing systemic exposure risk (Choi et al., 2002). The presence of both PAINS (quinone_A) and Brenk (hydroquinone) structural alerts warrants attention regarding potential reactivity. The quinone moiety specifically raises concerns about oxidative stress induction and protein reactivity, mechanisms associated with skin sensitization (Aptula et al., 2006). However, the low lipophilicity and limited skin permeation may mitigate these risks by restricting exposure to viable epidermal cells. The absence of predicted CYP enzyme inhibitions suggests minimal potential for drug interactions, enhancing aloesin's safety profile for widespread cosmetic application. The compound satisfies all druglikeness criteria and demonstrates favorable synthetic accessibility (3.07).

Mulberroside F (*Morus alba* L.)

Mulberroside F exhibits the most challenging physicochemical profile for cosmetic application among the evaluated compounds. Its high molecular weight (512.46 g/mol) and extreme hydrophilicity (consensus LogP -1.62) result in poor predicted skin permeation (Log Kp -10.35 cm/s), potentially limiting efficacy as a skin-lightening agent (Lee et al., 2002). The compound fails multiple druglikeness criteria, with violations of Lipinski (MW>500, NorO>10, NHorOH>5), Ghose (MW>480, WLOGP<-0.4), Veber (TPSA>140), Egan (TPSA>131.6), and Muegge (TPSA>150, H-acc>10, H-don>5) rules, reflecting potential challenges in formulation and bioavailability. The presence of PAINS (catechol_A) and Brenk (catechol) structural alerts indicates potential for oxidative reactivity and protein binding. Catechol moieties have been explicitly linked to skin sensitization through the formation of reactive quinones and subsequent haptenation of cutaneous proteins (Karlberg et al., 2008). However, the extremely low skin permeation potential may significantly mitigate these concerns by limiting exposure to viable epidermal cells. The high synthetic accessibility score (5.59) suggests potential challenges in commercial-scale production, which may impact the cost-effectiveness of cosmetic formulations.

Ellagic Acid (Various Plant Sources)

Ellagic acid has an intermediate molecular weight (342.21 g/mol) with moderate lipophilicity (consensus LogP 1.39). Its high TPSA (147.43 Å^2) and limited rotational flexibility (1 rotatable bond) contribute to moderate predicted skin permeation (Log Kp -6.73 cm/s), positioning it between glabridin and aloesin in terms of potential efficacy (Shimogaki et al., 2000). The presence of Brenk structural alerts (beta_keto_anhydride, more_than_2_esters) suggests potential for hydrolytic instability and reactivity. However, ellagic acid has an extensive history of use in cosmetics with minimal reported adverse events, suggesting these structural features may not translate to significant in vivo toxicity under normal use conditions (Yoshimura et al., 2005). Ellagic acid passes the Lipinski, Ghose, and Muegge criteria but fails the Veber and Egan due to a high TPSA, indicating potential limitations in membrane permeability. The compound demonstrates favorable synthetic accessibility (2.77), indicating its feasibility for commercial-scale production.

Integration of Safety and Efficacy Considerations

The comprehensive in silico analysis reveals a gradient of safety and efficacy profiles among the evaluated herbal skin-lightening agents:

1. **Glabridin** demonstrates the most favorable balance of predicted efficacy (optimal skin permeation) and safety (absence of structural alerts). The primary toxicological consideration involves potential CYP enzyme inhibition if significant systemic absorption occurs, necessitating careful consideration of concentration limits and application to intact skin only.
2. **Ellagic acid** presents a reasonable compromise between efficacy and safety, with moderate skin permeation and limited toxicological concerns despite structural alerts. Its extensive history of safe use in cosmetics provides additional reassurance regarding its suitability for skin-lightening applications.

3. **Aloesin** exhibits lower predicted skin permeation, which may limit its efficacy while also reducing systemic exposure. The presence of quinone-related structural alerts warrants consideration of stabilization strategies in formulation to minimize potential reactivity and sensitization.
4. **Mulberroside F** demonstrates the most challenging profile, with poor predicted skin permeation potentially limiting efficacy, and catechol-related structural alerts suggesting potential for sensitization if oxidation occurs. However, its extremely limited skin penetration may significantly mitigate toxicological concerns by restricting exposure to viable epidermal cells.

When compared to conventional synthetic skin-lightening agents like hydroquinone, mercury compounds, and topical corticosteroids—all associated with documented toxicity concerns including exogenous ochronosis, nephrotoxicity, and skin atrophy, respectively (Dlova et al., 2015)—these herbal alternatives offer potentially improved safety profiles, particularly when formulated with appropriate stabilization and at controlled concentrations.

Conclusion

This comprehensive in silico analysis provides critical insights into the safety and efficacy profiles of herbal skin-lightening agents, establishing a foundation for evidence-based selection and formulation strategies. Such platforms, when interpreted correctly by an expert in the field of toxicology and pharmacognosy, demonstrate significant utility in the preliminary toxicological assessment of cosmetic ingredients, identifying potential concerns that can be targeted for experimental validation. Among the evaluated compounds, glabridin emerges as the most promising candidate based on its favorable balance of predicted skin permeation and absence of structural alerts, though potential CYP inhibition warrants consideration in formulation design. Ellagic acid presents a reasonable alternative, with moderate skin permeation and an extensive safety history, while aloesin and mulberroside F may require specific formulation approaches to address their limited penetration and potential reactivity.

The computational methodology employed in this study extends beyond the specific compounds evaluated, establishing a framework applicable to diverse herbal bioactives across various therapeutic domains. This approach aligns with current regulatory trends toward reduced animal testing in cosmetic safety assessment, as previously demonstrated in our work with *Reynoutria japonica* Houtt. bioactives (Buyukyildirim et al., 2025). Future research directions should include experimental validation of these *in silico* predictions, with a particular focus on quantitative skin penetration studies, assessment of sensitization potential, and CYP inhibition at cosmetically relevant concentrations and exposure scenarios.

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A COMPREHENSIVELY COMPARISON OF TWO CLOSELY RELATED MUSCARI SPECIES AND THEIR CONSERVATION STATUS

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Abstract

The *Muscari* Miller genus in the Asparagaceae family comprises approximately 77 species worldwide. The genus has a wide distribution in the Caucasus, temperate Europe, Africa, and north-western, south-western, and central Asia. *Muscari* species are perennial bulbous plants native to Eurasia, predominantly blooming in spring with flowers that are typically blue, purple, or violet, and resemble grape clusters. In Türkiye, the numbers of recognized species have been increasing, with 52 currently identified. *Muscari turcicum* Uysal, Ertuğrul & Dural and *Muscari vuralii* Y. Bağcı & Doğu are two morphologically similar and closely related endemic species in Türkiye. Both species were recently described based on limited and localized populations, raising questions about their taxonomic boundaries due to the use of minimal distinguishing characters. This study aims to reveal the taxonomic relationships and boundaries of *M. turcicum* and *M. vuralii* taxa by comparing them with a broad concept using morphological, anatomical, palynological, chromosomal and molecular studies. In addition, it is aimed to clarify the taxonomic status of the taxa and determine their threat status. The analyses revealed that the two taxa discussed have significant common features, except for molecular data, and do not contain any significant differences in terms of the characters discussed. A study employing the *trnL* intron region to assess the genetic diversity and differentiation of these species found that gene flow (Nm) between populations ranged from 1.71 to 9.99, and genetic differentiation (G_{ST}) varied between 0.04 and 0.22. Additionally, both species exhibited high haplotype diversity (Hd: 0.7-0.8) and low nucleotide diversity (Pi: 0.004-0.005), suggesting rapid population expansion and the emergence of polymorphic regions during demographic growth. These findings indicate significant genetic overlap between the two species. Despite of the determined different haplotype diversity between two taxa, considering the high phylogenetical similarity and limited differences, it is proposed that *M. vuralii* be treated as a synonym of *M. turcicum* and classified as a subspecies under *M. turcicum*. We hope that this approach would simplify their taxonomic status and aid in conservation efforts.

Key Words: Endemic, Grape Hyacinth, Haplotype, Maternal Hereditary, Population, Türkiye.

1.Introduction

Muscari is a genus of bulbous plants belonging to the Asparagaceae family. The genus has a wide distribution in the Caucasus, temperate Europe, Africa, and north-western, south-western, and central Asia [1-9]. While the genus is represented by 77 species in the world [10], it is represented by 52 species, 35 of which are endemic in Türkiye [11]. When considering the density of the species number and endemism rate in *Muscari*, Türkiye is one of the main differentiation and gene centers [12-14]. *Muscari turcicum* Uysal, Ertuğrul & Dural and *Muscari vuralii* Bagci & Dogu are two morphologically similar and closely related endemic species in Türkiye. Both species were recently described based on limited and localized populations, raising questions about their taxonomic boundaries due to the use of minimal distinguishing characters. In their original descriptions, *M. turcicum* was classified as endangered (E) due to its restricted distribution, while *M. vuralii* was considered critically endangered

(CR) due to its even more localized range. The advent of DNA-based markers has revolutionized species identification techniques [15], and molecular genetic studies have provided numerous new techniques for reliable and easy identification of plant species. Many of these techniques have successfully been used to study the distribution and degree of variation in the gene pool of a species and to answer typical evolutionary and taxonomic questions [16-19]. The *trnT-F* region is located in the large single-copy (LSC) region of the chloroplast genome. Since the introduction of universal primers [20], *trnL* intron and adjacent *trnT-F* spacers have become widely used chloroplast markers for phylogenetic analyses in plants [21-22]. Specifically, the *trnL* intron, alone or in combination with other genomic regions, has been extensively used for genetic diversity analyses in plants and various taxonomic levels such as family, genus, and species [23-30]. The main focusing of this presentation are: (a) to determine intra- and inter population relationships and genetic diversity of *Muscari turcicum* and *Muscari vuralii*, two morphologically similar species growing in neigbour locations; (b) to investigate whether *M. turcicum* and *M. vuralii* represent distinct species; (c) to analyze anatomic, palynologic, karyologic and molecular relationships among these species and (d) to reconsider threat categories.

2.Material and Methods

2.1. Material

During the fieldwork, individuals belonging to populations of *Muscari turcicum* and *M. vuralii* were collected. The collected specimens were prepared as herbarium material and deposited in the KNYA herbarium. For molecular studies, leaves from individuals belonging to populations were placed in silica gel and dried, and DNA isolations were performed from healthy leaves dried in silica gel. The materials for which DNA isolation was carried out and used for PCR amplification are listed in Table 1. Additionally, taxa obtained from GenBank (NCBI) were included in the data matrix. Bulbs collected from the field were germinated, and root tips were taken for cytological studies. Seeds were obtained from fruit-bearing samples collected from the field, and seed surface analyses were conducted.

Table 1. Taxa and localities used in the study

TAXA	POPULATIONS	LOCATIONS
Muscari turcicum	Population 1	Konya, Bozkır, Üçpınar Köyü, Tufan Deresi, Yazı yaylası, serpantin anakaya, 37° 01' 247" K, 32° 10' 630" D, T. Uysal 3290
Muscari turcicum	Population 2	Konya-Hadim, Dedemli köyü, Çat deresi-Eğrigöl yayla yolu, güney yamaç, 36° 57' 799" K, 32° 15' 566" D, 2147 m, T. Uysal 3284
Muscari turcicum	Population 3	Konya-Hadim, Dedemli Köyü, Çat deresi-Eğrigöl yayla yolu, eğimli kalker yamaçlar, 1796 m, T. Uysal 3272
Muscari turcicum	Population 4	Konya, Bozkır, Üçpınar Köyü, Tufan Deresi, Yazı yaylası, serpantin anakaya, 37° 00' 297" K, 32° 11' 600" D, T. Uysal 3289
Muscari turcicum	Population 5	Konya, Hadim-Dedemli-Eğrigöl yolu, Kalker kayalıklar üzeri, 2083 m, 36° 57' 102" K, 32° 14' 025" D, T. Uysal 3285A
Muscari vuralii	Population	Karaman, Sarıveliler, Göktepe-Eski Alanya yolu, At alanı mevki, 1842 m, 36 °38' 577" K, 32° 31' 166" D, T. Uysal 3292; Karaman, Sarıveliler, Göktepe, Karalar yaylası batısı, nemli ıslak çayırlıklar, 1840 m, 23 iv 2018, K. Ertuğrul 5544 (KNYA).

2.2. Micro-Morphological Methods

A) Seed Surface Analyses; Seeds obtained from fruit-bearing specimens collected during the fieldwork were coated with gold to become conductive and to obtain detailed images. Detailed surface morphology of mature seeds belonging to the taxa was obtained using Scanning Electron Microscopy (SEM), revealing their taxonomic significance. Seeds were passed through alcohol series and then air-dried. They were mounted onto metal stubs covered with double-sided adhesive tape under a stereomicroscope. The stubs were gold-coated to make the seeds conductive and to ensure visibility on the SEM screen. The microstructural features of the seeds were visualized at different magnifications (99X, 139X, 113X, 500X, 1.00 KX, and 5.00 KX) using the ZEISS LS-10 scanning electron microscope (SEM) located at S.Ü. İLTEK.

B) Palynological Methods; In the examination of pollen morphology under light microscopy, the method described by Wodehouse (31) was employed. The anthers of the plant were placed onto a clean slide, and a few drops of ethyl alcohol were added. Heating was applied to evaporate the alcohol, thus removing substances like resin and gum from the pollen grains. Subsequently, a mixture of safranin-glycerin-gelatin was added to the slide, and a coverslip was placed on top. The preparation was inverted and left to dry. After 15 days, the dried slides were examined. Observations were conducted using 100× oil immersion objective lenses, and the pollen grains were photographed. Measurements of pollen grains were made using the software KAMERAM 2.1. For scanning electron microscopy (SEM), the pollen grains were directly transferred onto aluminum stubs and coated with a 5 nm gold layer using a sputter coater. These specimens were then microphotographed using a Zeiss Evo LS 10 SEM housed in the Advanced Research and Development Center at Selçuk University.

2.3. Anatomical Methods

In field conditions, a portion of the collected specimens was transferred into plastic bottles filled with 70% ethyl alcohol. These specimens were preserved under laboratory conditions. Root, stem, and leaf tissues from these specimens were cut into small pieces and subjected to the paraffin embedding method [32]. Dehydration series were applied to remove water from the tissues, after which the specimens were saturated with paraffin in an incubator at specific temperatures. Following paraffin infiltration, xylene was evaporated from the specimens. Paraplast blocks were prepared using molds, and sections with a thickness of 14–20 μm were obtained. The staining of the sections was performed through a sequential staining series, using safranin-fast green double staining. The sections were mounted with coverslips using Entellan. For tissues that could not be sectioned via the paraffin method, manual sections were taken using a razor blade. Some of these sections were examined directly, while the rest were stained with safranin. A Leica DM 1000 light microscope was used for microscopic examination, and the microphotographs were captured using a Canon EOS 450 D camera attached to the microscope.

2.4. Cytological Method

Bulbs belonging to the taxa collected during the fieldwork were germinated, and chromosome counts were performed. Chromosome investigations were carried out using the squash technique, with counts conducted at the somatic metaphase stage [33]. For this, the meristems of root tips from germinated bulbs were used. To observe metaphase chromosomes, the root tips were first treated with 8-hydroxyquinoline at low temperature, then fixed in Carnoy's solution. Prior to staining, the material was hydrolyzed with 5 N HCl at room temperature for one hour and stained with 1% aceto-orcein. After obtaining suitable metaphase spreads, chromosome images were captured using an Olympus BX53 microscope equipped with an Olympus DP72 digital camera. For permanent preparation, samples were treated with nitrogen, passed through alcohol series, and mounted with Entellan. Karyotype measurements of the studied taxa were performed using the KAMERAM program, and taxa were compared in terms of karyomorphological characteristics based on various symmetry indices [34-36].

2.5. Molecular Method

Total DNA isolation was performed using the 2X CTAB method described by Doyle and Doyle (37) and modified by Soltis et al. (38) and Cullings (39). For the *trnL* intron region, the trnc-d primers and protocol were used [20]. Raw DNA sequences were checked and manually corrected using Chromas software. Sequences were aligned with BioEdit v.7.0.5.3 software [40] and MEGA XI software [41]. For molecular diversity analysis, DnaSP 6 was used to calculate haplotype diversity (Hd), nucleotide diversity (Pi), gene flow (Nm), genetic differentiation (F_{ST}, and the related statistics G_{ST}, N_{ST}) values [42-43]. Haplotype networks were constructed using the median-joining algorithm in the Network software [44]. Population structure and molecular variance (AMOVA) analyses were performed with Arlequin software, and the significance of F_{ST} values was tested with 1023 permutations [45-46].

3.Results and Discussion

3.1. Morphological and Taxonomical Findings

The species in question are taxa with quite high morphological similarities, identified more than two decades ago from a very close geography. In the prologue, *Muscari vuralii* was compared with *Muscari turcicum*, *M. coeleste* and *M. macbaethianum*, which are morphologically more different from *M. vurali*, and *M. turcicum* was not included in the study. However, it is more closely related to *M. turcicum* in terms of both its distribution area and morphological characteristics (leaves, scape, perigon shape and color as well as fruit). In both previous [4, 47] and recent revisional and taxonomic studies [48-49] on the *Muscari* genus, morphological characters such as leaf shape and number, scape, raceme, perigon shape and stamen arrangement are prominent in species distinction, and it is seen that there is a considerable similarity between the two taxa in terms of these diagnoses (Figure 1). *M.turcicum* has a relatively wider distribution compared to *M. vuralii*, but the distribution area of both taxa can be evaluated as the sloping slopes or plains of the high mountain floor of the central Taurus, and especially doline for *M. vuralii*. In our opinion, this unique habitat of *M. vuralii* plays an important role in the rather isolated and limited distribution of the taxon (Figure 2).

In light of basic morphological data, it has been presented a determination key for *M. turcicum* and *M. vuralii* (Figure 1); we can see from key that two taxa are separated with **very limited character!** It is noteworthy that the constriction at the mouth of the perianth tube and the backward curling of the lobes are similar in both taxa, and it is also seen that they are similar in terms of stamen arrangement and fruit characteristics. Although the original description of the *M. turcicum*, it was reported that the leaves were patent, the determination that the leaves of the later discovered Eğrigöl population were ascending revealed that there was an overlap between the two taxa in terms of this feature.

1.Fertile flowers pure white or slightly puplish at the base of perianthe tubes, leaves patent or rarely ascending………………………………………………………………………………… ***turcicum***
1.Fertile flowers sky blue or whitish till half of perianthe tubes, leaves ascending … ***vuralii***

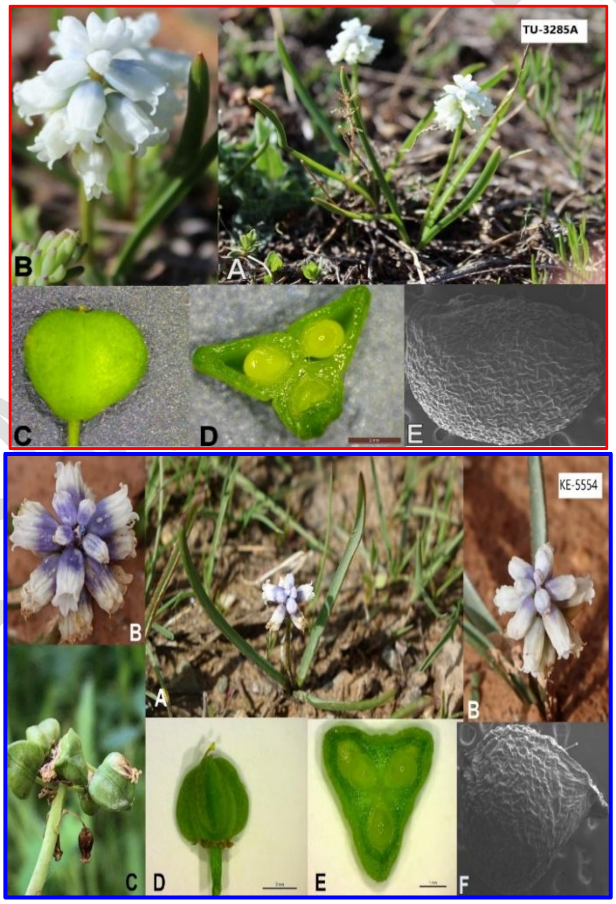

Figure 1. *Muscari turcicum* and *M. vuralii* habit and detailed views.
A: Habitat, B: Raceme, C-D: Fruit and cross section, E: Seeds

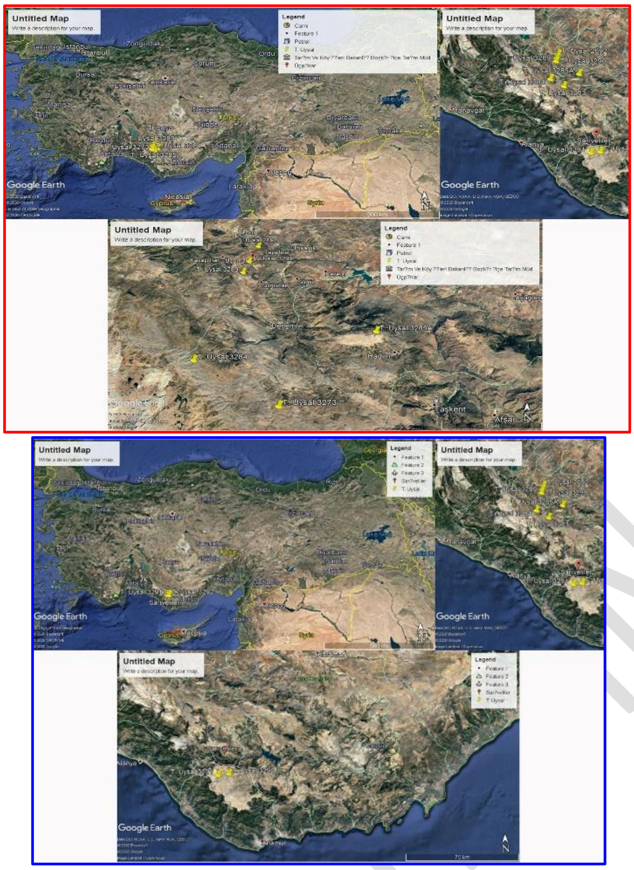

Figure 2. Distribution map of *Muscari turcicum* and *M. vuralii*

3.2. Micromorphological Findings

A) Seed Surface findings

In this study, the taxa *Muscari turcicum*, *M. vuralii*, and *Muscari bourgaei* Baker were examined using scanning electron microscopy (SEM). The micromorphological features and ornamentations observed in the seed structures of the taxa were determined. Seed surface analyses clearly distinguish *M. vuralii* and *M. turcicum* taxa from *M. bourgaei*, while revealing that the two species are quite similar (Figure 3). ***M. turcicum***: Seeds are 1.72 × 1.27 mm, ovoid, with dense surface folds and an irregular reticulate pattern, black in color, with a small and orbicular hilum. ***M. vuralii***: Seeds are 2.41 × 1.71 mm, sac-like or triangular, with a triangular hilum, dense surface folds and an irregular reticulate pattern, black in color. ***M. bourgaei***: Seeds are 1.95 × 1.48 mm, ovoid, with folded and alveolate surface ornamentation, black in color, with a small and orbicular hilum.

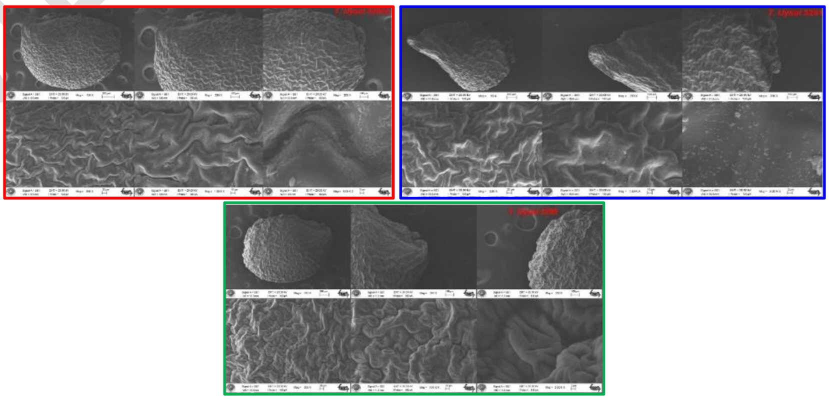

Figure 3. SEM micrographs of seeds of *Muscari turcicum*, *M. vuralii* and *M. bourgaei*

B) Palynological findings

M. turcicum: Pollen grains are monads, heteropolar, monosulcate with elliptic equatorial and polar views (Figure 4). The long axis (A) ranges from 24.86–30.75 µm (Mean±SD: 27.13±1.55 µm), the short axis (B) ranges from 18.79–23.17 µm (Mean±SD: 20.98±1.25 µm), and the A/B ratio is 1.29, indicating a subprolate shape. Sulcus length is 22.66 µm and width is 2.83 µm, extending from the distal to the proximal end. The exine is semitectate, 1.30 µm thick, with reticulate-perforate sculpturing. The intine is thin, measuring 0.70 µm. Lumina width is 1.11 µm (Figure 5) (Table 2). ***M. vuralii***: Pollen grains are monads, heteropolar, monosulcate with elliptic equatorial and polar views (Figure 4). The long axis (A) ranges from 27.00–30.81 µm (Mean±SD: 28.52±1.4 µm), the short axis (B) ranges from 17.89–22.54 µm (Mean±SD: 20.73±1.5 µm), and the A/B ratio is 1.37, indicating a prolate shape. Sulcus length is 20.41 µm and width is 4.88 µm, extending from the distal to the proximal end. The exine is semitectate, 1.29 µm thick, with reticulate-perforate sculpturing. The intine is thin, measuring 0.69 µm. Lumina width is 0.82 µm (Figure 5) (Table 2).

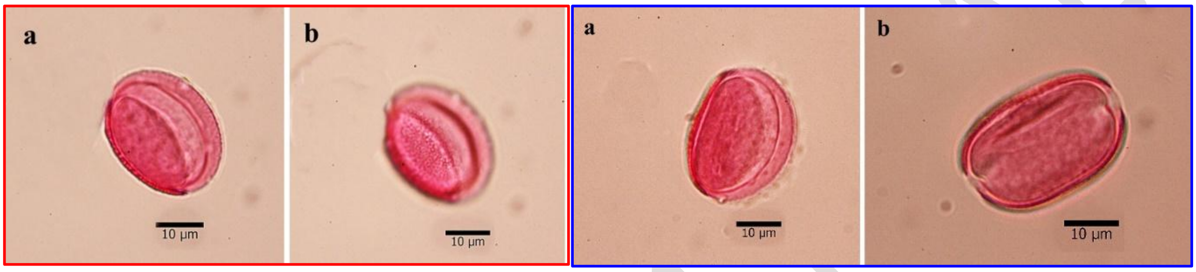

Figure 4. The light microscope photographs of *M. turcicum* (TU-3273) and and *M. vuralii* (TU-3292)

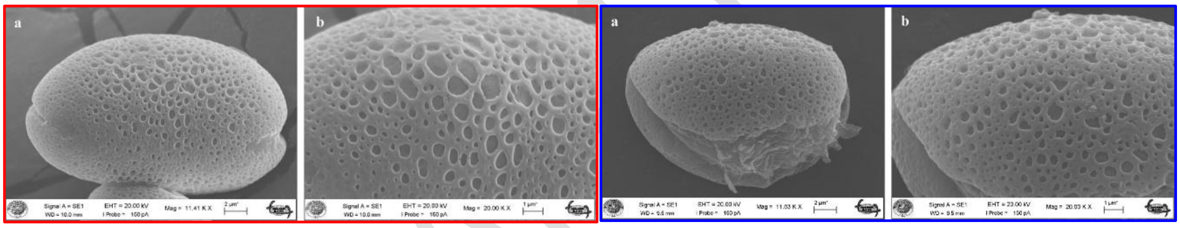

Figure 5. The SEM photographs of *M. turcicum* (TU-3273) and *M. vuralii* (TU-3292)

Table 2. The measurements of pollen grains in studied *Muscari* species (all values in µm except A/B, Slg: length of sulcus, Slt: width of sulcus, E: exine, I: intine, PS: pollen shape, SP: subprolate, P: prolate, PS: prolate-spheroidal, OS: Oblate-spheroidal, L:width of lumina, PT: Pollen type according to equatorial view, D: D-shaped; D1:Short sulcus at proximal surface, D2:long sulcus at proximal surface, E: Elliptical shaped, T: Triangular shaped, S: Spindle shaped).

Taxa/Pollen characters	**Long axis (A)**		**Short axis (B)**		**A/B**	**PS**	**Slg**	**Slt**	**E**	**I**	**L**	**PT**
	Min-Max	Mean	Min-Max	Mean								
M. vuralii	27-30.81	28.52±1.4	17.89-22.54	20.73±1.5	1.37	P	20.41	4.88	1.29	0.69	0.82	E
M. turcicum	24.86-30.75	27.13±1.55	18.79-23.17	20.98±1.25	1.29	SP	22.66	2.83	1.3	0.7	1.11	E

3.3. Anatomical Findings

Root Anatomy: ***M. turcicum***: The epidermis layer is uniseriate and located at the outermost region of the transverse root section, followed by a uniseriate exodermis (Figure 6a). An 8-layered cortex occupies the area towards the root center. The endodermis and pericycle consist of uniseriate parenchymatic cells. The phloem and xylem elements are arranged alternately within the vascular system. Three metaxylem strands are present at the center of the root (Figure 6b). ***M. vuralii***: The epidermis and exodermis layers function as protective tissues and are located on the outermost region of the root cross section (Figure 6-a). The epidermis consists of a single row of oval-shaped cells, while the exodermis is two-layered. The cortex consists of 9 layers and occupies a wide area. The endodermis and pericycle are composed of uniseriate parenchymatic cells. The phloem and xylem elements are arranged alternately. Five metaxylem strands are located at the center of the root (Figure 6-b) (Table 3).

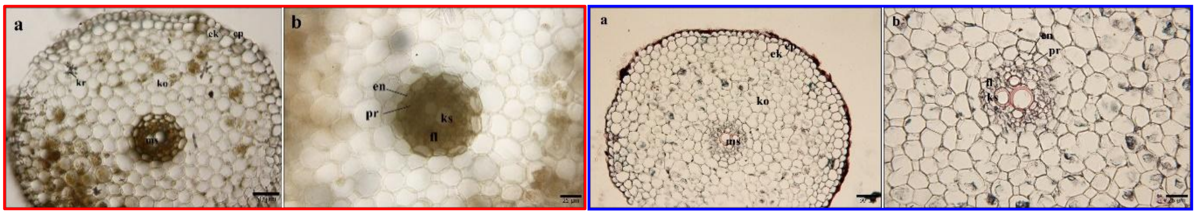

Figure 6. Transverse sections of the root of *M. turcicum* and *M. vuralii*. ep: epidermis, ek: exodermis, ko: cortex, ms: central vascular system, en: endodermis, pr: pericycle, fl: phloem, ks: xylem, kr: crystal**.**

Scape Anatomy: ***M. turcicum***: The outermost layer of the scape cross section is a uniseriate epidermis covered with a cuticle (Figure 7a,b). The cortex consists of 5 layers of parenchymatic cells. Four layers of continuous sclerenchymatic cells are present in the scape. Vascular bundles begin just beneath the sclerenchyma ring and increase in size toward the center. The vascular bundles are closed collateral type and number 11 (Figure 7-c). The pith region consists of parenchymatic cells (Figure 7-c). ***M.vuralii***: In transverse sections of the scape, the outermost layer is a uniseriate epidermis covered by a cuticle (Figure 7-a,b). The cortex consists of 5 layers of parenchymatic cells. Four continuous layers of sclerenchymatic cells are present. Vascular bundles start just beneath the sclerenchyma ring and increase in size toward the center. The vascular bundles are of the closed collateral type and total six in number (Figure 7-c). The pith region is composed of parenchymatic cells (Figure 7-c) (Table 3).

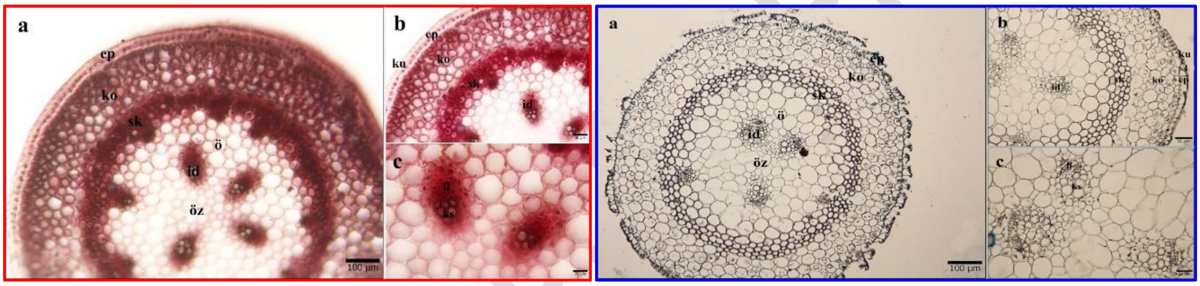

Figure 7. Transverse sections of the scape of *Muscari turcicum* and *M. vuralii*. A. General view. ep: epidermis, ko: cortex, sk: sclerenchyma, id: vascular bundle, ö: pith cell. B. Close-up view of epidermis, cortex, and sclerenchyma. ku: cuticle. C. Vascular bundles and pith. fl: phloem, ks: xylem.

Leaf Anatomy: ***M.turcicum***:Transverse sections of the leaf show that a uniseriate epidermis is present on both surfaces (Figure 8-a,b,c). Epidermal cells are occasionally interrupted by stomata. The mesophyll layer consists of two types of parenchymatic cells. Palisade parenchyma is arranged in two layers on both surfaces of the leaf, while spongy parenchyma is located in the central part of the mesophyll (Figure 8-a). Vascular bundles are arranged in a single row. Lacunar spaces are observed in the mesophyll. Stomata are mesomorphic on both leaf surfaces (Figure 8-b,c). ***M.vuralii***: Leaf cross sections reveal that a uniseriate epidermis is present on both surfaces (Figure 8-a,b). Epidermal cells are occasionally interrupted by stomata. The mesophyll layer is composed of two types of parenchymatic cells. Palisade parenchyma forms a single layer on both surfaces, while spongy parenchyma is located centrally (Figure 8-a). Vascular bundles are arranged in a single row. The stomata on both surfaces are of the mesomorphic type (Figure 8-b) (Table 3).

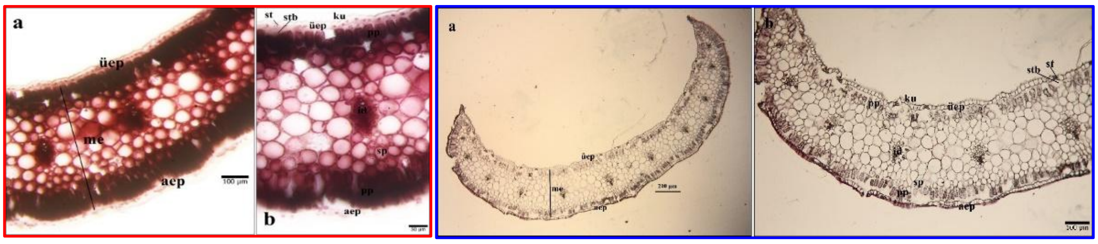

Figure 8. Transverse sections of *Muscari turcicum* and *M. vuralii* leaves. A. General view. üep: upper epidermis, me: mesophyll, aep: lower epidermis. B. Close-up view. ku: cuticle, pp: palisade parenchyma, sp: spongy parenchyma, id: vascular bundle, la: lacuna, st: stoma, stb: stomatal cavity.

Table 3. Anatomical features of the examined *Muscari* taxa

Species/Organ	**Root**		**Scape**			**Leaf**	
	Cortex parenchyma (rows)	Metaxylem number	Cortex	Sclerenchyma	Vascular bundles	Mesophyll type	Shape
M. vuralii	9	5	5	4	6	Equifacial	Linear
M. turcicum	8	3	5	4	11	Equifacial	Linear

According to general aspect to anatomies of the studies taxa, we can say that there are very minor differences between them. The observed differences are seen fairly limited with the number of vascular bundles and metaxylem. The remaining anatomical characters are seen absolutely similar. Therefore, we can conclude that two taxa are further resembled in terms of anatomical features.

3.4. Cytological Findings

The chromosome numbers of *Muscari turcicum*, *M. vuralii* and *M. bourgaei* were determined as 2*n*= 18, indicating that they possess diploid chromosome sets, with a basic chromosome number of *x*=9. The chromosomes of the studied species are composed of metacentric and submetacentric types. It was determined that *Muscari turcicum* and *M. vuralii* share the same karyotype formula (10m+8sm). Chromosome lengths ranged between 1.27 µm and 4.79 µm. In *Muscari turcicum* (CV_{CL}: 35.678, CV_{CI}: 13.846, AI: 4.94), *M. vuralii* (CV_{CL}: 39.475, CV_{CI}: 17.614, AI: 6.953), and *M. bourgaei* (CV_{CL}: 45.771, CV_{CI}: 21.072, AI: 9.645), an increase in chromosome length coefficient of variation (CV_{CL}) and chromosomal index coefficient of variation (CV_{CI}) was found to be positively correlated with increased asymmetry indices (AI). Further chromosomal measurements and karyotype symmetries are provided in Table 4 and 5, and the metaphase images, idiograms, and karyograms are presented in Figure 9.

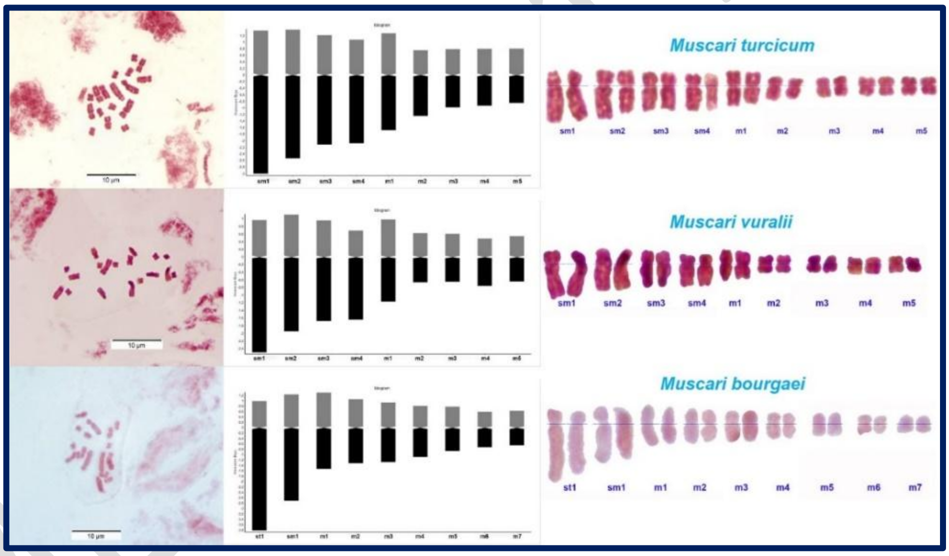

Figure 9. Metaphase, idiogram, and karyogram images of *M. turcicum*, *M. vuralii*, and *M. bourgaei*

M. turcicum: The chromosome number of the species was determined as 2*n*=18. According to the karyotype analysis, the taxon comprises metacentric and submetacentric chromosomes. The basic chromosome number is *x*=9. Details on the chromosome formula and asymmetry indices are provided in Table 4 and 5. ***M. vuralii***: The chromosome number was determined as 2*n*=18. Karyotype analysis indicated the presence of metacentric and submetacentric chromosomes. The basic chromosome number is *x*=9. Chromosome formula and asymmetry indices are detailed in Table 4 and 5. ***M. bourgaei***: The chromosome number was determined as 2*n*=18. According to karyotype analysis, the taxon comprises metacentric, submetacentric, and subtelocentric chromosomes. The basic chromosome number is *x*=9. Further data on the chromosome formula and asymmetry indices are given in Table 4 and 5.

Table 4. Karyotype formula according to Levan et al. (1964) and characteristic parameters of the studied *Muscari* taxa. PL-ploidy level; R-range; SC-the shortest chromosome length; LC-the longest chromosome length; p-mean length of the short arm; q-mean length of the long arm; CL-mean length of the chromosome; CI-mean centromeric index; TCL the total chromosome length of the haploid complement; m-metacentric and sm-submetacentric; CF: Chromosome formula; Satellite: S; B-chromosome: B; SD-standard deviation.

Collector number	Taxa	2n	R (SC-LC) (µm)	Ratio (SC/LC)	p (µm) mean (±SD)	q (µm) mean (±SD)	CL (µm) mean (±SD)	TCL (µm)	CI mean (±SD)	CF
T.Uysal 3272	*M. turcicum*	18	2.30 - 6.32	2.749	1.50 (±0.36)	2.45 (±1.08)	3.96 (±1.41)	35.595	40 (±0.06)	10m+8sm
T.Uysal 3292	*M. vuralii*	18	2.09 - 6.34	3.037	1.42 (±0.38)	2.34 (±1.16)	3.76 (±1.48)	33.851	40 (±0.07)	10m+8sm
T.Uysal 3299	*M. bourgaei*	18	1.27 - 4.79	3.766	0.92 (±0.23)	1.55 (±0.99)	2.47 (±1.13)	22.75	41 (±0.09)	14m+2sm+2st

Table 5. The karyotype indices of *Muscari* taxa. A_1: intrachromosomal asymmetry index, A_2: interchromosomal asymmetry index, CV_{CL}: coefficient of variation of chromosome length, CV_{CI}: coefficient of variation of centromeric index, AI: karyotype asymmetry index, M_{CA}: mean centromeric asymmetry.

Collector number	Taxa	A_1	A_2	CV_{CL}	CV_{CI}	AI	M_{CA}
T.Uysal 3272	*M. turcicum*	0.326	0.357	35.678	13.846	4.94	24.05
T.Uysal 3292	*M. vuralii*	0.309	0.395	39.475	17.614	6.953	24.47
T.Uysal 3299	*M. bourgaei*	0.279	0.458	45.771	21.072	9.645	25.50

3.5. Molecular Findings

Alignment of *trnL* intron region data

Sequences belonging to the *trnL* intron were obtained for 84 individuals from six populations of *Muscari turcicum* and *M. vuralii*. The aligned data matrix, generated using BioEdit and MEGA X, is 573 nucleotides long. Within the aligned data matrix, the conserved regions (C: 537), variable regions (V: 36), parsimony-informative sites (Pi: 17), and singleton sites (S:19) were identified through the MEGA X program. Additionally, haplotypes of the species were detected using DnaSP, revealing a total of 10 haplotypes: 2 for *Muscari turcicum* (H7–H8), 6 for *M. vuralii* (H1–H6) and 4 for (*M. macbeathianum* and *M. anatolicum* (H9-H10) (Figure 10).

Haplotype (gene) and nucleotide diversity values were calculated to determine the genetic variation of *M. turcicum* and *M. vuralii* populations (Table 6). At the same time, genetic differentiation parameters G_{ST}, N_{ST} and F_{ST} were calculated. While the highest values of haplotype and nucleotide diversity were observed in the 4th population of *M. turcicum*, the lowest values were detected in the 3rd population. At the species level, it was determined that haplotype diversity was high (Hd: 0.713092-0.81818) and nucleotide diversity was low (Pi: 0.004252-0.00530), respectively. It was reported that this population with a small effective population size, high haplotype diversity and low nucleotide diversity experienced a rapid expansion and most polymorphic regions may have emerged during this demographic expansion. AMOVA analysis is consistent with Nei's results showing a high degree of population differentiation in genetic structure (Fst=1.00000), and it was concluded that 100% of the variance is among the populations (Table 6).

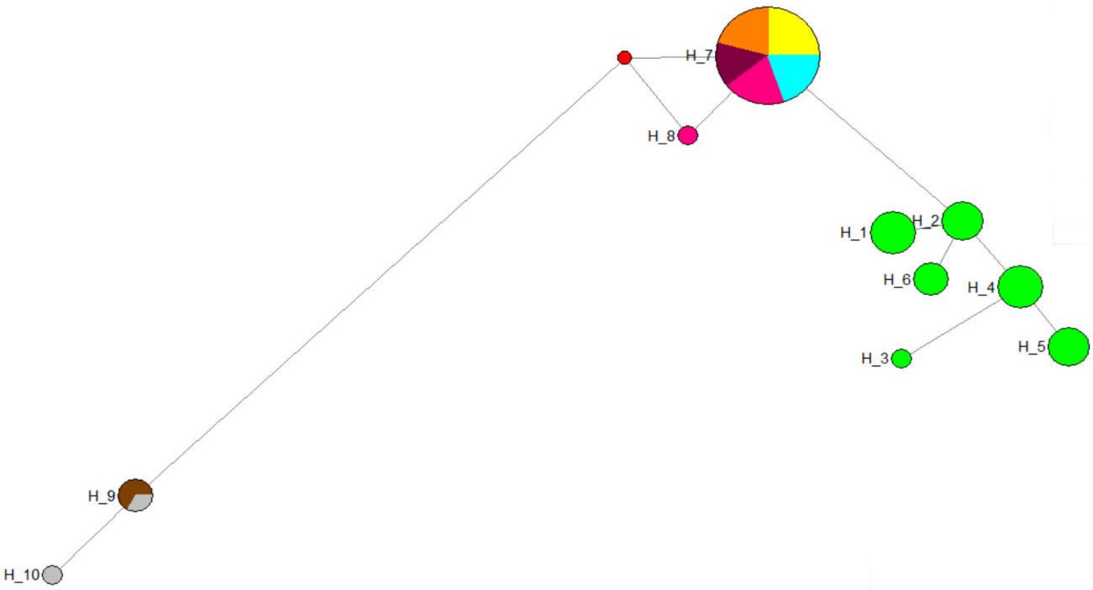

Figure 10. A Network analysis of *Muscari* taxa showing the haplotype diversity based on *trnL* intron sequences

Table 6. Genetic structure analyses performed for *M. turcicum* and *M. vuralii*

	Ki-Kare (Genetic differentiation)	**Hs (Hbk 1992)**	**G_{ST}**	**N_{ST}**	**F_{ST}**
Mturcicumpop1-Mturcicumpop2	P<0.001	0,84817	0,00696 (Nm:71,34)	71,34 (Nm:3,54)	0,12337 (Nm:3,55)
Mturcicumpop1-Mturcicumpop3	P<0.001	0,65879	0,15939 (Nm:2,64)	0,33454 (Nm: 0,99)	0,33443 (Nm:1,00)
Mturcicumpop1-Mturcicumpop4	P<0.001	0,98977	0,00120 (Nm:416,29)	0,20633 (Nm: 1,92)	0,20554 (Nm:1,93)
Mturcicumpop1-Mturcicumpop5	P<0.001	0,60110	0,18489 (Nm:2,20)	0,31176 (Nm:1,10)	0,31152 (Nm:1,11)
Mvuraliipop-Mturcicumpop1	P<0.001	0,85812	0,05386 (Nm:8,78)	0,52136 (Nm:0,46)	0,52000 (Nm:0,46)
Mvuraliipop-Mturcicumpop2	P<0.001	0,80081	0,10908 (Nm:4,08)	Nst: 0,57646 (Nm: 0,37)	Fst: 0,57542 (Nm: 0,37)
Mvuraliipop-Mturcicumpop3	P<0.001	0,69192	0,20031 (Nm: 2,00)	Nst: 0,65586 (Nm: 0,26)	Fst: 0,65536 (Nm: 0,26)
Mvuraliipop-Mturcicumpop4	P<0.001	0,88636	0,04767 (Nm: 9,99)	Nst: 0,52357 (Nm: 0,45)	Fst: 0,52185 (Nm: 0,46)
Mvuraliipop-Mturcicumpop5	P<0.001	0,65559	0,22667 (Nm: 1,71)	Nst: 0,61781 (Nm: 0,31)	Fst: 0,61716 (Nm: 0,31)
AMOVA analysis					
Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	
Among groups	1	171.308	5.42337 Va	74.10	
Among populations within groups	33	120.371	1.89594 Vb	25.90	
Within populations	46	0.000	0.00000 Vc	0.00	
Total	80	291.679	7.31930		
Fixation Indices F_{SC}:1.00000 F_{ST}:1.00000 F_{CT}: 0.74097					
[DnaSP 6 (43) and Arlequin (46)]					
Populations of taxa		**Haplotype diversity (Hd)**		**Nucleotide diversity (Pi)**	
Mturcicum pop1		0,97222		0,00525	
Mturcicum_pop2		0,76923		0,00293	
Mturcicum_pop3		0,43939		0,00084	
Mturcicum_pop4		1,00000		0,00815	
Mturcicum_pop5		0,38462		0,00409	
Mvuralii_pop		0,81818		0,00530	

Nielsen (50) emphasized that the low percentage of total variation within the populations is an indicator of high differentiation among the populations. In line with these results, we can say that the populations of *M. turcicum* and *M. vuralii* taxa are differentiated from each other according to *trnL* intron. Habitat restricted species found in isolated populations generally tend to be genetically homogeneous at the population level [51-52], which is unmatched in this study. As concern this mismatch, small sized populations of *M. turcicum* and *M. vuralii* are likely to have experienced strong genetic drift. Genetic drift of these taxa may lead to a decrease in diversity within the populations and an increase in differentiation between the populations. Although multiple haplotypes were observed within all, the nucleotide differences among these haplotypes were minimal. This case suggests that these populations might have undergone population bottlenecks followed by rapid expansions, allowing the retention of new mutations.

Taxonomic positions and conservation status of taxa

In conclusion, based on morphological, anatomical, palynological and karyological data, *Muscari turcicum* and *M. vuralii* appear highly similar. In a study on the action plan or threat status to be carried out for the protection of any species, it is important to clarify the taxonomic status and boundaries of the taxon first. In this study, a broad and comprehensive analysis was performed *on M. vuralii* and *M. turcicum* taxa, and it was determined that the taxa showed significant similarities except for molecular genetic analyses. Analyses to determine genetic variation based on the chloroplast gene region (*trnL* intron) showed that the *M. vuralii* had higher haplotype diversity and that this situation was related to the population continuing to speciation. Based on this, it was thought that the *M. vuralii* population could actually be considered as a population that still continues to evolve, belonging to the *M. turcicum*. On the other hand, *M. turcicum* is seen as a more stable in terms of haplotype diversity and has completed its speciation, and it is seen that it has expanded its distribution area (Figure 10). Therefore, it is thought that this situation is related to the species being exposed to genetic drift. In addition, it is suggested that the two taxa considered according to this intron region are more closely related to the rest from a phylogenetic perspective and that they do not need to be considered as separate species any more, considering the overlapping of other characters, and that they should be considered as a separate subspecies under the *M. turcicum*. With the taxonomic approach made here, the *M. vuralii* was arranged as *M. turcicum* subsp. *vuralii* (Bağcı and Doğu) Uysal Comb. and Stat. Nov.

Now that we have decided on the taxonomic status of the taxa considered, our separate observations regarding the threat status of the taxa have revealed that both subspecies should be evaluated as E and CR, respectively.

Muscari turcicum subsp. *turcicum* (**E**)
EOO-Extend of occurence/ Dispersal area is around 257 km square
AOO-Area of occupation / Life area is around 28 km square
Muscari turcicum subsp. *vuralii* (**CR**)
EOO-Extend of Occurence/ Dispersal area is around 6 km square
AOO-Area of Occupation / Life area is around 12 km square
Since the calculated distribution area value is smaller than the living area, EOO should also be calculated as 12 square km.

Therefore, we contributed that the threat categories should be evaluted as Endangered and Critically Endangered for every taxa, respectively. However, the distribution and habitat values for the *M. turcicum* subsp. *vuralii* is too critic and it is considered to be under greater threat. Therefore, more sensitivity should be shown.

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THE DETERMINATION OF GENETIC RELATIONSHIPS AND DIVERSITY AMONG *MUSCARI NEGLECTUM* AND *MUSCARI ARMENIACUM* POPULATIONS BY USING ISSR MARKERS

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Abstract

Muscari Miller (Asparagaceae) species have been used in folk medicine for centuries as diuretic, expectorant, anti-nausea, anti-wart and anti-rheumatic. The species are also used as food for humans and animals in Türkiye. *Muscari neglectum* Guss. ex Ten. and *Muscari armeniacum* Leichtlin ex Bakerin are the most widely distributed species within the *Muscari* genus. Species are sometimes confused with dry samples by taxonomists. In fact, sometimes populations of species overlap and it becomes very difficult to separate dried samples from each other. In this study, populations of the targeted species were characterized with ISSR markers. Information on intra- and inter-species genetic variation and differentiation was attempted to be explained. According to molecular variance analysis (AMOVA), since the variation between populations (88.21%) is higher than the variation within populations (11.79%), it is thought that the species are quite differentiated from each other in point of genetic features.

Key Words: Asparagaceae, Genetic differentiation, Genetic diversity, Population genetic analysis, Türkiye.

1.Introduction

Muscari Miller is distributed geographically from South-Eastern Europe to the Caucasus, from South-Western Asia to North Africa [1-10]. While the genus is represented by 77 species in the world [11], it is represented by 52 species, 35 of which are endemic in Türkiye [12]. *Muscari*, classified within the Asparagaceae (sensu latu.) family, is one of the taxonomically problematic genera. One of the problems encountered within the genus is that taxonomists sometimes confuse dried specimens of the widely distributed *Muscari neglectum* Guss. ex Ten. and *Muscari armeniacum* Leichtlin ex Bakerin. In fact, sometimes populations of the species overlap and it becomes very difficult to distinguish dried specimens from each other. Taxa belonging to the genus are grown as ornamental plants in parks and gardens, and since the taxa contain secondary metabolites, they are also evaluated as medicinal and aromatic plants [13]. The leaves, flowers and flower buds of the *M. neglectum* are consumed raw, boiled, grilled or pickled [14-15].

The bulbs of the species have chest stimulant, anti-inflammatory, anti-allergic and aphrodisiac effects. The fruits of the species have been used in the treatment of rheumatic diseases [16-19]. Additionally, M. neglectum has been an important plant in the dyeing industry since ancient times [15]. The *M. armeniacum* is suitable for human consumption as food [20-22]. It is a honey plant that attracts butterflies and bumblebees to the garden due to its pleasant smell [23-24]. In fact, it has found application area in perfumery and cosmetics with this feature. It is known that the species supports wound healing when used externally, has antiseptic properties, and provides an anesthetic effect in dermatological inflammatory processes, wounds and burns [1, 24-27]. In this study, populations of the species were characterized with ISSR markers to overcome the uncertainties in the classification of *Muscari neglectum* and *Muscari armeniacum*, which are similar in morphological features and whose dried specimens may be confused by some taxonomists.

2.Material and Methods

This study includes natural populations of *M. armeniacum* (14 populations), *M. neglectum* (14 populations), *M. microstomum* P.H.Davis & D.C.Stuart (1 population), *M. anatolicum* Cowley & Özhatay (3 populations) and *M. kerkis* Karlén (one population) distributed in different regions of Türkiye (Table 1).

Table 1. Localities of the studied taxa and populations of these taxa

Collector number	Taxa	Localities
1.*T.Uysal*-3418 (1-5)	*M. neglectum*	**Aydın**, Aydın karayolu, İzmir'den 15 km. yolun sağındaki orman açıklıkları, 175m, 01 iv 2018 (KNYA).
2. *T.Uysal* -3318 (1-5)	*M. neglectum*	**Konya,** Konya-Seydişehir'den Bozkır'a 32 km kala, yolun kenarındaki orman açıklıkları, 1600-1650m, 24 iii 2018 (KNYA).
3. *T.Uysal* -3404 (1-5)	*M. neglectum*	**Balıkesir,** Balıkesir-Edremit, Mehmetalan köyü yolu, zeytinlik bahçeleri açıklıkları, taşlık, kayalık, 78m, 31 iii 2018 (KNYA).
4. *T.Uysal* -3695 (1-5)	*M. neglectum*	**Erzurum**, Erzurum-Artvin yolu, tortuma 15-20 km kala *Astragalus microcephalus* birlikleri açıklıkları, 2022m, 12 v 2018 (KNYA).
5. *T.Uysal* -3766 (1-5)	*M. neglectum*	**Gaziantep**, Kampüs alanı, seyir tepe, güneybatı yamaçlar, orman açıklıkları, 985 m, 17 iii 2019 (KNYA).
6. *T.Uysal* -3856 (1-5)	*M. neglectum*	**Kastamonu**, Tosya, Ilgaz dağı etekleri, Ermelik köyü civarı, bağ bahçe açıklıkları, 905m, 08 iv 2019 (KNYA).
7. *T.Uysal* -3866 (1-5)	*M. neglectum*	**Kayseri,** Kayseri-Malatya yolu, Tohma çayı, Beypınar köyü civarı, kremsi-sarı kalker kayalıklar ve açıklıkları, Kaynarca köyüne varmadan 500 km önce, 1680-1750 m, 20 iv 2019 (KNYA).
8. *T.Uysal* -3873 (1-5)	*M. neglectum*	**Malatya,** Malatya-Akçadağ, Levent kalyonu, Çayözü köyünün güneydoğu yamaçları, 20 iv 2019 (KNYA).
9. *T.Uysal* -3907 (1-5)	*M. neglectum*	**Sivas**, Suşehri'nden 5 km (Koyulhisar'a doğru), kumul tepe açıklıkları, 730-740m, 23 iv 2019 (KNYA).
10. *T.Uysal* -4174 (1-5)	*M. neglectum*	**Mersin,** Anamur, Ermenek yayla yolu, Ermenek-Kazancı candır yayla arası Ortagöl dönüşü Kızılalan, 1490 m, 09 v 2021 (KNYA).
11. *T.Uysal* -4192 (1-6)	*M. neglectum*	**Konya**, Beyşehir; Huğlu- Akseki yolu, Çayırlık alan, 1400-1450m, 23 iv 2021 (KNYA).
12. *T.Uysal* -4214 (1-5)	*M. neglectum*	**Konya,** Derbent girişi, eğimli yamaçlar, 1650m, 29 iv 2021 (KNYA).
13. *T.Uysal* -4274 (1-5)	*M. neglectum*	**Van**, Edremit; Bahçeler, erik, dut, ıslah edilmiş ağaç gölgelikleri, 08 v 2021 (KNYA).
14. *T.Uysal* -3772 (1-6)	*M. neglectum*	**Şanlıurfa,** Birecik, Birecik'in 5 km güneyi (Özel Birecik ortaokulunun karşı yamaçları), 450 m, *Pistacia* ağaçlarının yukarı yamaçları, 18 iii 2019 (KNYA).
1) *T.Uysal* -3312 (1-5)	*M. armeniacum*	**Konya,** Seydişehir- Manavgat Yolu, Madenli Köyü girişi, 1430m, 24 iii 2018 (KNYA).
2) *T.Uysal* -3336 (1-5)	*M. armeniacum*	**Antalya,** Antalya-Alanya, Sarımut yaylası civarı, 1090m, 24 iii 2018 (KNYA).
3) *T.Uysal* -3487 (1-5)	*M. armeniacum*	**Antalya,** Antalya -Karamıklı yaylası, İbradı, 1565 m, 11 iv 2018 (KNYA).
4) *T.Uysal* -3630 (1-5)	*M. armeniacum*	**Kayseri,** Pınarbaşı Bünyan mesire yeri 1430 m, 29 iv 2018 (KNYA).
5) *T.Uysal* -3828 (1-5)	*M. armeniacum*	**Bolu,** Mengen-Çubuk köyünden Devrek'e-Karasu köprüsüne doğru, orman açıklıkları, meyilli yamaçlar, 495m, 06 iv 2019 (KNYA).
6) *T.Uysal* -3829 (1-5)	*M. armeniacum*	**Zonguldak,** Devrek, Halilbeyoğlu köyü, Bağbahçe açıklıkları, 165m, 06 iv 2019 (KNYA).
7) *T.Uysal* -3836 (1-5)	*M. armeniacum*	**Bartın,** Safranbolu yolu, İnceçay köyü çevresi, bozuk serpentin, meyilli yamaçlar, 937m, 06 iv 2019 (KNYA).
8) *T.Uysal* -3851 (1-5)	*M. armeniacum*	**Kastamonu,** Taşköprü, Bekdemirekşi köyü, kavak bahçeleri açıklıkları, 745m, 07 iv 2019 (KNYA).
9) *T.Uysal* -3904 (1-6)	*M. armeniacum*	**Gümüşhane,** Artabel milli parkı yolu (Gülaçar köyü), Torul-Şiran yolu, Gülaçar köyüne 24 km kala (Demirkayrak köyü civarı), kayalık açıklıklar, 1050m, 23 iv 2019 (KNYA).
10) *T.Uysal* -4191 (1-5)	*M. armeniacum*	**Konya,** Beyşehir; Derebucak Yenişerbademli yolu, çam-ardıç orman açıklıkları, taşlık-kayalık alanlar, 1345m, 23 iv 2021 (KNYA).
11) *T.Uysal*-4246 (1-5)	*M. armeniacum*	**Kayseri,** Develi; Kulpak köyünden Erciyes Dağına çıkarken batı yamaçları, bozuk meşe açıklıkları, taşlık kayalık sekiler 1450-1550m, 06 v 2021 (KNYA).
12) *K.Ertuğrul*-5500 (1-5)	*M. armeniacum*	**Osmaniye,** Kadirli, Bağdaş Yaylası, Yirce Yaylası çıkışı, *Pinus, Cedrus* ve *Abies* altları, 1300 m.
13) *K.Ertuğrul*-5503 (1-5)	*M. armeniacum*	**Osmaniye,** Kadirli, Bağdaş, Yince Yolu, Malkaya Mevkii, 1590 m
14) *H.Demirelma*-3368 (1-5)	*M. armeniacum*	**Ankara,** Ankara -Kızılcahamam Soğuksu Milli Parkı, yanıksırtı (*Pinus* sp ve *Quercus*), 1321m.
***T.Uysal* -3845 (1-5)**	*M. microstomum*	**Kastamonu,** Taşköprüye 8 km kala, çam-meşe orman açıklıkları, 775-780m, 06 iv 2019 (KNYA).
***T.Uysal* -3787 (1-6)**	*M. anatolicum*	**Isparta,** Kızıldağ milli parkı göl yolu, *Q.coccifera* kayalık açıklıkları,1450m, 22 iii 2019 (KNYA).
***T.Uysal* -3862 (1-5)**	*M. anatolicum*	**Niğde,** Halaç-Kılavuz yolu, 1930 m, 19 iv 2019
***K.Ertuğrul*-5574 (1-5)**	*M. anatolicum*	**Karaman,** Ayrancı Çat köy Aslanköy arası, Bozkır 2060 m
***T.Uysal* -3461 (1-5)**	*M. kerkis*	**Muğla,** Muğla-Fethiye-Deniz yolu Nif-Çal arası, Çal dağının güney yamaçlar, döküntülü serpantin üzeri 1210 m, 02 iv 2018 (KNYA).

2.1. DNA isolation and ISSR-PCR amplification

Leaf samples taken from individuals (at least five) of different populations belonging to taxa were stored in silica gel to be used in molecular analyses and DNA isolation of all individuals was performed [28-30]. ISSR-PCR amplifications were performed according to the protocol of Li et al. (31).

2.2. Statistical analyses

For the analysis of ISSR data, bands from gel images will be scored as present (1) and absent (0) and a data matrix was created. To determine genetic diversity and differentiation levels, Polymorphic band percentage, Nei's (32) gene diversity (h), Shannon index value showing the level of diversity in each population [(I)Lewontin (33)] and Genetic Differentiation Coefficient (G_{ST}) were calculated using the POPGENE-Version 1.32 program [34]. A dendogram was created to determine the relationships between taxa according to the similarity index in the NTSYS pc 2.1 [35] program and principal element analysis (PCA) was performed to determine the two-dimensional scaling of the populations belonging to the taxa. To determine the variation between and within populations, molecular variance (AMOVA) analyses were evaluated with the Arlequin program [36].

3.Results and Discussion

Initially, 25 ISSR primers were screened and only 5 primers (UBC834, UBC812, UBC840, UBC810, UBC841) formed a scorable band in the analyzed taxa and their populations. ISSR profiles are given in Figure 1 and detailed information is given in Table 2. A total of 248 ISSR bands were obtained and the bands ranged from approximately 200-1800 bp (Figure 1).

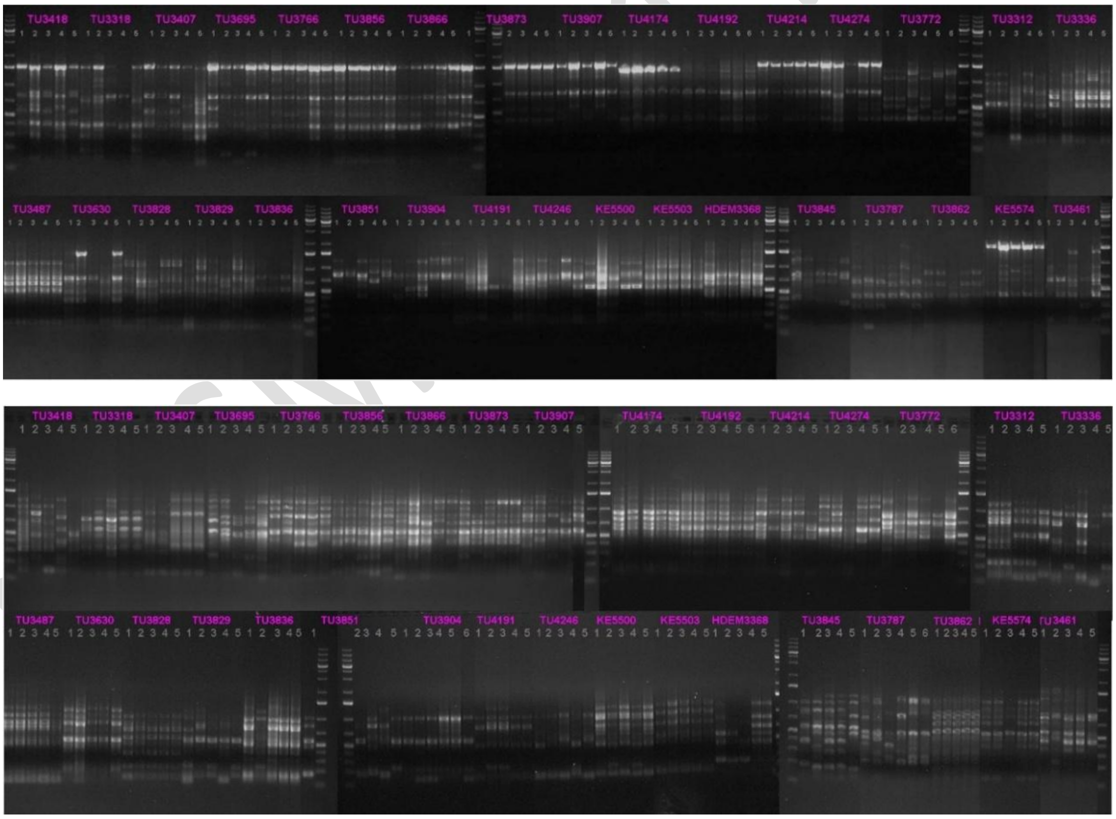

Figure 1. ISSR patterns of *M.armeniacum, M.neglectum* and closely related taxa (UBC834 ve UBC840)

Nei's gene diversity (h), Shannon's information index (I), and percentage of polymorphic loci (PBY) are important parameters for assessing genetic diversity. According to genetic diversity analyses performed at the species level, Nei's gene diversities (h) are 0.08, percentage of polymorphic loci varies between 33.47-38.31% and Shannon index varies between 0.12-0.14. It was determined that they did not differ significantly in terms of genetic diversity on species level (Table 2). Previous studies have shown that the percentage of polymorphic bands based on ISSR markers in members of the Asparagaceae family, such as *Muscari adilii* M.B. Güner & H. Duman [97.08%; (37)], *Muscari neglectum* (Iran) [96.40%; (38)], *Asparagus officinalis* L. [%82.01; (39)] and *Polygonatum verticillatum* (L.) All. [85.49%; (40)], ranged from 82.01% to 97.08%. We can conclude that *M.neglectum* and *M.armeniacum* have relatively low percentages of polymorphic bands at the species level compared to these findings.

According to Table 2, the highest genetic identity is observed between Konya (12-TU4214) and Van (13-TU4274) populations (0.9847), while the lowest identity is observed between Kastamonu (6-TU3856) and Mersin (10-TU4174) populations (0.8609) among *M. neglectum* populations (Tables 1 and 2). These two [Konya (TU4214) and Van (TU4274)] populations have similar living conditions. Both populations are distributed in regions with cold winter climates and under the subalpine zone (below 1800 m). Of the other two populations [Kastamonu (TU3856) and Mersin (TU4174)], one is distributed in the Black Sea region and the other in the Mediterranean region, and it is thought that the populations are affected by different climatic conditions (Tables 1 and 2).

The highest genetic identity is observed between Osmaniye (12-KE5500 and 13-KE5503) populations (0.9741), while the lowest identity is observed between Bartın (7-TU3836) and Ankara (14-HDEM3368) populations (0.8908) among the *M. armeniacum* populations. We can say that these two populations [Osmaniye (KE5500 and KE5503)] are closer due to their similar living conditions. These two populations were taken from plateaus and similar altitudes. The largest distance (0.1156) is between the other two populations [Bartın (TU3836) and Ankara (HDEM3368)] and this distance between the populations is thought to be due to the different habitat structure (serpentine slopes and forest) (Tables 1 and 2). Among the analyzed species (*M. neglectum* and *M. armeniacum*), it is seen that some populations sampled from the same region [Konya (TU4192 ve TU4214; 0.9713) ve Antalya (TU3336 ve TU 3487); 0.9560] are not closer or some populations sampled from different regions [*M.neglectum*: Mersin (TU4174)-Konya (TU4192); 0.9726 and *M.armeniacum*: Bolu (TU3828)-Zonguldak (TU3829); 0.9648] are not farther/more different.

In *M. neglectum*, while the distance between Konya (TU4192-south and TU4214-north) populations is thought to be due to different temperature conditions, the closeness of Mersin (TU4174) and Konya (TU4192) populations may be due to similar temperature conditions. A similar situation applies to *M. armeniacum*. Antalya [TU3336 (1090m) and TU3487 (woodland, streamside, 1595m)] populations grow in different altitudes and habitats, while Bolu (TU3828)-Zonguldak (TU3829) populations both grow in similar habitats (forestland, vineyard) and temperatures (Black Sea region). Previous studies support our results. Diao et al. (41) reported that the level of genetic differentiation of four populations of *Miscanthus floridulus* (Labill.) Warb. ex K.Schum. & Lauterb. from a favorable environment was higher than that of *M. floridulus* populations from upland and barren environments. Zhang et al (42) reported that the precipitation, slope, wind, soil fertility and other conditions were quite different among 12 populations of *Miscanthus sinensis* Andersson, which may have caused more differences in genetic diversity levels.

It has been reported that the growth characteristics of leafing before flowering may inhibit wind pollination to a certain extent, and rainy weather during flowering may restrict pollinator activity [43], and these factors may increase the probability of homozygosity and reduce the probability of recombination, which will promote self-pollination and inbreeding, thus increasing the similarity of individuals within a population and consequently increasing differentiation among populations [44-45]. It has been reported that the genetic diversity and genetic structure of plant populations are closely related to factors such as effective population size, reproductive system, natural selection and life history characteristics (including life form, ecological tolerance, seed dispersal and gene flow) [45-47].

Table 2. Intra-population genetic diversity and among population differentiation parameters *of M.armeniacum* and *M.neglectum* populations calculated with the POPGENE program [Nei (48) Molecular Evolutionary Genetics (p. 176-192)]. PBS=Polymorphic locus; PBY= Percentage of polymorphic loci; h=Nei's (49) gene diversity; I=Shannon index [Lewontin (33)], ne=Effective number of alleles [Kimura and Crow (50)], Genetic differentiation coefficient (G_{ST}), Gene flow(Nm), na=Observed number of alleles.

	PB	PBY (%)	na	ne	h(SD)	I(SD)	Ht	Hs	G_{ST}	Nm
M. neglectum (14 populations-72 individuals)	95	38.31	1.3831 (0.4871)	1.1380 (0.2666)	0.0871 (0.1481)	0.1404 (0.2180)	0.0868 (0.0218)	0.0141 (0.0012)	0.8373	0.0971
M. armeniacum (14 populations-71 individuals)	83	33.47	1.3347 (0.4728)	1.1284 (0.2599)	0.0810 (0.1454)	0.1294 (0.2153)	0.0810 (0.0212)	0.0138 (0.0014)	0.8301	0.1023

Nei's Original Measures of Genetic Identity and Genetic distance Nei (1972) Am. Nat. 106:283-292)
Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

M. neglectum

pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	****	0.9108	0.9267	0.9262	0.9497	0.9009	0.9342	0.9141	0.9207	0.8975	0.8962	0.9086	0.9081	0.9006
2	0.0935	****	0.9498	0.9223	0.9178	0.8863	0.9319	0.9178	0.9319	0.8979	0.8979	0.9129	0.9043	0.9004
3	0.0761	0.0515	****	0.9224	0.9346	0.9096	0.9539	0.9414	0.9346	0.8896	0.8908	0.9094	0.9090	0.8917
4	0.0767	0.0809	0.0808	****	0.9538	0.9140	0.9513	0.9206	0.9295	0.8984	0.9046	0.9226	0.9163	0.8994
5	0.0517	0.0858	0.0677	0.0473	****	0.9366	0.9635	0.9360	0.9409	0.8999	0.9040	0.9070	0.9108	0.9248
6	0.1044	0.1207	0.0947	0.0899	0.0655	****	0.9425	0.9239	0.9083	**0.8609**	0.8738	0.8804	0.8850	0.9016
7	0.0681	0.0705	0.0472	0.0500	0.0371	0.0592	****	0.9628	0.9583	0.9044	0.9115	0.9243	0.9262	0.9253
8	0.0898	0.0858	0.0604	0.0827	0.0661	0.0792	0.0379	****	0.9494	0.8977	0.8941	0.9109	0.9122	0.9119
9	0.0826	0.0705	0.0677	0.0731	0.0609	0.0962	0.0426	0.0520	****	0.9295	0.9242	0.9376	0.9327	0.9245
10	0.1082	0.1077	0.1170	0.1071	0.1055	**0.1497**	0.1004	0.1080	0.0731	****	**0.9726**	0.9631	0.9535	0.9206
11	0.1096	0.1077	0.1156	0.1003	0.1009	0.1349	0.0926	0.1119	0.0789	**0.0278**	****	**0.9713**	0.9686	0.9109
12	0.0959	0.0911	0.0950	0.0805	0.0976	0.1274	0.0787	0.0933	0.0644	0.0376	**0.0291**	****	**0.9847**	0.9174
13	0.0964	0.1006	0.0954	0.0874	0.0934	0.1221	0.0767	0.0919	0.0696	0.0476	0.0319	**0.0154**	****	0.9107
14	0.1047	0.1049	0.1146	0.1060	0.0782	0.1036	0.0777	0.0923	0.0785	0.0827	0.0933	0.0862	0.0935	****

M.armeniacum

pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	****	0.9302	0.9282	0.9346	0.9290	0.9336	0.9244	0.9507	0.9308	0.9282	0.9399	0.9271	0.9229	0.8973
2	0.0724	****	**0.9560**	0.9290	0.9346	0.9438	0.9248	0.9382	0.9179	0.9204	0.9252	0.9043	0.9170	0.8951
3	0.0745	**0.0450**	****	0.9424	0.9310	0.9463	0.9196	0.9320	0.9197	0.9152	0.9152	0.9037	0.9070	0.8963
4	0.0677	0.0736	0.0593	****	0.9435	0.9547	0.9223	0.9469	0.9344	0.9202	0.9352	0.9194	0.9136	0.9091
5	0.0736	0.0676	0.0715	0.0581	****	**0.9648**	0.9370	0.9364	0.9297	0.9330	0.9309	0.9041	0.9237	0.8927
6	0.0687	0.0579	0.0552	0.0464	**0.0358**	****	0.9403	0.9492	0.9398	0.9313	0.9404	0.9125	0.9269	0.9047
7	0.0786	0.0782	0.0838	0.0808	0.0651	0.0616	****	0.9288	0.9086	0.9389	0.9107	0.8961	0.9084	**0.8908**
8	0.0505	0.0638	0.0705	0.0546	0.0658	0.0522	0.0738	****	0.9546	0.9333	0.9490	0.9115	0.9122	0.9086
9	0.0717	0.0856	0.0837	0.0679	0.0728	0.0621	0.0958	0.0464	****	0.9304	0.9312	0.9195	0.9332	0.9228
10	0.0745	0.0830	0.0886	0.0831	0.0693	0.0711	0.0631	0.0690	0.0721	****	0.9495	0.9232	0.9255	0.9247
11	0.0619	0.0777	0.0887	0.0670	0.0716	0.0614	0.0935	0.0524	0.0713	0.0518	****	0.9407	0.9444	0.9233
12	0.0757	0.1006	0.1012	0.0840	0.1008	0.0915	0.1097	0.0926	0.0840	0.0799	0.0611	****	**0.9741**	0.9062
13	0.0803	0.0866	0.0976	0.0904	0.0794	0.0759	0.0960	0.0919	0.0691	0.0774	0.0572	**0.0262**	****	0.9072
14	0.1084	0.1108	0.1095	0.0953	0.1135	0.1001	**0.1156**	0.0959	0.0804	0.0783	0.0798	0.0985	0.0974	****

Table 3. Molecular variance analysis (AMOVA) results for *M. neglectum* and *M. armeniacum* populations [36, 51-52]

AMOVA

Groups		Source of variation	df	Sum of squares	Variance components	Percentage of variation
1.Grup: ***M.negletum*** TU3418_AYDIN TU3318_KONYA TU3407_BALIKESIR TU3695_ERZURUM TU3766_GAZIANTEP TU3856_KASTAMONU TU3866_KAYSERI TU3873_MALATYA TU3907_SIVAS TU4174_KARAMAN TU4192_KONYA TU4214_KONYA TU4274_VAN TU3772_SANLIURFA	**2.Grup:** ***M.armeniacum*** TU3312_KONYA TU3336_ANTALYA TU3487_ISPARTA TU3630_KAYSERI TU3828_BOLU TU3829_ZONGULDAK TU3836_BARTIN TU3851_KASTAMONU TU3904_GUMUSHANE TU4191_KONYA TU4246_KAYSERI KE5500_OSMANIYE KE5503_OSMANIYE HDEM3368_ANKARA	Among groups	1	558.287	7.13718 Va	39.14
		Among populations within groups	26	1243.542	8.94657 Vb	49.07
		Within populations	115	247.233	2.14986 Vc	11.79
		Total	142	2049.063	18.23360	
		Fixation Indices F_{SC}: 0.80626 **F_{ST} : 0.88209** F_{CT}: 0.39143				

Groups *M.negletum*		Source of variation	df	Sum of squares	Variance components	Percentage of variation
1.Grup: EGE TU3418_AYDIN TU3407_BALIKESIR **2.Grup: İÇ ANADOLU** TU3318_KONYA TU4192_KONYA TU4214_KONYA TU3866_KAYSERI TU3907_SIVAS TU4174_KARAMAN	**3.Grup: DOĞU ANADOLU** TU3695_ERZURUM TU3873_MALATYA TU4274_VAN **4.Grup: GÜNEYDOĞU ANADOLU** TU3766_GAZIANTEP TU3772_SANLIURFA **5.Grup: KARADENİZ** TU3856_KASTAMONU	Among groups	4	238.761	1.05714 Va	9.01
		Among populations within groups	9	413.036	8.50529 Vb	72.46
		Within populations	58	126.133	2.17471 Vc	18.53
		Total	71	777.931	11.73715	
		Fixation Indices F_{SC}: 0.79638 **F_{ST}: 0.81472** F_{CT}: 0.09007				
Groups *M.armeniacum*		**Source of variation**	**df**	**Sum of squares**	**Variance components**	**Percentage of variation**
1.Grup: AKDENİZ TU3336_ANTALYA KE5500_OSMANIYE KE5503_OSMANIYE TU3487_ISPARTA **2.Grup: İÇ ANADOLU** TU3312_KONYA TU4191_KONYA TU3630_KAYSERI	TU4246_KAYSERI HDEM3368_ANKARA **3.Grup: KARADENİZ** TU3828_BOLU TU3829_ZONGULDAK TU3836_BARTIN TU3851_KASTAMONU TU3904_GUMUSHANE	Among groups	2	121.129	0.75487 Va	6.93
		Among populations within groups	11	470.616	8.01958 Vb	73.58
		Within populations	57	121.100	2.12456 Vc	19.49
		Total	70	712.845	10.89902	
		Fixation Indices F_{SC}: 0.79056 **F_{ST}: 0.80507** F_{CT}: 0.06926				

According to UPGMA analyses derived from ISSR patterns, *M. neglectum* and *M. armeniacum* are clearly different and the most genetic distance among them are about 15%. The created dendogram showed that each species is positioned with own populations within one clade and with a distance of no more than ten percent (Figure 2). Coming data from our Popgene analyses indicated that the populations of *M. neglectum* and *M. armeniacum* are clearly separated from each other and this separation is also supported by high genetic differentiation (G_{ST}: 0.8373 and 0.8301), low gene flow (Nm: 0.09 and 0.1023) and high percentage of variation between populations (AMOVA: 81.47 and 80.51; Table 3). It has been reported that low total variation within populations is an indicator of high differentiation between populations [53]. In addition, the dendogram clearly shows the distinction of the analyzed *Muscari* species and supports the distinction of *M. armeniacum, M. neglectum, M. anatolicum* and *M. microstomum* species in seed surface [54] and palynology [55] studies, as well as in phylogenetic studies [56-58]. In addition, the dendogram also shows the distinction between *M. neglectum* and *M.kerkis*. Morphologically, *M. kerkis* is distinguished from *M. neglectum* by the looseness of the inflorescence during flowering. The distinction between these two species is supported by palynological studies [55]. The two-dimensional scale diagram (Figure 3) determined by PCA supports UPGMA tree cluster analysis.

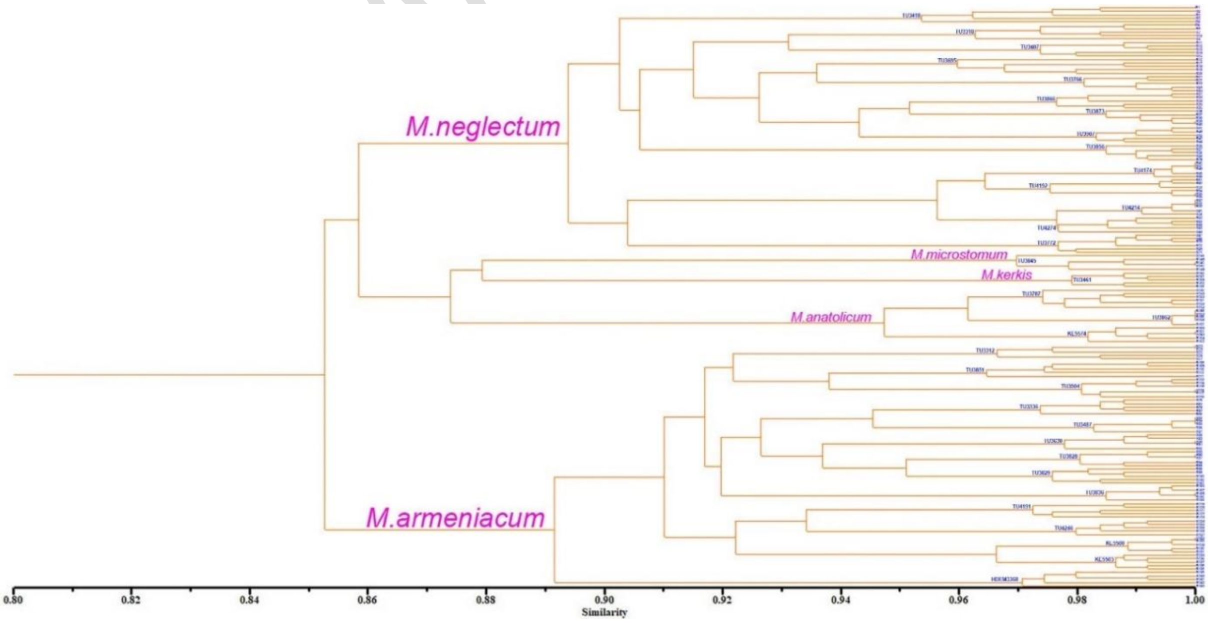

Figure 2. UPGMA cluster of *M.armeniacum, M.neglectum* and closely related taxa based on ISSR

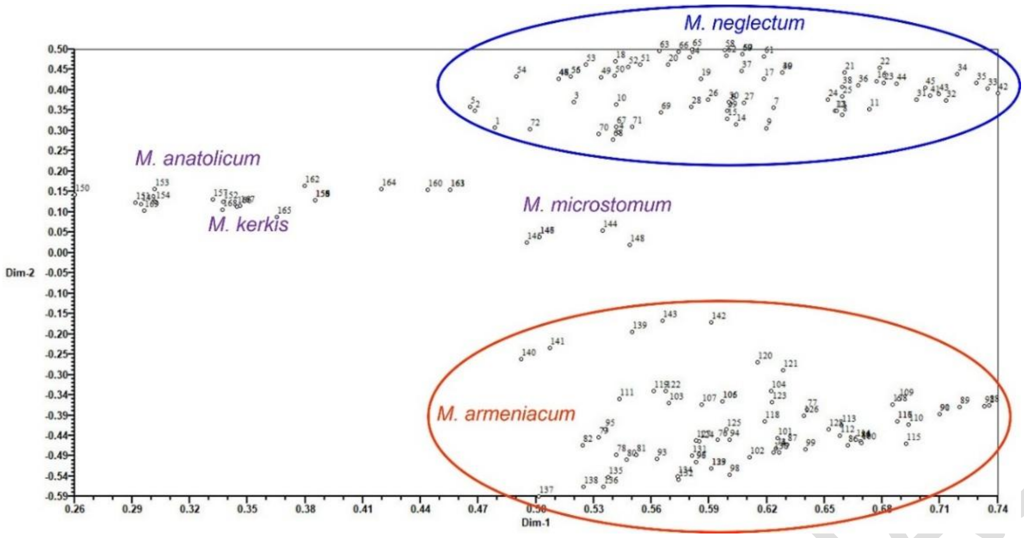

Figure 3. The two-dimensional scaling resulting from PCA analysis of *M.armeniacum*, *M.neglectum* and closely related taxa based on ISSR

As a result of this study, we concluded that ISSR markers can be used effectively in distinguishing *Muscari* species and populations, especially those that are difficult to distinguish. Moreover, information on genetic variation is of vital importance in the conservation and threat status of such taxonomic groups with a large number of regional and local endemic taxa. This study indicates that there is a high genetic variation among different populations of two widely distributed species under study. We can say that the high level of genetic differentiation between populations of *M. armeniacum* and *M. neglectum* species is probably largely due to the difference in habitats. In addition, factors such as heat, cold, temperate, humidity and altitude together with habitat suggest that different evolutionary mechanisms operate in affecting the differentiation of populations from each other (Tables 2 and 3; Figure 2). However, information on genetic variation for other local and regional endemic taxa of the group is still a mystery. We aim to focus on genetic variation studies on these taxa in future studies and we believe that molecular markers such as ISSR are quite suitable in this regard.

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KARYOMORPHOLOGICAL AND PALYNOLOGICAL CHARACTERISTICS OF THREE ENDEMIC *HYACINTHELLA* (ASPARAGACEAE) TAXA IN TURKIYE

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Abstract

In this study, the karyomorphological and palynological features of three *Hyacinthella* Schur: (H. lazulina K.Perss. & Jim Perss., *H. venusta* K.Perss., and *H. campanulata* K.Perss. & Wendelbo), endemic to Türkiye, were examined, and the basic chromosome numbers (*x*) of the genus *Hyacinthella* were confirmed. Plant bulbs were collected from their natural habitats in April 2019. Root tips obtained from the bulbs were used for chromosome analysis, employing the squash method with acetocarmine staining. After pretreatment, fixation, and staining, permanent preparations were made. Metaphase cells with clear morphology were photographed under a research microscope, and karyotypic parameters (chromosome measurements, arm ratio, asymmetry indices) were determined using the KAMERAM software. For palynological studies, pollen grains were analyzed using both light microscopy (LM) and scanning electron microscopy (SEM), and interspecific comparisons were made. The somatic chromosome numbers were determined as 2*n*=22 for *H. lazulina* and *H. venusta*, and 2*n*=18 for H. campanulata, supporting the base chromosome numbers of *x*=9 and 11 for the genus. The karyotypes of all three taxa were similar, with exclusively metacentric chromosomes. Asymmetry index (AI) values were also closely related: 0.477 (*H. lazulina*), 0.554 (*H. venusta*), and 0.532 (*H. campanulata*). In all three taxa, pollen grains were found to be prolate in shape with tectate exine structure and reticulate-perforate sculpturing. However, muri showed interspecific variation: muri were regular and uniform in *H. venusta*, small but regularly arranged in *H. lazulina*, and appeared large and irregular in *H. campanulata*. Palynological observations further revealed distinguishing features among the species. These findings provide significant insights into the cytogenetic and palynological diversity of *Hyacinthella*, contributing to the taxonomic and evolutionary understanding of this genus.

Key Words: Chromosome asymmetry, Chromosome number, Hyacinthaceae, Karyotype, pollen.

1. Introduction

The genus *Hyacinthella* Schur is a small bulbous group within the Asparagaceae family, previously assigned to Hyacinthaceae and Liliaceae in classical classifications [1-2]. According to updated taxonomic frameworks, Hyacinthella is now firmly placed in the subfamily Scilloideae of Asparagaceae sensu lato, which encompasses several ornamental and ecologically significant monocots [2]. Globally, the genus comprises approximately 18 species, predominantly distributed in Southeastern Europe and Western Asia [3]. Türkiye represents the center of diversity for the genus, hosting 12 species—10 of which are endemic—resulting in an endemism rate exceeding 80% [4-5]. These taxa typically inhabit rocky slopes, forest clearings, or steppe-like vegetation across the Irano-Turanian phytogeographic region. Despite the genus' taxonomic richness and endemism, detailed cytogenetic and palynological investigations remain scarce. Earlier cytological studies primarily focused on reporting chromosome numbers, identifying counts of 2*n*=16, 18, 20, 22, 24, and even 60 across various taxa [6-8]. However, comprehensive karyomorphological descriptions, including chromosome morphology, symmetry indices, and idiogram generation, have only recently been initiated. Palynological studies are similarly limited in scope. While Tekin & Meriç (9) provided preliminary insights into the pollen morphology of H. acutiloba, broader, comparative studies involving multiple taxa were lacking until Şahin & Eroğlu (10) published a comprehensive analysis using both LM and SEM imaging across 11 *Hyacinthella* taxa. Their findings underscored the diagnostic potential of pollen features such as aperture type, muri configuration, and lumen size. Given this background, the current study aims to: (1) elucidate the karyomorphological profiles of *H. lazulina*, *H. venusta*, and *H. campanulata*, (2) analyze pollen micro-morphology using LM and SEM, (3) compare the findings with previously published cytological and palynological data to refine species boundaries and contribute to taxonomic clarification within the genus.

2. Material and Methods

Three *Hyacinthella* species were collected during their flowering season in April 2019 from distinct habitats in Central Anatolia (Table 1, Figure 1). The specimens were identified based on taxonomic descriptions provided by Persson & Wendelbo (6) and deposited in the Herbarium of Selçuk University with voucher codes KE5624 (*H. lazulina*), KE5622 (*H. venusta*), and KE5629 (*H. campanulata*).

Figure 1. General habitus of the studied taxa

Table 1. Collection sites and voucher numbers of studied *Hyacinthella* taxa.

Taxa	Location	Altitude (m)	Voucher
H. lazulina	Karaman, Çakırdağ–Pelitlik region	1160	K.Ertuğrul 5624
H. venusta	Konya, Barçın yayla	1650	K.Ertuğrul 5622
H. campanulata	Konya, Altınapa dam area	1240	K.Ertuğrul 5629

2.1. Karyological Analysis

Chromosome counts were performed on somatic metaphases using the squash technique. Fresh primer roots of bulbs were selected and used for the chromosomal counts. First, the samples were placed into 0.002 M 8-hydroxyquinoline at 4 °C for 8 h and then fixed with Carnoy for 24 h at a low temperature. Before staining, they were hydrolyzed with 5 M HCl for 1 h at room temperature, stained with 1% aceto-orcein, and mounted in 45% acetic acid. The slides were mounted in Euparal as a permanent mounting medium using Bowen's method (11). At least 10 metaphase plates were examined for each taxon; the best ones were photographed (100×) with a digital camera (Olympus DP-72) mounted on an Olympus BX53 microscope. The chromosome nomenclature was done as proposed by Levan et al. (12), with the symbols m designating the metacentric chromosomes. Karyotype asymmetry was determined using the mean centromeric indices, the ratio of the shortest/ longest pair, and according to the A_1 and A_2 indices [13]. Moreover, CV_{CI} (the coefficient of variation of the centromeric index), CV_{CL} (the coefficient of variation of the chromosome length) and AI (karyotype asymmetry index) were counted according to the method proposed above [14].

2.2. Palynological Analysis

Pollen grains were extracted from fresh flowers and processed using Wodehouse (15) and Erdtman (16) protocols. Stained grains were mounted in glycerin jelly and examined with a Leica DM1000 light microscope. Scanning Electron Microscopy (SEM) was performed at Selçuk University R&D Lab. Pollen grains were sputter-coated with gold and visualized using SEM (ZEISS Sigma 300). Terminology for morphological traits followed Punt et al. (2007). Measured traits included: Polar axis (P), equatorial diameter (E), A/B ratio (shape index), Aperture type, Exine and intine thicknesses, ornamentation type (murus thickness, lumen shape and size, sulcus membrane).

3. Results and Discussion

3.1. Karyological findings

Somatic chromosome numbers were 2*n*=22 for *H. lazulina* and *H. venusta*, and 2*n*=18 for *H. campanulata*. All chromosomes were metacentric (Figure 2, Tables 2 and 3). This is consistent with previous chromosome counts reported by Persson & Persson (7) and Johnson & Brandham (17), though this study provides the first detailed karyomorphological metrics for these taxa.

Chromosome lengths ranged from 0.83-1.81 µm. *H. campanulata* had the shortest mean total karyotype length (9.554 µm), while *H. venusta* and *H. lazulina* had comparable lengths (11.781 µm and 11.782 µm, respectively). Relative symmetry was highest in *H. lazulina*, suggesting a more conservative karyotype structure.

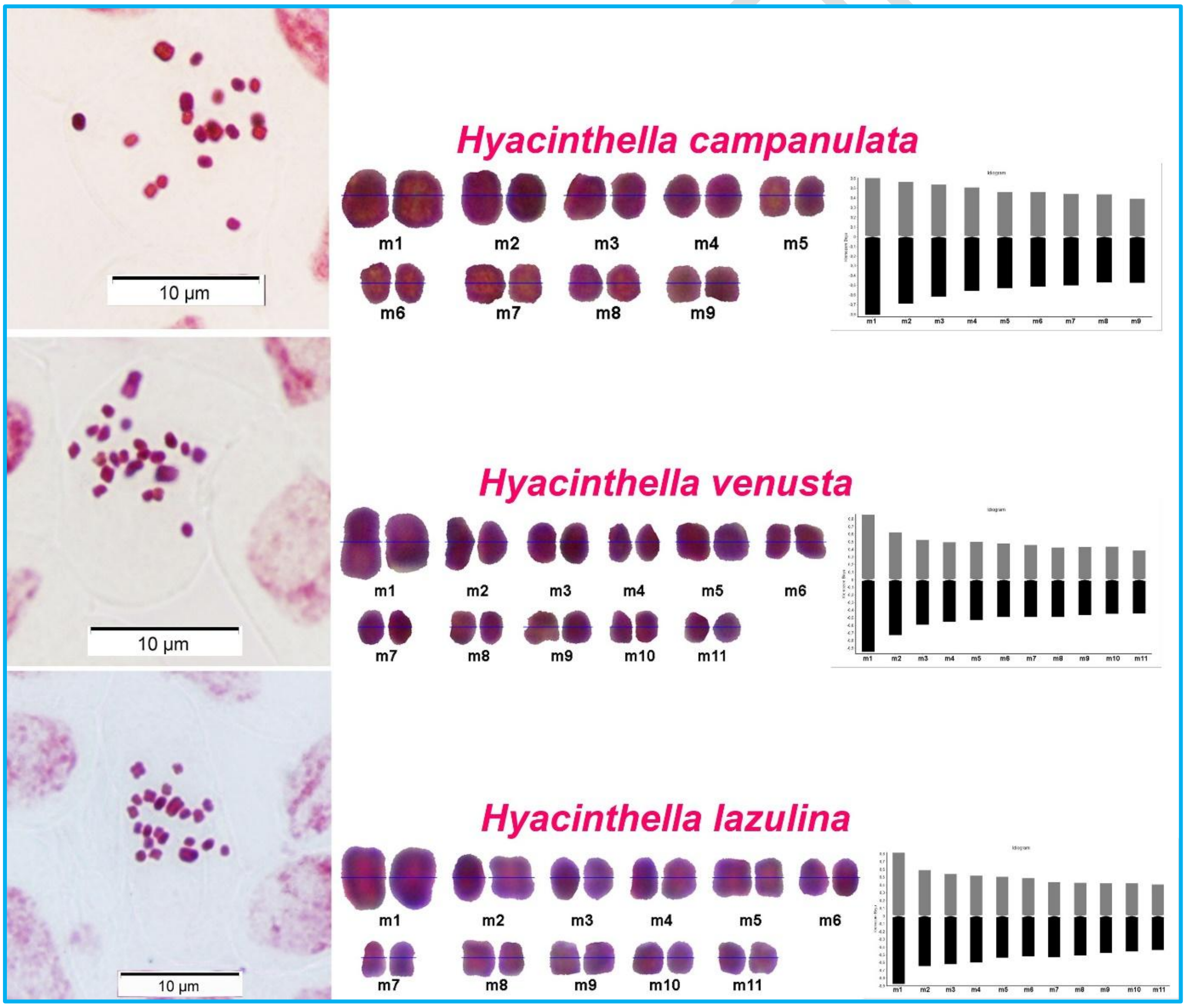

Figure 2. Metaphase, idiogram, and karyogram images of *Hyacinthella taxa*

Table 2. Karyotype formula according to Levan et al. (12) and characteristic parameters of the studied *Hyacinthella* taxa. PL-ploidy level; R-range; SC-the shortest chromosome length; LC-the longest chromosome length; p-mean length of the short arm; q-mean length of the long arm; CL-mean length of the chromosome; CI-mean centromeric index; TCL the total chromosome length of the haploid complement; m-metacentric and sm-submetacentric; CF: Chromosome formula; Satellite: S; B-chromosome: B; SD-standard deviation.

Collector number	Taxa	2n	R (SC-LC) (µm)	Ratio (SC/LC)	p (µm) mean (±SD)	q (µm) mean (±SD)	CL (µm) mean (±SD)	TCL (µm)	CI mean (±SD)	CF
TU3513	*H. campanulata*	2n	0.86 - 1.41	1.63	0.49 (±0.06)	0.57 (±0.11)	1.06 (±0.17)	9.554	46 (±0.02)	18m
KE5622	*H. venusta*	2n	0.83 - 1.81	2.174	0.51 (±0.12)	0.56 (±0.15)	1.07 (±0.27)	11.781	47 (±0.01)	22m
KE5624	*H. lazulina*	2n	0.85 - 1.69	1.999	0.51 (±0.11)	0.56 (±0.12)	1.07 (±0.23)	11.782	47 (±0.01)	22m

Table 3. The karyotype indices of *Hyacinthella* taxa. A_1: intrachromosomal asymmetry index, A_2: interchromosomal asymmetry index, CV_{CL}: coefficient of variation of chromosome length, CV_{CI}: coefficient of variation of centromeric index, AI: karyotype asymmetry index.

Collector number	Taxa	A_1	A_2	CV_{CL}	CV_{CI}	AI
TU3513	*H. campanulata*	0.141	0.159	15.857	3.354	0.532
KE5622	*H. venusta*	0.095	0.251	25.128	2.205	0.554
KE5624	*H. lazulina*	0.104	0.213	21.304	2.239	0.477

3.2. Palynological findings

All three species exhibited monosulcate, heteropolar, prolate pollen grains with tectate, reticulate-perforate exine (Table 4, Figure 3).

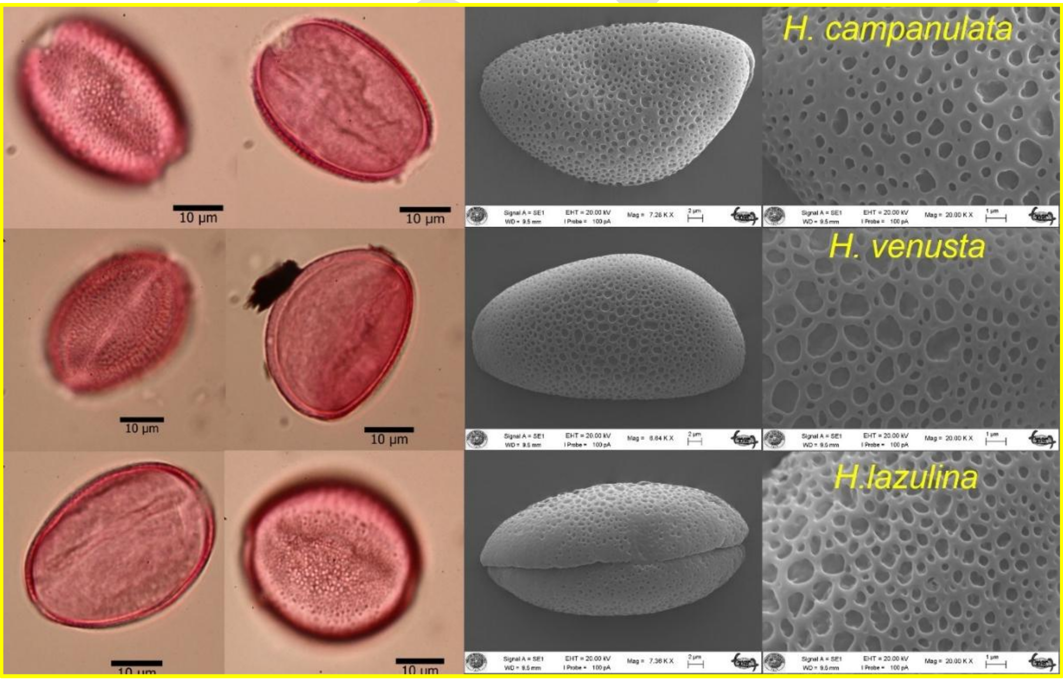

Figure 3. The SEM and the light microscope photos of studied *Hyacinthella* taxa

Table 4. Summary of pollen morphological characters

Taxa	Polar Axis (µm)	Equatorial Axis (µm)	Exine (µm)	Intine (µm)	Ornamentation	Muri
H. lazulina	35.95 ± 2.07	27.83 ± 1.89	1.20 ± 0.15	0.72 ± 0.10	Reticulate-perforate	Small, regular
H. venusta	39.54 ± 1.53	27.08 ± 1.93	1.36 ± 0.20	0.81 ± 0.15	Reticulate-perforate	Uniform, regular
H. campanulata	34.95 ± 4.41	25.69 ± 2.30	1.21 ± 0.17	0.77 ± 0.15	Reticulate-perforate	Large, irregular

The data align with Şahin & Eroğlu (10), who reported monosulcate, tectate pollen with similar size ranges and ornamentation across 11 *Hyacinthella* taxa. *H. campanulata*'s distinctly irregular muri pattern supports its differentiation from the other taxa. The somatic chromosome numbers determined in this study (2*n*=22 for *H. lazulina* and *H. venusta*; 2*n*=18 for *H. campanulata*) are in agreement with earlier reports by Persson & Wendelbo (6), Persson & Persson (7), and Johnson & Brandham (17). However, unlike those studies, the present work provides the first karyomorphological descriptions for these species, including chromosome lengths, arm ratios, and asymmetry indices. The exclusive presence of metacentric chromosomes in all three taxa corroborates with findings from *H. acutiloba*, *H. siirtensis*, *H. micrantha*, and *H. campanulata* reported by Kiran et al. (8), which also noted predominantly symmetrical karyotypes. Notably, while *H. lazulina* and *H. venusta* shared identical chromosome numbers and karyotype formulas (22m), their AI values—0.477 vs. 0.554—suggest a slight increase in karyotype asymmetry in *H. venusta*. This may point to chromosomal reorganization events (e.g., pericentric inversions or differential condensation) affecting the relative arm lengths of homologous pairs.

The lowest AI value observed in *H. lazulina* implies a more conserved and primitive karyotype structure compared to the other taxa, in accordance with karyotype evolution theories that associate increased asymmetry with derivation [14,18]. Palynological traits observed under LM and SEM likewise support species-level differentiation. All taxa possessed monosulcate, tectate pollen with reticulate-perforate ornamentation, consistent with the general pollen architecture of the Asparagaceae family [10, 19]. However, distinct differences were noted in the muri structure and lumen size: *H. lazulina*: small and regular muri, narrow lumina, *H. venusta*: regular, polygonal muri, moderate lumen diameter and *H. campanulata*: large, irregular muri and broader lumina.

These micro-morphological variations echo the results of Şahin & Eroğlu (10), who highlighted sulcus membrane ornamentation and muri architecture as significant characters in delimiting *Hyacinthella* species. In particular, the irregular and expanded muri observed in *H. campanulata* may represent a derived condition, as suggested for *H. nervosa* and *H. hispida* by the same authors. Furthermore, our findings of prolate pollen in all three species are congruent with previous morphological reports [9] for *H. acutiloba*. However, in contrast to earlier studies which described the pollen as simply "reticulate", our identification of "reticulate-perforate" ornamentation refines the descriptive accuracy, aligning better with modern palynological standards [20]. When integrating karyomorphological and palynological data, the differentiation of *H. campanulata* from *H. lazulina* and *H. venusta* is evident at both cytogenetic and micromorphological levels. The latter two species, despite sharing karyotypic symmetry and chromosome number, still exhibit distinct pollen features—particularly in exine thickness and pollen axis ratios—supporting their recognition as separate taxa.

4. Conclusion

This study contributes novel karyomorphological and palynological data for three endemic *Hyacinthella* species from Türkiye.

The key findings are summarized as follows:

Chromosome numbers: 2*n*=22 for *H. lazulina* and *H. venusta*, 2*n*=18 for *H. campanulata*, confirming base chromosome numbers of *x*=11 and x=9 respectively.

Karyotype formulas: All species exhibited symmetrical, metacentric karyotypes (22m or 18m).

Pollen morphology: All species had monosulcate, tectate, prolate pollen with reticulate-perforate sculpturing, but varied in muri size and ornamentation regularity.

The results are consistent with and expand upon those of Kiran et al. (8) and Şahin & Eroğlu (10), illustrating how integrated cytogenetic and palynological approaches can be used to elucidate relationships among morphologically similar taxa. We recommend that future studies incorporate molecular cytogenetics (e.g., FISH or GISH) and DNA barcoding to further resolve phylogenetic relationships and evolutionary dynamics within *Hyacinthella*.

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CHEMICAL PROFILING AND BIOACTIVITY ASSESSMENT OF *SATUREJA* SPP.: INSIGHTS FROM SPME AND IN VITRO ANTIMICROBIAL ASSAY

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Abstract

The genus *Satureja* (Lamiaceae) comprises aromatic species that are particularly rich in volatile secondary metabolites, many of which are linked to notable biological activities such as antimicrobial effects. Endemic to the Mediterranean region, the genus is distributed across Southern Europe, North Africa, and Western Asia. Turkey, in particular, represents a centre of diversity, with 17 species, including seven endemics. In the present study, the volatile profiles of two *Satureja* (*S. cilicica* and *S. cuneifolia*) species were analysed using Solid Phase Microextraction (SPME) coupled with Gas Chromatography-Mass Spectrometry (GC-MS). As a result of SPME analysis, it was determined that α-Terpinene, α-Phellandrene, and dl-Limonene compounds, which are compounds typically seen in *Satureja* species and other aromatic Lamiaceae members, were present in similar proportions in the content. Additionally, the antimicrobial potential of ethanol and water extracts of plant species was evaluated in vitro against some pathogenic Gram-positive (*Staphylococcus aureus* (MRSA), *Sarcina lutea*, *Bacillus cereus*, *Listeria monocytogenes*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Proteus mirabilis*) bacterial and fungal strains (*Candida parapsilosis* and *Candida albicans*) using broth microdilution method. When the antimicrobial activity results were evaluated, it was concluded that the extracts had different MIC (Minimum Inhibitory Concentration) values on different microorganisms and that ethanolic extracts were more effective than water extracts in terms of antimicrobial activity. The outcomes support the role of specific volatiles in antimicrobial efficacy and point to *Satureja* species as promising candidates for further phytochemical and pharmacological research.

Key Words: Antimicrobial activity, Kayakekiği, Kınalıkekik, Lamiaceae, volatile compounds.

1.Introduction

Using plants for medicinal purposes represents a practice deeply rooted in human history, one that continues to exert considerable influence on contemporary healthcare paradigms. As the World Health Organisation reported, an estimated 80% of the global population depends on phytotherapeutic approaches for meeting primary healthcare needs [1]. Although traditional herbal medicine systems have historically predominated in regions such as Asia, Africa, and Latin America, recent decades have witnessed a marked resurgence of interest in natural products across industrialised nations, including Europe and North America [2]. This renewed engagement is largely driven by the recognition of herbal medicines as possessing comparatively fewer adverse effects, alongside increasing apprehension regarding the limitations and side effects associated with synthetic pharmaceuticals. Moreover, substantial progress in phytochemical and pharmacological research has provided robust scientific validation for the therapeutic potential of numerous plant species, particularly their antioxidant, antimicrobial, and anti-inflammatory activities, thereby reinforcing the integration of botanical therapeutics within modern biomedical frameworks [3].

The genus *Satureja* (Lamiaceae) comprises aromatic species that are particularly rich in volatile secondary metabolites, many of which are linked to notable biological activities. There are around 43 species of *Satureja* around the globe; the majority are found in Mediterranean nations, with the remaining species ranging from North Africa and Western Europe to the Altai-Western Himalayas and Xinjiang [4-5]. Even though *Satureja* species are mostly found in the Mediterranean basin, the Caucasus, east of Türkiye, west of Iran, and the border between northern Iraq, Türkiye has the most species (17), seven endemics, compared to other countries [6-8]. In this study, two species belonging to the *Satureja* genus, *S.cilicica* and *S.cuneifolia*, were examined. *S.cilicica* is an endemic species and is known among the public as “kınalı kekik”. *S. cuneifolia* is widely distributed and is known among the public as “dağ kekiği”. There are various biological activity studies such as cytotoxic, antioxidant and antimicrobial on both species but this studies were focused on mostly the essential oils of this species. The purpose of this study was to characterise the volatile compounds of *Satureja cilicica* and *Satureja cuneifolia* by solid-phase microextraction (SPME) and to evaluate the antimicrobial activity of their extracts.

2.Material and Methods

2.1. Collection of the plant materials

Plant specimens were collected from their natural habitats and dried without directly sunlight. When they completely dried, they were pulverised and prepared for the analyses. Sample materials were stored in the herbarium of Selçuk University.

2.2. SPME (solid-phase microextraction) analyses

Plant powders were used directly for SPME analysis. A 10 ml vial was filled with diluted ground material and capped. For analysis, a 50/30 µm DVB/CAR/PDMS HS-SPME fiber was selected. Using a Restek Rxi-5 MS capillary column, SPME was immediately injected into the headspace of the vial to adsorb volatile chemicals before being directly injected into the Shimadzu QP2010ULTRA GC-MS instrument. The SPME-GC-MS technique was used to analyze volatile components of lupin (injector temperature was 250 °C). Before peak area integration, SPME fiber chromatograms of all samples were subjected to noise reduction. Then, the peak areas of the parts of the chromatogram were integrated. Compounds were identified by comparison with the W9N11 library.

2.3. Preparation of the plant extracts

Powdered plant materials were used to prepare the extracts. To prepare the extract, firstly, Soxhlet extraction was carried out using ethanol for 6-8 hours. Then, the other extraction was carried out using the maceration method with water. After, the solvents of the obtained extracts were evaporated and they stored at -20 °C until use.

2.4. Microorganism strains

To determine antimicrobial activity within the scope of the study; *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 25933, *Klebsiella pneumoniae* ATCC 70603, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Listeria monocytogenes* NRRL-B-33314, *Salmonella enteritidis* ATCC 13076, *Sarcina lutea* ATCC 9341, *Bacillus cereus* ATCC 11778, standard bacterial strains and *Candida albicans* ATCC 26555 and *Candida parasilopsis* yeast strains were used. These strains were obtained from Selçuk University, Faculty of Science, Department of Biology, Microbiology Research Laboratory.

2.5. Antimicrobial analyses

Water and ethanol extracts were tested on a variety of bacterial and fungal strains to assess their antibacterial properties. The antibacterial activity was assessed using the broth microdilution technique. For this aim, strains of microorganisms were inoculated into Brain-Heart Infusion Broth (Merck) medium and incubated at 37°C for the whole night. The bacterial suspension's concentration in sterile saline was brought down to 0.5 McFarland turbidity (10^8 cfu/ml). The inoculum's ultimate concentration in the wells was set at 5×10^5 colony-forming units. Each well of a sterile 96-well microplate was filled with Hinton-Mueller broth medium. The initial wells of the microplates were filled with extract solutions created at a concentration of 25 mg/ml. The extracts were then diluted twice (6.25–0.0030 mg/ml) by dividing the solutions among the remaining wells. Each well then received 100 µl of culture suspension. After using gentamicin as a positive control, the plates were incubated at 37 °C for a whole day. Each well was filled with a 2,3,5-triphenyl tetrazolium chloride (0.5%) solution after the incubation time, and the wells were then incubated once more for 30 minutes at 37 °C. The last well that showed no discernible development after this time frame—that is, no pink-red color—was designated as the MIC (Minimum Inhibition Concentration).

3. Results and Discussion

3.1. SPME Results

In SPME analyses, the major chemical constituents in *Satureja cilicica* and *Satureja cuneifolia* were detected (Table 1). Overall, both species predominantly contain monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpene hydrocarbons. These groups are characteristic of many species in the Lamiaceae family and are commonly associated with biological actions such as antimicrobial, anti-inflammatory and antioxidant effects. Notably, compounds such as m-Mentha-4,8-diene, Alpha-Terpinene, Beta-Myrcene, and dL-Limonene represent the monoterpene hydrocarbon fraction, while 5-Caranol and Carvomenthol are classified among the oxygenated monoterpenes. Sesquiterpene hydrocarbons, including Caryophyllene, Bicyclogermacrene, Alpha-Humulene, and Beta-Bisabolene, are also significant, indicating a complex and diverse chemical profile.

Table.1 Major compounds of the studied *Satureja* species according to SPME results

	Compound	***S. cilicica* (% Area)**	***S. cuneifolia* (% Area)**
1	Carvomenthol	6.33	-
2	m-Mentha-4,8-diene, (1S,3S)-(+)-	4.46	5.18
3	5-Caranol	4.99	4.00
4	3-Octen-5-yne,2,7-dimethyl	2.94	2.38
5	Alpha-Humulene	2.92	0.09
6	Beta-bisabolene	-	3.01
7	Caryophyllene	0.39	2.22
8	Bicyclogermacrene	1.28	1.36
9	Alpha-Terpinene	1.26	1.33
10	Beta-Myrcene	1.16	1.21
11	dL-Limonene	0,97	1.16
12	Alpha-Pinene	0.74	0.72
13	Beta-Pinene	0.28	0.24

Overall, while the chemical profiles of *S. cilicica* and *S. cuneifolia* share several similarities, distinct quantitative differences in key compounds suggest species-specific variations that could influence their potential pharmacological applications. These findings underscore the importance of chemotaxonomic studies for better understanding the diversity and bioactivity potential of the genus *Satureja*. Our study results are consistent with previous studies in terms of content, but there are differences in quantity. For example, a study analyzing the essential oil of *S. cuneifolia*, identified Carvacrol as the dominant component along with p-cymene and thymol. One another study reported that p-cymene and carvacrol were the primary compounds in *S. cuneifolia* essential oil [9-12].

3.2. Antimicrobial Assay Results

Antimicrobial effects of water and ethanol extracts of *Satureja cilicica* and *Satureja cuneifolia* species was determined by broth microdilution method according to Zengin et al.[13]. Obtained results are shown in Table 2. According to these results, it was determined that the water extract of *Satureja cilicica* was effective at a doses of 0.78 mg/ml against *S. aureus*, 1.56 mg/ml against *P. mirabilis* and 3.12 mg/ml against *L. monocytogenes* bacteria, while it did not show any activity against other bacterial species studied. Again, while water extract was found to be effective against *C. parapsilosis* at a dose of 3.12 mg/ml, no antifungal effect was observed on *C. albicans*. When we look at the ethanol extract of *Satureja cilicica*, it has been determined that there is no effect on only *E. coli* and *S. enteritidis* among the studied bacteria, but it has an effect on all other standard bacteria at doses of 0.19 – 3.12 mg/ml. It showed strong antibacterial activity against *B. cereus* and *L. monocytogenes* with MIC value of 0.19

mg/ml. While it was determined that it was effective against *C. parapsilosis* at a dose of 0.39 mg/ml, no antifungal effect was found on *C. albicans*. It was observed that the ethanol extract of *Satureja cilicica* showed higher antimicrobial activity than the water extract.

Table 2. MIC values of the studied *Satureja* extracts

Tested Microorganisms	***MIC Values of Satureja cilicica* extracts (mg/ml)**		***MIC Values of Satureja cuneifolia* extracts (mg/ml)**		**Gentamicin MIC Values (mg/ml)**
	Water	**Ethanol**	**Water**	**Ethanol**	
Esherichia coli ATCC 25922	-	-	-	-	0.0001
Pseudomonas aeruginosa ATCC 27853	-	3.12	-	3.12	0.0001
Klebsiella pneumonia ATCC 70603	-	1.56	-	-	0.0012
Staphylococcus aureus (MRSA)ATCC 43300	0.78	0.39	0.78	0.39	0.0001
Salmonella enteritidis ATCC 13076	-	-	-	-	0.0001
Sarcina lutea ATCC 9341	-	0.39	1.56	0.78	0.00003
Proteus mirabilis ATCC 25933	1.56	1.56	1.56	1.56	0.0003
Bacillus cereus ATCC 11778	-	0.19	-	0.39	0.00003
Listeria monocytogenes NRRL-B-33314	3.12	0.19	3.12	0.39	0.0003
Candida parapsilosis	3.12	0.39	3.12	0.39	0.0001
Candida albicans ATCC 26555	-	-	-	-	0.0003

When we look at the water extract of *Satureja cuneifolia*, it has been determined that it has antibacterial activity against *L. monocytogenes* at doses of 3.12 mg/ml and against *S. lutea* and *P. mirabilis* at doses of 1.56 mg/ml. The highest activity was detected against *S. aureus* standard bacteria at a dose of 0.78 mg/ml. No activity could be detected against other standard bacterial strains studied. While it was found to be effective against *C. parapsilosis* at a dose of 3.12 mg/ml, no antifungal effect was observed on *C. albicans*. When we evaluated the ethanol extract, it was observed that while there was no activity against *E. coli*, *K. pneumoniae* and *S. enteritidis* standard bacteria, it had antibacterial activity at doses of 0.39 – 3.12 mg/ml on other standard bacteria used. While it was determined to be effective against *C. parapsilosis* at a dose of 0.39 mg/ml, no antifungal effect was found on *C. albicans*. Again, when ethanol and water extract were compared, it was concluded that the ethanol extract showed a stronger antimicrobial activity than the water extract on the studied strains. In conclusion, *Satureja cilicica* and *Satureja cuneifolia* possess considerable antimicrobial potential, particularly in their ethanolic extracts and mainly against Gram-positive bacteria and certain fungal strains. The stronger activity of ethanol extracts emphasizes the role of solvent polarity in the extraction of bioactive constituents. These findings support the potential use of these species as natural antimicrobial agents, particularly against resistant Gram-positive pathogens. These findings are consistent with previous research, which showed that *S. cuneifolia* essential oil showed antimicrobial activity against various pathogens [14-15].

4.Conclusion

According to the findings, *Satureja cilicica* and *Satureja cuneifolia* are both intriguing sources of bioactive chemicals that might be used to create natural antibacterial drugs. More research is necessary in light of the reported biological activity and chemical variety, especially to identify and describe the precise chemicals causing the effects.

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BIOLOGICAL ACTIVITY ASSESSMENT OF DIFFERENT PARTS OF *HEDYSARUM ANATOLICUM*

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Abstract

The genus *Hedysarum* L. is represented by ca. 32 species in Türkiye, 18 of which are endemic. *Hedysarum anatolicum* Amirahm. & Kaz.Osaloo is a plant species endemic to Türkiye, belonging to the family Fabaceae. This species grows naturally in temperate climate conditions in a local of the Southeast region of Türkiye. It is thought that *H. anatolicum* could be a scientific importance due to its limited distribution area. In this study, methanol extracts from the leaves and flowers of *H. anatolicum* were prepared, and the antioxidant potentials of the obtained extracts and their effects on genotoxicity and acetylcholinesterase inhibitory activity were investigated. In this scope, antioxidant activity was evaluated by the DPPH radical scavenging method, genotoxicity was evaluated by the *Allium cepa* method and acetylcholinesterase (AChE) inhibition was measured spectrophotometrically. The results revealed that the flower extract showed stronger antioxidant activity than the leaf extract. Similarly, in agreement with the antioxidant findings, AChE inhibitory activity was higher in the flower extract. In the *Allium cepa* test, leaf extract caused a decrease in mitotic index at all applied concentrations, while flower extract caused a decrease in mitotic index only at the highest concentration. These results suggest that the flower extract of *H. anatolicum* has significant biological activity and may be a promising candidate for future pharmacological studies.

Key Words: AChE, Fabaceae, Pharmacological potential, ROS, Türkiye.

1.Introduction

The medicinal use of plants is probably as old as the existence of humanity. The World Health Organization (WHO) estimates that approximately 80% of the world's population relies on traditional medicine as a first line of medical intervention. Many of the plant species used for this purpose have been found to contain therapeutic substances that can be extracted and used in the preparation of drugs, but the plant itself can also be used directly or as a pharmaceutical extract [1]. Since synthetic drugs were rare and difficult to obtain 250 years ago, many plants with various medicinal benefits were used as sources of drugs [2]. The method of drug discovery probably emerged through trial and error, establishing a cause-and-effect relationship between the use of plants or different plant parts and correlating the desired result [3]. The genus *Hedysarum* L. (Fabaceae) includes about 200 species of annual and perennial herbaceous plants, found in Europe, Asia, North America, and North Africa [4-5]. One of the *Hedysarum's* important centres of diversification is thought to be Central Asia [5-6]. In Central Asia, there are 81 species in total, 46 of which are endemic. When the situation of the *Hedysarum* genus in Türkiye is evaluated, it is known that it is represented by approximately 32 species, 18 of which are endemic. *Hedysarum* species are widely recognised for their use as fodder, honey, and medicinal plants. Numerous *Hedysarum* plants have significant physiologically active chemicals that are crucial for developing novel medications with therapeutic effects against neurological, cardiovascular, oncological, and viral disorders [7-12]. In addition, according to current research, biological substances included in medicinal *Hedysarum* species exhibit antidiabetic, antioxidant, immunomodulatory, and anticancer properties [7, 9, 11, 13]. The leaves and roots have been shown to contain 155 physiologically active compounds, including different amino acids, carbohydrates, alkaloids, sterols, flavonoids, isoflavones, xanthones, tannins, and volatile oils [8-12].

Rich in xanthones, magniferin, and oligomeric catechins, species in the *Hedysarum* and *Multicaulia* sections are useful resources for the synthesis of multifunctional biologically active compounds and aid in the creation of novel, potent herbal medications with antiviral and antibacterial qualities [9-12, 14-16]. At the same time, the application area of *Hedysarum* species in traditional medicine is quite wide. The root parts of *H. polybotrys* are widely used in traditional Chinese medicine to treat various diseases. The dried root of *Hedysarum polybotrys* contains a bioactive phytoestrogen isoflavone called calycosin, which has been shown to have low toxicity to healthy cells, induce apoptosis in cancer cells, and have anti-metastatic potential in a variety of tumors [17]. Our study has been focused on the investigation of the endemic member of the genus *Hedysarum anatolicum* Amirahm. & Kaz.Osaloo.

Hedysarum anatolicum is a plant species endemic to Türkiye, belonging to the family Fabaceae. This species grows naturally in temperate climate conditions in a local of the Southeast region of Türkiye. It is thought that *H. anatolicum* could be a scientific importance due to its limited distribution area. This study aimed to comparatively reveal the antioxidant, genotoxic and acetylcholinesterase inhibitory potential of the methanol extracts prepared from two different parts (leaf and flower) of the *Hedysarum anatolicum*.

2.Material and Methods

2.1. Collection of the plant material

The plant material *Hedysarum anatolicum* was collected from its natural habitat (C4: Karaman; Ermenek, Göktepe plateau, sparse *Cedrus* forest clearings, marly soils, 1710 meters, 16.07.2004; Ibid KE5366 & T. Uysal, 19.06.2017) and brought to Selçuk University, Faculty of Science, Department of Biology, Plant Biology Laboratory. After the identification of the plant by Prof. Dr. Tuna UYSAL, the plant material was cleaned for preliminary preparation, dried in an environment away from direct sunlight and made ready for extraction.

2.2. Preparation of the extracts

After the plant material was dried, the leaf and flower parts were separated and powdered separately. The powdered materials were extracted with a Soxhlet apparatus. Methanol was used as the solvent in the extraction. The plant material was subjected to extraction for approximately 6-8 hours until the solvent became completely transparent. After the obtained extract was filtered with filter paper, it was removed from the solvent at 40 °C using a Rotary Evaporator. The obtained crude extracts were stored at -20 °C as stock solutions. The leaf extract was coded as HL, and the flower extract as HF. The extract yield was calculated using the following formula.

$$\% \text{ yield} = (A1 \times 100) / A2$$

A1 indicates the weight of the extract obtained, and A2 indicates the dry plant weight used at the beginning of the extraction.

2.3. Antioxidant Activity (DPPH Test)

The radical scavenging activity of the obtained extracts was determined using the DPPH test. The DPPH free radical scavenging test was performed in a 96-well microplate. 100 µl (0.156- 5 mg / mL) of the extracts prepared with different concentrations of methanol were added to 100 µL of 0.01% DPPH solution and the plate was incubated in the dark at room temperature for 30 minutes. At the end of the period, the absorbance value in the wells was read with an Elisa reader at 490 nm. Ascorbic acid was used as a standard at different concentrations. DPPH radical scavenging activity (%) was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = [(A_{control} - A_{extract}) / A_{control}] \times 100$$

2.4. Genotoxicity Determination (*Allium cepa* assay)

The genotoxic activity of the extracts was determined using the *Allium cepa* test. Healthy onions of approximately equal size were germinated in pure water, and root tips approximately 1 cm long were treated with different extracts. To examine mitosis and possible anomalies, the obtained root tips were placed in Carnoy's fixative after the extract treatment and stored at +4 °C. To examine cells, root tips were hydrolysed in 5N HCl at room temperature and then stained using aceto-orcein dye. Afterwards, crushed preparations were prepared and area scanning was performed on the preparations to determine whether there were any anomalies.

2.5. Acetylcholinesterase Inhibition Test

The inhibitory effect of the extracts was determined by using a kit. The inhibitory effect of both extracts on AChE was revealed by following the manufacturer's procedure given in the kit. The absorbance values of the extracts were compared with the control group.

3.Results and Discussion

Antioxidant Activity Determination Test Results

The absorbance values obtained after the DPPH test were compared to the control, and the % inhibition values of *Hedysarum anatolicum* extracts were calculated. The graphs and the slope equations of the graphs are given below (Figure 1). According to the data we obtained as a result of the study, when the antioxidant potential of the extracts was evaluated with the DPPH test, we can say that the flower extract has a lower IC_{50} value than the leaf extract, therefore, it is more effective than the leaf extract in terms of antioxidant potential. The IC_{50} values obtained as a result of DPPH analyses were calculated as 1.09 ± 0.015 mg/ml for the leaf extract and 0.868 ± 0.012 mg/ml for the flower extract, respectively. The obtained data revealed that the flower extract had a greater radical scavenging effect than the leaf extract, but both extracts had relatively less antioxidant potential than ascorbic acid.

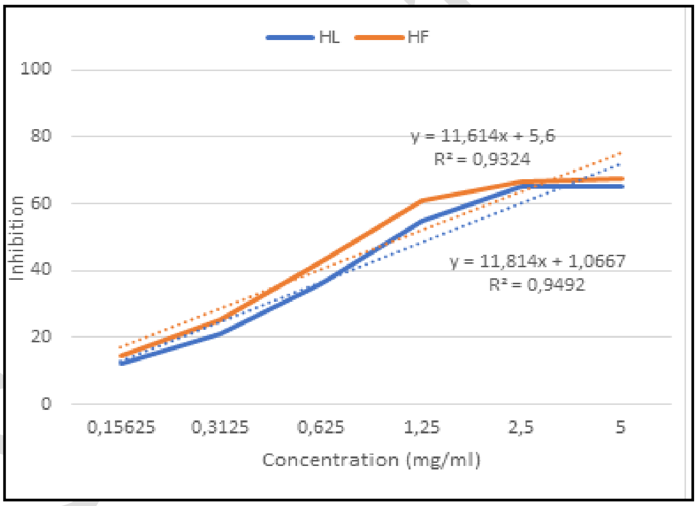

Figure 1. DPPH inhibition graphs of *Hedysarum anatolicum* extracts (HL: leaf extract; HF: flower extract).

Limited studies on the antioxidant potential of plants belonging to the *Hedysarum* genus have been conducted in the last 10 years, these studies have examined the antioxidant activities of different plant parts, extraction methods and extraction solvents [18-21]. Based on previous studies, *Hedysarum* species appear to have different antioxidant potential levels, but different methods and solvents appear to alter the antioxidant capacity.

Acetylcholinesterase Inhibitory Activity Results

The acetylcholinesterase inhibitory activities of the extracts were studied via kit and a graph was created based on the absorbance results. The graph created is given below (Figure 2). Based on the data obtained after absorbance measurement, it was determined that both extracts had acetylcholinesterase inhibitory effects and the flower extract was effective than the leaf extract.

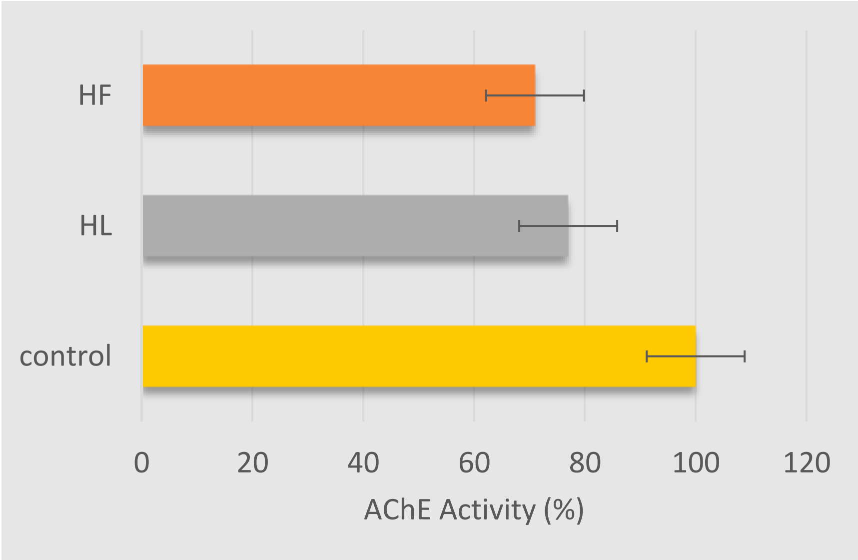

Figure 2. Acetylcholinesterase inhibition graph of extracts compared to control (HL: leaf extract; HF: flower extract)

As far as we know, there is one study on the AChE inhibitory potential of *Hedysarum* species. In the relevant study, it was reported that leaf methanol extracts exhibited strong AChE inhibitory activity [20]. The results of this study are consistent with our study in terms of the AChE inhibitory effect of leaf extracts, but also reveal the need for further studies in this area. Since the investigation of plant extracts that selectively inhibit AChE is of great importance to find new and more potent AChE inhibitors, studies on this subject have gained great importance.

Genotoxicity assay (*Allium cepa*) Results

It was determined that the leaf extracts caused a significant decrease in the mitotic index compared to the control group due to the increase in concentration, but the flower extracts caused a significant decrease in the mitotic index only at the highest concentration (Table 1). When we make a general evaluation, it is seen that the extracts do not have a considerable negative effect on the mitotic index compared to the control group.

Table 1. Mitotic indexes and chromosome anomalies at different cell cycle phases

Concentration	Total cell number	Mitotic Index ± SD	Prophase (%)		Metaphase (%)		Ana-Telophase (%)	
			Total	Anormal	Total	Anormal	Total	Anormal
Control	3460	42.23 ± 1.16	73.58	0.6	18.28	0.6	6.34	0.6
HL (0,4mg/ml)	3201	25.08 ± 1.30*	63.47	1.1	16.66	12.2*	5.85	0.81
HL (0,2mg/ml)	3001	28,42 ± 1.34*	70.28	1.20	13.36*	8.20*	4.8	1.40
HL (0,1mg/ml)	3361	35.37 ± 1.20*	67.68	1.23	16.26*	7.32*	7.2	0.31
HF (0,4mg/ml)	3479	27.62 ± 1.37*	69.33	1.07	11.28	9.52*	6.2	1.2
HF (0,2mg/ml)	3428	35.52 ± 1.26	66.47	1.32	13.11*	8.54*	5.50	0.54
HF (0,1mg/ml)	3563	37.27 ± 1.47	73.68	1.47	12.65*	8.65*	3.22*	0.33

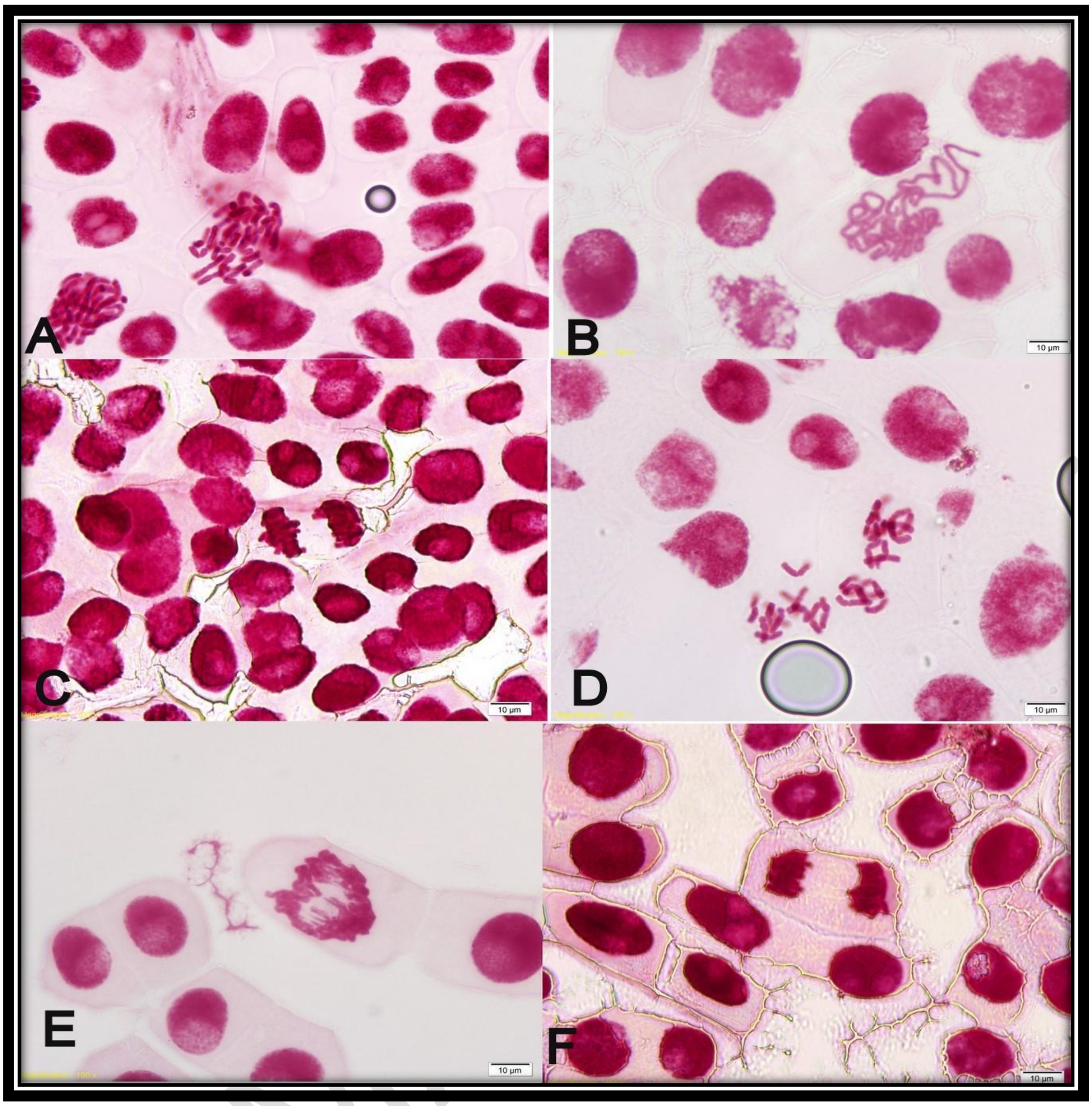

Figure 3. Some anomalies observed in *Allium cepa* experiment
(A-D: c-mitosis; B-E: prophase anomaly; C-F: stickiness in anaphase.

On the other hand, the chromosome anomalies caused by the extracts are at a very low level. When the preparations to which the extract was applied were examined, no significant change was detected in the total prophase percentage. This situation shows that the extracts do not cause statistically significant side effects in the prophase phase. When the metaphase phase is examined, a decrease depending on the concentration was observed after the extract application. The fact that both the total percentage decrease and the abnormality percentage increase were observed in all extracts in the metaphase phase shows that the concentration-dependent chromosome anomalies occur most frequently in the metaphase phase (Figure 3).

When we evaluated the general results, it was revealed that the flower extract showed stronger antioxidant activity than the leaf extract. Similarly, in line with the antioxidant findings, AChE inhibitory activity was higher in the flower extract. In the *Allium cepa* test, leaf extract decreased the mitotic index at all applied concentrations, while the flower extract decreased the mitotic index only at the highest concentration.

4.Conclusion

The results of this study suggest that the flower extract of *H. anatolicum* has a biological potential and may be a promising candidate for future studies. As a conclusion, following this preliminary screening, we plan to identify the compounds in the extract and determine further biological activities in our ongoing studies.

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RESEARCH OF SOME CHARACTERISTICS OF THE *Cerinthe minor* PLANT

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Abstract

Cerinthe minor L. (Boraginaceae) is among worth-mentioning traditionally used plants in Turkey. *C. minor* belongs to the tribe Lithospermeae Dumort, and genus *Cerinthe* L., which is known as a small genus that includes approximately ten species distributed in the circum-Mediterranean region and Central Europe, more specifically, from the Atlantic region of Morocco to the western parts of the Irano-Turanian region As of today, four Cerinthe species (*C. minor, C. retorta* Sm., *C. major* L., and *C. glabra* Mill.), and two *C. minor* subspecies (*C. minor* subsp. *auriculata*, and *C. minor* subsp. *minor*) have been identified in Turkey. The above-ground parts of the plants were collected from Van Yuzuncu Yil University, Medicinal and Aromatic Plants Garden in 2022. In this study; total ash and dry matter ratio, macro-micro and heavy metal contents were examined. As a result of the study; total ash content was determined as 16.02% and dry matter content as 92.14%. Some of the macro and micro mineral contents were determined as potassium (K), 38.58 g kg^{-1}, calcium (Ca), 35.25 g kg^{-1}, magnesium (Mg), 3.57 g kg^{-1}, iron (Fe), 505.51 mg kg^{-1}, zinc (Zn), 31.98 mg kg^{-1} and copper (Cu) content was determined as 11.32 mg kg^{-1}. In terms of heavy metal content was determined as arsenic (As) as 0.707 mg kg^{-1}, lead (Pb) as 1.13 mg kg^{-1}, chromium (Cr) as 1.44 mg kg^{-1} and cobalt (Co) content was determined as 0.017 mg kg^{-1}.

Key Words: *Cerinthe minor* L., medicinal plant, chemical content, mineral elements

1. Introduction

Cerinthe minor L. has been traditionally used for both culinary and medicinal purposes in Turkey; however, its pharmacognostical properties have not been investigated to a great extent. Throughout history, medicinal plants have played an important role in the prevention and treatment of diseases. Although the use of herbal medicines has decreased with the rise of synthetic drugs in the last two centuries, today, interest in plant-based treatments and traditional methods is increasing again, especially in developed countries. The World Health Organization (WHO) reported that more than 80% of the world's population safely uses medicinal plants as a priority in health care (Schippmann, Leaman, & Cunningham, 2002). Cerinthe minor L. (Boraginaceae) is among the important plants traditionally used in Türkiye. The genus Cerinthe L. is a small group of about ten species, spreading from Morocco on the Atlantic coast to the western parts of the Irano-Turanian region, especially around the Mediterranean and Central Europe (Selvi, Cecchi, & Coppi, 2009). Turkey holds a significant place globally in terms of plant diversity. Numerous studies have been carried out on Turkey's flora so far. It is estimated that the flora of Turkey includes around 12.000 taxa. Among these, 3649 taxa are endemic to Turkey, resulting in an endemism rate of approximately 32% (guner et al., 2012). This rate is quite remarkable, especially when compared to other European countries; for instance, the endemism rate is 14.9% in neighboring Greece and only 2.9% in France (Bagcıvan and Taskın, 2020). Several factors contribute to Turkey's high endemism rate, including its location at the crossroads of three phytogeographical regions, its rapidly changing topography over short distances, its varied geological structure, the coexistence of different soil types, the influence of multiple climate types, and the significant geological changes it has undergone over time (Polat, 2020).

Figure 1. *Cerinthe minor* plants

As of today, four Cerinthe species (*C. minor, C. retorta*, *C. major,* and *C. glabra*) and two C. minor subspecies (*C. minor* subsp. *auriculata* and *C. minor* subsp. *minor*) have been identified in Turkey (Güner et al., 2012; Jetter & Riederer, 1999; Zengin et al., 2016). *C. minor and C. minor* subsp. *auriculata*, commonly known as "Hışhış otu," are traditionally consumed as food in several ours provinces such as Tunceli, Batman, and Kars (Doğan & Tuzlacı, 2015; Kadıoğlu et al., 2020; Yeşil & İnal, 2019). Additionally, C. minor leaves are brewed into a decoction to treat edema, while a mouthwash made from the branches of *C. minor* subsp. *auriculata* is used for gum wound healing in folk medicine (Korkmaz & Karakuş, 2015; Kadıoğlu, Kadıoğlu & Sezer, 2021). Despite its widespread use in Turkish folk medicine, *C. minor* remains largely unexplored pharmacognostically. However, species belonging to the genus Cerinthe have been determined to contain toxic alkaloids in some studies (El-Shazly and Wink, 2014; Mroczek, Baj, Chrobok, and Glowniak, 2004). *Cerinthe* species are known to contain beneficial compounds such as antioxidants, they are also reported to possess toxic alkaloids (Ersoy et al., 2022).

In this study; it was aimed to determine the amount of total dry matter, ash contents and macro-micro nutrients and heavy metal contents of *Cerinthe minor* species, which is an important medicinal and aromatic plant.

Figure 2. Different *Cerinthe minor* variety

2.Material and Methods

Above-ground parts of plants were collected from Van Yuzuncu Yil University, Medicinal and Aromatic Plants Garden in 2022. In this study; total ash and dry matter ratio, macro and micro mineral element concentrations and heavy metal contents were examined. Ash and dry matter amount of plant samples were determined according to Kaçar and Inal (2008). 2 grams of pre-ground samples were weighed, placed in porcelain crucibles and placed in a muffle furnace. In the muffle furnace, dry burning process was applied to the samples at 500-550 0C for 4 hours.

The amount of ash and dry matter formed as a result of the burning process was calculated with the following formula:

Ash, %=[(K-D)/(B-D)]*100

D: Tare weight of the ash container

B: Weight of the plant sample with the ash container

K: Weight of the ash container with the ash

The determination of the dry matter and water content of the plant samples was made according to Kaçar and İnal (2008). 3-5 grams of plant samples were weighed and dried in an oven at 105 0C for 3-5 hours.

The dry matter and water amounts of the samples were calculated with the following formula:

Dry matter, %=[(K-D)/(B-D)]*100

D: Tare weight of the drying container

B: Weight of the air-dried plant sample with the drying container

K: Weight of the plant sample dried at 105 0C with the drying container

Atomic Absorption Spectrometer (AAS) device was used to determine the mineral contents of the plant materials (Hanlon, 1992). All analyzes carried out in the study were performed in triplicate and standard deviations were determined.

3.Results and Discussion

In this study, the total ash content of the *Cerinthe minor* plant was found to be 16.02%, while its dry matter content was measured at 92.14% (Table 1). Total ash analysis is one of the key chemical methods used to determine the amount of inorganic material in food. Ash represents the inorganic residue left behind after the organic components have been burned. In general, minerals in food are associated with organic substances such as proteins, fats, and carbohydrates. When food is heated to temperatures between 500 and 600 °C, water and volatile compounds are removed through evaporation, and the organic matter is combusted. The remaining residue contains mineral compounds in the form of oxides, sulfates, phosphates, silicates, or chlorides, depending on the combustion conditions and the composition of the original food sample

Dry matter in plants refers to all plant components excluding water and plays a crucial role in various functions such as growth, metabolism, nutrient storage, and industrial applications. Through the process of photosynthesis, plants synthesize organic compounds by combining water and carbon dioxide using sunlight. These organic compounds form the fundamental building blocks of a plant's dry matter. Dry matter is essential for the plant's growth and development. Its content typically varies depending on factors such as the plant’s age, species, environmental conditions, and cultivation practices. In previous studies, the dry matter content of various plants has been reported to range between 7.50% and 91.21% (Yüksel et al., 2020; Yıldırım et al., 2021; Tunçtürk and Tunçtürk, 2025). As for total ash content, it was reported to be 6.30% in *Cephalaria schrader ex Roemer & Schultes G.C. setosa Boiss. & Hohen.*, 6.21% in *Cichorium intybus L.*, 5.93% in *Centaurea karduchorum Boiss.* and 24.08% in *Symphytum officinale* (Tunçtürk et al., 2007; Tunçtürk et al., 2018; Tunçtürk et al., 2019).

Table 1. Total ash and dry matter content (%) of *Cerinthe minor* plant

PARAMETERS	CONTENTS
Total ash content (%)	16.02 ±0.520
Dry matter ratio (%)	92.14 ±0.550

In this study, the macroelement contents of the plant were determined as follows: potassium (K) at 38.58 g/kg, calcium (Ca) at 35.25 g/kg, and magnesium (Mg) at 3.57 g/kg. Regarding microelements, the iron (Fe) content was measured at 505.51 mg/kg, manganese (Mn) at 61.443 mg/kg, zinc (Zn) at 31.98 mg/kg, and copper (Cu) at 11.32 mg/kg (Table 2). In a study conducted by Turan (2014) on approximately 50 different plant species, the highest concentrations of Zn (31.63 mg/kg), Fe (848.74 mg/kg), and Na (3150.31 mg/kg) were found in comfrey, while K, Ca, and Mg levels were recorded at 4044.77, 11691.50, and 1182.04 mg/kg, respectively. Similarly, Maiti et al. (2016) reported that the concentrations of Fe, Cu, and Zn in 44 traditionally used medicinal plant species ranged from 98.28 to 3973.55 mg/kg, 4.17 to 33.88 mg/kg, and 9.49 to 216.31 mg/kg, respectively. These findings are consistent with the results of our study.

Elements such as calcium (Ca), magnesium (Mg), and sodium (Na) are vital for the health of both humans and animals, while trace elements like copper (Cu), zinc (Zn), manganese (Mn), and molybdenum (Mo) are also essential, albeit in smaller quantities. In the absence of these micronutrients, fundamental biological processes such as growth and reproduction may be halted. Deficiencies can lead to physiological abnormalities and increase susceptibility to diseases (33). According to Kabata-Pendias & Pendias (2001), the tolerance limits for copper, zinc, manganese, and molybdenum in the mature leaf tissues of a wide range of plant species are 20–100 mg/kg for Cu, 100–400 mg/kg for Zn, 400–1000 mg/kg for Mn, and 10–50 mg/kg for Mo; levels exceeding these ranges may result in toxicity. The mineral content of plants is influenced by several factors, including genetic structure, environmental and growing conditions, soil characteristics, water availability, and seasonal variation. Consequently, a high degree of variability in mineral composition can be observed among different plant species and even among different plant parts (28). Previous studies have reported nutrient element concentrations in medicinal plants as follows: magnesium (Mg) ranging from 1.17 to 86.43 g/kg, potassium (K) from 4.34 to 557.91 g/kg, and calcium (Ca) within a wide range of 0.03 to 777.52 g/kg (Kabata-Pendias, 2001; Koca et al., 2009; Akgünlü, 2012).

In this study, the concentrations of several trace and heavy metals were determined as follows: nickel (Ni) at 1.72 mg/kg, aluminum (Al) at 1595.99 mg/kg, arsenic (As) at 0.707 mg/kg, cobalt (Co) at 0.017 mg/kg, chromium (Cr) at 1.447 mg/kg, and lead (Pb) at 1.137 mg/kg. In their comprehensive study on trace elements and heavy metal concentrations in various plant species, Kabata-Pendias and Pendias (2001) reported the toxic limits for aluminum as 30.983–368.877 mg/kg, arsenic—one of the most toxic trace elements—as 5–20 mg/kg, cadmium (Cd) as 5–30 mg/kg, cobalt as 15–50 mg/kg, chromium as 5–30 mg/kg, and lead within a tolerance range of 30–300 mg/kg. Certain metals, such as lead (Pb), cadmium (Cd), cobalt (Co), mercury (Hg), and nickel (Ni), are classified as heavy metals and can exhibit toxic effects when their concentrations exceed specific thresholds (Kayalar et al., 2014; Özkan, 2004). On the other hand, elements such as iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), molybdenum (Mo), and nickel (Ni) can serve as essential micronutrients for both plants and animals and are generally non-toxic when present within permissible limits (Bedir, 2010).

Table 2. Macro-micro and heavy metal contents of *Symphytum officinale* plant

MINERALS	CONTENTS		
Mg(g/kg)	3.579	±	0.274
K(g/kg)	38.584	±	0.181
Ca(g/kg)	35.253	±	0.961
Fe (mg/kg)	505.51	±	5.85
Mn (mg/kg)	61.443	±	0.901
Zn (mg/kg)	31.98	±	1.847
Cu (mg/kg)	11.327	±	0.778
Ni (mg/kg)	1.721	±	0.552
Al (mg/kg)	1595.99	±	1492.8
As (mg/kg)	0.707	±	0.345
Co (mg/kg)	0.017	±	0.145
Cr (mg/kg)	1.447	±	1.516
Pb (mg/kg)	1.137	±	1.039

Additionally, chromium, nickel, and lead levels in soil are generally considered to be within normal limits when they range between 10–100 mg/kg, and cadmium (Cd) is considered acceptable when present at levels below 1 mg/kg. According to Yıldız (2001), extractable heavy metal concentrations in soils may cause toxic effects when they exceed 1 mg/kg for cadmium, 10 mg/kg for cobalt, 0.1 mg/kg for copper, 10 mg/kg for selenium, 0.5–1 mg/kg for vanadium, and 100 mg/kg for nickel. Our findings are consistent with previous research: the concentrations of chromium (Cr), cadmium (Cd), and cobalt (Co) in medicinal plants and wild vegetables were reported to range from 0.10 to 425.0 mg/kg (Yıldız, 2004; Eşetlili et al., 2014), 0.007 to 0.47 mg/kg (Eşetlili et al., 2014), and 0.05 to 1.35 mg/kg (Canbay and Zerrin, 2015), respectively. In a study involving approximately 50 different plant species, Turan (2014) reported the concentrations of copper (Cu), manganese (Mn), lead (Pb), cadmium (Cd), chromium (Cr), and nickel (Ni) as 5.44, 55.32, 0.15, 0.26, 2.17, and 1.95 mg/kg, respectively.

4.Conclusion

With the growing popularity of herbal medicines, the safe consumption of traditionally used medicinal plants should be supported by comprehensive pharmacognostic studies that explore their chemical composition and pharmacological effects. Although *Cerinthe* species are known to contain beneficial compounds such as antioxidants, they are also reported to possess toxic alkaloids. Therefore, gaining a deeper understanding of the potential toxicity of plants used in the pharmaceutical industry is crucial. The results of this study indicated that *Cerinthe minor* falls within acceptable tolerance limits for heavy metals, with concentrations of arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), and lead (Pb) all well below established toxicity thresholds. It is essential to promote the conscious consumption of plants, as the accumulation of certain elements particularly heavy metals in living organisms can pose significant health risks.

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SOME RESEARCH ON *POLYGONUM ORIENTALE* L. (≡*PERSICARIA ORIENTALIS*; POLYGONACEAE) PLANT

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Abstract

The Polygonaceae family, which has the highest distribution in the Northern Hemisphere, is represented by 52 genera and an estimated 1552 taxa in the world. These family members are defined as herbs, shrubs, rarely climbers or trees. There are 10 genera under the Polygonaceae family in Turkey. Poligonum orientale, which is located under Polygonum, one of the largest genera of the Polygonaceae family, is accepted in many international references under the name *Persicaria orientalis*. The plants were collected from Van Yüzüncü Yıl University, Medicinal and Aromatic Plants Garden in 2021. In this study; antioxidant activity, total phenolic and flavonoid substance amounts and dualex values (nitrogen balance index (NBI), chlorophyll content, flavonol and anthocyanin content) were examined. In the results of working; The amount of antioxidant substance was determined as 137.73 µmol TE/g, total phenolic substance (202.87 mg GAE/g) and total flavonoid substance amount was determined as 7.82 mg QE/100 g. The data obtained in terms of Dualex values (dx) such as Nitrogen Balance Index (NBI), chlorophyll content, flavonol and anthocyanin content are respectively; It was determined as 13.70 mg/g, 23.86 mg/cm2, 1.74 dx and 0.08 dx.

Key Words: *Polygonum orientale* L., medicinal plant, antioxidant, phenolic, flavonoid, dualex value

1.Introduction

The Polygonaceae family, commonly known as the knotweed or smartweed family, is predominantly distributed across the Northern Hemisphere. It encompasses approximately 52 genera and an estimated 1.552 taxa globally. Members of this family are primarily herbaceous plants, with some species being shrubs, and rarely climbers or trees. In Turkey, the Polygonaceae family is represented by 11 genera, including Polygonum, one of its largest genera. Within this genus, Polygonum orientale is noteworthy. While many international references list this species under the name Persicaria orientalis , the International Plant Names Index (IPNI) and the Plant List of Turkey consider Persicaria orientalis a synonym of Polygonum orientale. Originally described by Carl Linnaeus in 1753, Polygonum orientale was later transferred to the genus Persicaria by Édouard Spach in 1841. This species is native to a broad region extending from India to the Russian Far East and reaching into northern and eastern Australia . Due to its non-native status in Turkey, it is considered an alien species within the country's flora. Moreover, in certain regions, it is recognized as an invasive plant. It is commonly seen in the provinces of Ankara, Rize, Sakarya, Adapazarı and Urfa in our country (Ozkan and Yazlik (2020).

Polygonum orientale is a branching annual, 30 to 100 centimeters in height. Flowers are white. Studies have suggested antioxidant, radical scavenging, cardioprotective, antihyperglycemic, melanogenesis inhibitory, bone healing, phytoremediative, antinociceptive, anti-inflammatory, antidiarrheal, cytotoxic, thrombolytic properties (Anonymus b).

Known hazards of *Polygonum orientale*: Although no specific mention has been made for this species, there have been reports that some members of this genus can cause photosensitivity in susceptible people. Many species also contain oxalic acid (the distinctive lemony flavour of sorrel) - whilst not toxic this substance can bind up other minerals making them unavailable to the body and leading to mineral deficiency. Having said that, a number of common foods such as sorrel and rhubarb contain oxalic acid and the leaves of most members of this genus are nutritious and beneficial to eat in moderate quantities. Cooking the leaves will reduce their content of oxalic acid. People with a tendency to rheumatism, arthritis, gout, kidney stones or hyperacidity should take especial caution if including this plant in their diet since it can aggravate their condition.

Figure 1. *Polygonum orientale* plants

Medicinal use of Prince's Feather: Polygonum orientale extracts have been shown to have anti-inflammatory and pain-relieving properties. These effects support its traditional use in the treatment of inflammatory diseases such as rheumatoid arthritis (Gou et al., 2017). The flowers of the plant have been used in traditional medicine as a blood thinner and circulatory enhancer. In addition, the leafy stems have been used in the treatment of hernia, and a decoction obtained by boiling the ripe fruits has been used in the treatment of conditions such as hepatitis, ulcers, and abdominal bloating (Anonymus a, 2025). Polygonum orientale is a plant rich in flavonoids. These compounds can protect cells from oxidative stress by fighting free radicals and may have anti-aging effects (Anonymus b, 2025). The seeds of the plant have been used traditionally as a carminative, antipyretic, and thirst quencher. In addition, their diuretic effects may help eliminate edema in the body. It has been suggested that plants belonging to the genus Polygonum may have antitumor properties. However, more research is needed to validate these effects in humans. There is information that hair products, especially those derived from the P. multiflorum species, can turn white hair into black.

Edible parts of Prince's Feather: Roots, Flowers, fruit, leaves, whole plant. The young shoots are a standard vegetable in some country (Indo-China).

Propagation of the herb: Seed - sow spring in a cold frame. Germination is usually free and easy. When they are large enough to handle, prick the seedlings out into individual pots and plant them out in the summer if they have reached sufficient size. If not, overwinter them in a cold frame and plant them out the following spring after the last expected frosts.

Cultivation of Prince's Feather: Roadsides, near houses and wastelands, also commonly cultivated in gardens, from sea level to 3000 metres (Anonymus, 2025a).

Figure 2. Cosmetic products derived from *Polygonum spp.*

In this study; It was aimed to determine the dualex values, antioxidant activity amount, and total phenolic and flavonoid substance amounts of *Polygonum orientale* species, which is an important medicinal and aromatic plant.

2.Materials and Methods

The plants were collected in 2021 from the Medicinal and Aromatic Plants Garden at Van Yüzüncü Yıl University. Prior to harvesting, leaf-level measurements were taken in real-time and non-destructively using the Dualex Scientific+ device (FORCE-A, France), which is equipped with a leaf clip sensor.

This device measures chlorophyll content (µg/cm²), nitrogen balance index (NBI) (mg/g), flavonol, and anthocyanin levels (dx). Harvesting was carried out using sterile scissors, cutting the plants at the point where the stem meets the soil. For subsequent analyses on dried samples, the plant materials were dried in an oven at 40°C and stored under suitable conditions. In the study, total antioxidant activity was determined using the FRAP (ferric reducing antioxidant power) method described by Lutz et al. (2011); total phenolic content was measured according to Obanda and Owuor (1997); and total flavonoid content was assessed using the method developed by Quettier et al. (2000).

3.Results and Discussion

Antioxidants are a group of chemical substances that are produced by body cells and also taken with food (especially from herbal products). The most important antioxidants taken with food are beta-carotene and vitamins E and C. In addition, other antioxidant substances are lycopene, eugenol, resveratrol, myrcetin, silymarin and some sesquiterpene lactone, triterpene, steroid and tannin compounds (Wang et al., 1996; Aruoma, 2003).

It is known that medicinal plants provide great support to the body's defense system and that the use of these plants in the treatment of liver and bile disorders, as well as their antioxidant effects, plays a preventive role in the onset of many illnesses and diseases. In this study, the amount of antioxidant capacity was determined as 137.73 µmol TE/g KA, the phenolic substance content was determined as 202.87 mg GAE/g KA, and the flavonoid substance content was determined as 7.82 mg QE/100g KA (Figure 1).

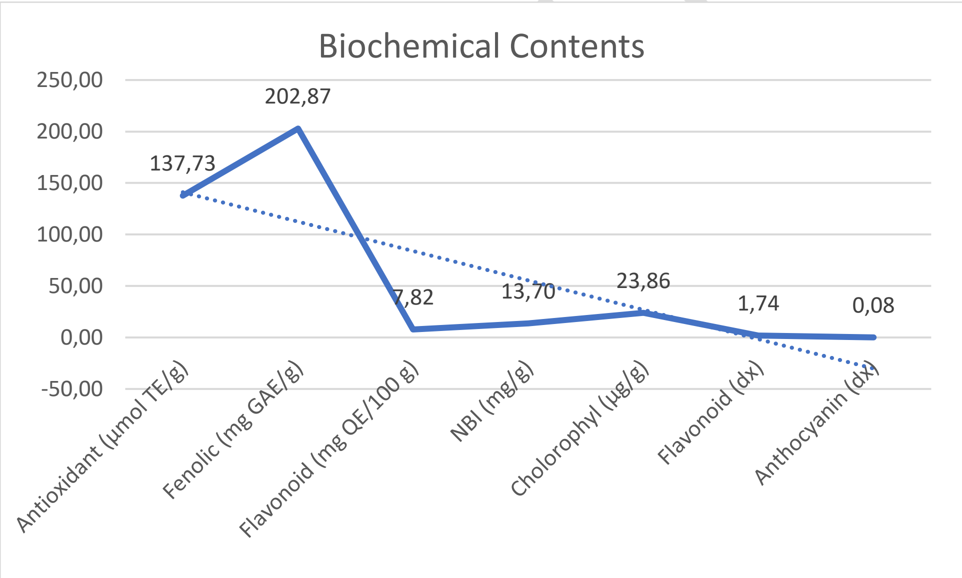

Figure 3. Some biochemical contents of *Polygonum orientale* plant

In the study conducted by Wei et al., (2009), the IC50 value in P. orientale was 4.11 mmol/L, which shows that taxifolin has a very potent antioxidant activity. The extract was found low in antioxidant property. The absorbance was taken at 517nm by UV Spectrophotometer. The IC50 values were found in leafextract and shoot extract are (1244.58µg/ml and (1506µg/ml). For ascorbic acid IC50 value was (139.19µg/ml). Cytotoxic activity of the crude extract by using Brine Shrimp Lethality Bioassay. Vincristin sulphate was used as standard. LC50 value of leaf extract was found (6.85µg/ml) and (9.7104µg/ml) for the shoot extract. In conclusion, the extract of the experimental plants have mild antimicrobial, low antioxidant and good cytotoxic properties (İslam et al. 2016). Free radicals and reactive oxygen species (ROS) occur during metabolic events that occur in our bodies throughout our lives. Oxidative stress disrupts the balance between ROS production and antioxidant defense and causes oxidative degradation, which causes antioxidant defense mechanisms (A, C, E, glutathione, ubiquinone, flavonoids, etc.) to enzymatic (catalase, superoxide dismutase, glutathione peroxidase, etc.) It has been shown that deficiency as well as ROS and excessive activation play a role in the emergence of many diseases such as heart and nerve diseases, diabetes, asthma and rheumatism (Gok and Serteser, 2003).

This study by Gou et al. (2017) supported the fact that P. orientale is traditionally applied by the local people for inflammatory disorders such as rheumatoid arthralgia and rheumatoid arthritis. In other study, Total antioxidant capacities and phenolic contents of *Polygonum capitatum*, *Polygonum chinensis*, *Polygonum cuspidatum*, and *Polygonum multiflorum* were 74.60, 53.66, 56.22, and 14.34 mmol trolox/100 g dry weight (DW), and 8.69, 4.15, 6.33, and 1.27 g gallic acid/100 g DW, respectively, significantly higher than those of five dietary vegetables, fruits and spices (spring onion, broccoli, orange, carrot, and ginger) (Huang et al.2008). ***Polygonum orientale*** (*P. orientale*) has been reported to possess antioxidant, anti-inflammatory, analgesic, anti-myocardial ischemic, and vasodilatory activities (Liang et al., 2013, 2014). Additionally, evidence suggests that the fruit of *P. orientale*, known as *Fructus Polygoni orientalis*, can help alleviate indigestion and relieve pain (Zhai et al., 2006). Previous studies have shown that the water extract of *P. orientale* is rich in flavonoids, particularly isoorientin, orientin, vitexin, and quercetin (Huang et al., 2014), all of which are known for their anti-inflammatory and analgesic properties (Da Silva et al., 2010; Gorzalczany et al., 2011; Napimoga et al., 2013; Robbins et al., 2014). However, the underlying mechanisms responsible for these anti-inflammatory and analgesic effects remain unclear. Therefore, further research is needed to identify the main effective fractions, active compounds, and the precise mechanisms by which P. orientale exerts its anti-inflammatory and analgesic activities (Gou et al., 2017).

In this study; NBI (chlorophyll/phenolic) content was determined as 13.70 mg/g, chlorophyll content as 23.86 microgram/cm2, flavonol content as 1.74 (dx) and anthocyanin content as 0.08 (dx). The Dualex™ was developed by Goulas et al. (2004) in cooperation with FORCE-A to non-destructively measure leaf phenolic content to evaluate a correlation with the nitrogen status of plants. Cerovic et al., (2005), described that phenolics increased under nitrogen deficiency. At the same time, chlorophyll responded in an opposite manner. Cartelat et al. (2005) suggested that the chlorophyll/phen ratio expressed as NBI was a better indicator for leaf nitrogen concentration as each parameter alone (Overback et. al., 2018). Phenolics including the major group of flavonoids are most important groups of secondary metabolites and bioactive compounds in plants (Kim et al., 2003) and their synthesis is highly correlated with light because of their function as UV-protective pigments (Li et al., 1993). A rapid increase in flavonoid biosynthesis is generally observed under high light conditions, which reflects the important role of flavonoids in photoprotection (Schmelzer et al., 1988; Zoratti et al., 2014). First results of Barthod et al. (2007) showed that the Dualex™ derived UV absorbance of leaf epidermis increased significantly with increasing light and the data from the instrument could be a good indicator for the amount of phenolics in the leaves (Overback et. al., 2018).

4.**Conclusion**

Many species of Polygonum are rich in bioactive constituents, which contribute to a wide range of medicinal properties. In this study, it was found that the P. orientale plant, which has anti-inflammatory and analgesic effects in terms of health, contains antioxidant and phenolic components, but since the basic mechanism of this plant has not been fully elucidated, further research is needed to determine the main effective fractions, active compounds and mechanisms that reveal its effects on health.

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PHYTOPHARMACEUTICAL POTENTIAL AND BREEDING CHALLENGES OF SEA BUCKTHORN (*Hippophae Rhamnoides* L.): A REVIEW OF AN UNDERUTILIZED HORTICULTURAL RESOURCE

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Abstract

Sea buckthorn (*Hippophae rhamnoides* L.), a deciduous and dioecious shrub native to Europe and Asia, has garnered significant attention due to its rich phytochemical profile and broad spectrum of medicinal applications. The berries, seeds, and leaves of sea buckthorn contain high concentrations of bioactive compounds, including vitamins C and E, flavonoids, carotenoids, phytosterols, and essential fatty acids such as omega-3, -6, -7, and -9. These constituents offer antioxidant, anti-inflammatory, immunomodulatory, and wound-healing properties, positioning sea buckthorn as a valuable phytopharmaceutical crop. Despite its therapeutic promise, the crop remains underutilized in modern horticultural systems, particularly in countries like Türkiye, where its natural habitat suggests potential for adaptation and cultivation. This review highlights the current understanding of sea buckthorn's medicinal value while emphasizing the major challenges limiting its widespread breeding and commercialization. Key breeding constraints include dioecy, long juvenile phase, thorniness, and the lack of early sex identification markers. Moreover, limited genomic resources and inadequate propagation methods hinder the selection and improvement of elite cultivars. Recent advances in biotechnology such as tissue culture, molecular marker-assisted selection, and transcriptomic tools, offer promising strategies to overcome these challenges. Integrating conventional and biotechnological approaches could accelerate the development of high-yielding, thornless, and nutraceutical-rich cultivars. By combining current knowledge, this review aims to support repositioning sea buckthorn as a valuable medicinal and horticultural resource, especially in arid and semi-arid regions where it can contribute to human health and sustainable agriculture.

Key Words: Sea buckthorn, Phytopharmaceuticals, Breeding challenges, Biotechnology, Underutilized medicinal plant

1.Introduction

Hippophae rhamnoides L., commonly known as sea buckthorn, is a hurdy deciduous shrub or small tree, native to the temperate regions of Europe and Asia. Also referred to as Siberian pineapple, sand thorn, and sea berry, it is notable for an extensively developed root system, which forms symbiotic associations with nitrogen-fixing *Frankia* species (Actinobacteria), enriching soil fertility even under marginal conditions (Liu et al., 2021; Singh, 2003). Typically ranging from 1 to 3 meters in height, sea buckthorn thrives in poor, erosion-prone soils and is widely utilized in Eurasia for soil and water conservation, land reclamation, and habitat restoration (He et al., 2016). Its exceptional tolerance to cold, drought, salinity, and alkalinity, combined with its ability to fix atmospheric nitrogen, makes it an ideal crop for use as a windbreak and for stabilizing marginal and degraded lands (Gupta & Suryakumar, 2011). In addition to its ecological significance, sea buckthorn offers high nutritional and economic value. All parts of the plant, including fruits, leaves, stems, roots, and even thorns, have been used in traditional medicine and as dietary supplements. The fruits are particularly valued for their rich content of flavonoids, carotenoids, sterols, essential fatty acids, and high levels of vitamins C and E (Mei et al., 2023; Ljubojević et al., 2022). These bioactive compounds contribute to a range of documented pharmacological effects, including anti-inflammatory, antimicrobial, cardioprotective, and hepatoprotective properties (Skalski et al., 2019; Gantait et al., 2024; Popović-Djordjević et al., 2022).

As a result, sea buckthorn is increasingly recognized as a valuable raw material in the pharmaceutical, cosmetic, and functional food industries (Tang et al., 2001). Owing to its remarkable tolerance to cold, drought, salinity, and alkalinity, along with its ability to improve soil fertility through nitrogen fixation, sea buckthorn has been promoted as a "miracle plant" in various cultural texts and is often referred to as the "Golden Bush" or "Green Gold" (Ahani et al., 2015; Stobdan et al., 2013). Since the 1940s, extensive research, especially in Russia, has led to the development of sea buckthorn-based products such as nutrient-dense foods and radiation-protective creams for cosmonauts (Piłat et al., 2015).

The global significance of sea buckthorn has grown rapidly, prompting the formation of the International Seabuckthorn Association in 1999 by countries such as China, India, and Canada to promote research and industry development. By 2016, the crop was cultivated across more than 633,000 hectares in 26 countries (Wang et al., 2022), reflecting growing awareness of its therapeutic and ecological benefits. Despite its potential, *H. rhamnoides* remains underutilized, particularly in commercial horticulture and structured breeding programs. The species faces significant challenges, including a dioecious reproductive system, poor seed germination, limited genomic tools, and a lack of elite cultivars adapted for large-scale cultivation (Dandin & Kumar, 2017; Singh, 2022). Addressing these limitations through modern breeding strategies, molecular approaches, and conservation efforts is crucial for unlocking the full potential of sea buckthorn as a resilient and economically valuable crop.

This review explores the phytopharmaceutical applications of sea buckthorn and highlights the current breeding challenges hindering its development. By evaluating both traditional uses and recent scientific advancements, the paper aims to provide a comprehensive understanding of this underutilized yet highly promising horticultural resource.

2.Importance of Underutilized Horticultural Plants

Underutilized horticultural plants hold significant potential to address global challenges such as food security, climate resilience, biodiversity conservation, and nutritional health. Despite being largely neglected in mainstream agriculture, these crops often exhibit unique adaptive traits such as drought, salinity, and poor soils, as well as resistance to pests and diseases (Santosh et al., 2024). They are rich in micronutrients, antioxidants, and bioactive compounds, thereby offering solutions to "hidden hunger" and undernutrition prevalent in many developing regions (Oloyede et al., 2023; Okigbo & Anyaegbu, 2021). Beyond their nutritional benefits, underutilized crops play a crucial role in preserving agrobiodiversity, supporting traditional knowledge systems, and enhancing the resilience of rural livelihoods, particularly among indigenous and marginalized communities (Meena et al., 2022; Rai et al., 2005). Their cultivation contributes to sustainable land use and diversification of farming systems, offering ecological services such as soil enrichment, pollinator support, and climate mitigation. Promoting the cultivation and consumption of these crops supports the United Nations Sustainable Development Goals (SDGs), especially those concerning zero hunger, good health and well-being, and responsible consumption and production. In the face of increasing climate change impacts and resource degradation, underutilized horticultural species offer a strategic alternative to vulnerable monoculture systems, enhancing the resilience and sustainability of agro-ecosystems (Sharma et al., 2018; Singh et al., 2020). As nutrient-dense, stress-resilient, and culturally embedded resources, these crops represent a vital yet untapped asset for sustainable agricultural development (Kour et al., 2018).

3.Phytopharmaceutical Potential of *Hippophae rhamnoides* L.

Sea buckthorn is increasingly recognized for its exceptional phytopharmaceutical value, owing to its rich profile of bioactive compounds distributed across its berries, seeds, leaves, and bark. These components include vitamins (A, C, E, K), flavonoids, carotenoids (such as β-carotene and zeaxanthin), polyunsaturated fatty acids (notably omega-3, -6, and the rare omega-7), phytosterols, tocopherols, phenolic acids, and various alkaloids (Bayır et al., 2024; Ilhan et al., 2021). Among these, vitamin C is particularly abundant, with concentrations reaching up to 600 mg/100 g, far surpassing that of many commercial fruits (Bayır et al., 2024). The berries also exhibit high flavonoid levels, ranging from 130.3 to 415.7 mg quercetin equivalents per 100 g fresh weight, and total phenolic contents of 120–340 mg gallic acid equivalents per 100 g, contributing to their strong antioxidant activity—measured up to 1720 μmol Trolox equivalents per 100 g using DPPH and FRAP assays (Ilhan et al., 2021; Moskalets et al., 2019). The amino acid profile of the fruit includes high levels of asparagine (427 mg/100 g) and proline (426.6 mg/100 g), with notable amounts of lysine, threonine, and methionine—making it a functional protein-rich supplement (Olas, 2016; Ercisli et al., 2007). Organic acids such as quinic (2.8%), malic (1.6%), and citric acid (0.16%) also contribute to the fruit's acidity and stability (Ranjith & Arumughan, 2009). Additionally, sea buckthorn oils represent a major therapeutic asset. The seed oil is rich in linoleic (30–40%) and α-linolenic acids (20–35%), conferring cardiovascular and anti-inflammatory benefits, while the pulp oil contains up to 54% palmitoleic acid (omega-7), a rare fatty acid in plants known for promoting skin regeneration and mucosal healing (Li et al., 2020; Mei et al., 2023). In addition, vitamin E levels in sea buckthorn oil range from 80 to 250 mg/100 g, further enhancing its antioxidant efficacy. Alkaloids such as hippophamide and related amine derivatives have also been identified, particularly in the leaves and roots, with preliminary indications of cardiovascular and neuroprotective effects, although these remain underexplored (Bayır et al., 2024). Historically, sea buckthorn has been extensively used in traditional Chinese, Tibetan, and Russian medicine to treat ulcers, skin wounds, coughs, and respiratory infections (Ahani & Attaran, 2022). Contemporary pharmacological studies support these traditional uses, confirming its antimicrobial, anti-inflammatory, antitumor, hepatoprotective, and cardioprotective activities, largely attributed to its potent antioxidant and flavonoid content (Moskalets et al., 2019; Ahani & Attaran, 2022). Its therapeutic applications have extended to modern dermatology and functional medicine, where sea buckthorn-derived products are now formulated into nutraceuticals, cosmeceuticals, wound-healing agents, and immuno-modulatory supplements.

Despite its vast medicinal promise, the commercial development and clinical standardization of *H. rhamnoides* remain challenged by significant phytochemical variability across subspecies, environments, and cultivation methods (Mei et al., 2023; Moskalets et al., 2019). Addressing these inconsistencies through targeted breeding and biochemical profiling is essential for unlocking the full potential of this underutilized horticultural resource. The essential oil and volatile profile of *Hippophae rhamnoides* L. varies substantially across different plant parts, subspecies, and cultivation types, reflecting its chemical richness and potential in food, cosmetic, and pharmaceutical applications. GC–MS analyses have identified over 100 volatile compounds, predominantly esters, alcohols, and oxygenated sesquiterpenes. Çakır (2004) reported that fruits contain high concentrations of ethyl dodecenoate (39.4%), ethyl octanoate (9.9%), and ethyl decanoate (5.5%), representing more than 94% of the total volatile oil. Slynko et al. (2019) further characterized essential oil profiles from berries, seedlings, and shoots, reporting distinct compositional differences. Berries were dominated by carboxylic acids and their esters (71.45%), especially 3-methylbutyl benzoate (11.63%), ethyl hexanoate (9.07%), and 3-methylbutyl 3-methylbutanoate (7.77%). In contrast, seedlings exhibited up to 72.9% phenylpropanoids, while shoots were rich in alkanes (29.86%) and oxygenated sesquiterpenes such as germacrene B and shyobunon. Oil yields varied by tissue type, ranging from 37.5 mg/kg in shoots to 265 mg/kg in berries.

Building on these findings, Meng et al. (2025) applied molecular sensory science to identify 21 aroma-active compounds critical to the fruity and winy aroma of *H. rhamnoides* ssp. *sinensis*. Key volatiles included ethyl isovalerate, ethyl caproate, 1-hexanol, phenylethyl alcohol, and α-terpineol. These compounds are derived from fatty acid oxidation, amino acid degradation, and carotenoid cleavage, involving precursors like oleic acid, linoleic acid, and phenylalanine. Complementing these findings, Socaci et al. (2013) used an ITEX-GC–MS method to profile 12 Carpathian varieties (wild and cultivated), identifying 43 volatile compounds, primarily short-chain ethyl esters and aromatic esters, which accounted for over 80% of the total volatiles. PCA enabled effective discrimination between varieties based on aroma fingerprints, validating volatiles as biomarkers for quality, freshness, and cultivar origin. Together, these studies confirm that *H. rhamnoides* possesses a rich and diverse essential oil composition, with esters dominating in fruits and phenylpropanoids in vegetative tissues. This volatile complexity enhances its potential in natural product development, cosmetics, functional foods, and aromatherapy.

4.Breeding Challenges of *Hippophae rhamnoides* L.

Sea buckthorn typically grows as a shrub or small tree reaching 2–6 meters in height. It features linear-lanceolate leaves, while its male flowers are apetalous and consist of 4 to 6 perianth segments. Female flowers are solitary and bear a single pistil. The plant produces aromatic drupaceous fruits, and its seeds are glossy brown with a hard seed coat and a starchy, creamy endosperm (Mozaffarian, 2005; Gupta & Suryakumar, 2011). Cytological and morphological studies confirm that *H. rhamnoides* is a diploid species (2n = 24) with extensive variability across its 8 to 11 recognized subspecies (Tang, 2002; Swenson & Bartish, 2002). These subspecies include *ssp. sinensis*, *ssp. mongolica*, *ssp. turkestanica*, and *ssp. fluviatilis*, each adapted to distinct ecological zones spanning from China, Russia, and Mongolia to Central and Eastern Europe (Jia et al., 2012; Yongshan et al., 2003). A comprehensive taxonomic synopsis also notes unresolved classification issues due to hybridization and environmental plasticity (Swenson & Bartish, 2002; Wang et al., 2022).

H. rhamnoides thrives in harsh climates, showing remarkable tolerance to cold (-40°C), drought, salinity, and alkalinity. (Singh, 2003). In Türkiye, it has been reported as a naturally growing species in the Coruh Valley (Artvin, Erzurum provinces), especially near Uzundere (lat. 40°33'N, long. 41°35'E, at ~1025 m elevation), where it forms dense thickets along riverbanks and steep slopes (Ercisli et al., 2008; Ercisli & Orhan, 2007). These habitats are ecologically significant, providing erosion control and wildlife support in a highly biodiverse region. Recent studies identified 96 wild genotypes of *H. rhamnoides* in Eastern Türkiye, showing remarkable morphological variation in berry shape, color (yellow to orange-red), and size, indicating high adaptation to local conditions (İlhan et al., 2021). These native populations are mainly classified under the subspecies *caucasica*, known for its resistance to harsh environmental factors and high phytochemical content. The species' distribution in Turkey aligns with areas experiencing cold winters and moderate summers, enabling its use in afforestation, windbreaks, and soil conservation efforts in marginal lands. Conservation efforts emphasize the potential of *H. rhamnoides* as a valuable non-timber forest product (NTFP) for local economies, supporting rural development and biodiversity conservation simultaneously (Sezen et al., 2015; Ilhan et al., 2021).

Despite its high phytopharmaceutical value, the genetic improvement and commercial breeding of *Hippophae rhamnoides* face several critical challenges. One of the most fundamental limitations is its dioecious nature, where male and female flowers are borne on separate plants, necessitating careful sex identification and pairing, which complicates controlled breeding and delays selection efficiency (Li et al., 2020). From a breeding and horticultural perspective, the male-to-female plant ratio is crucial. Because only female plants bear fruit, maintaining an optimal sex ratio (often 1 male:6–8 females) is essential for maximizing yield. Vegetative propagation and early sex determination are recommended strategies to ensure gender balance and orchard productivity (Rajchal, 2009; Murkute et al., 2011). Moreover, traditional breeding is hindered by the species' long juvenile phase and complex reproductive biology, with seed-propagated progenies exhibiting wide heterogeneity in phenotypic and phytochemical traits due to open pollination and the absence of clonal propagation systems (Bayır et al., 2024; Mei et al., 2023). Another major obstacle is the lack of comprehensive molecular tools and characterized genomic resources. Although some progress has been made in the use of microsatellite (SSR) markers to assess genetic diversity, molecular breeding and marker-assisted selection (MAS) remain underutilized in sea buckthorn programs (Li et al., 2020). In a study of 78 accessions using 23 SSR loci, significant genetic divergence was observed between *ssp. mongolica* and *ssp. sinensis*, indicating untapped variation that could be exploited for trait improvement. However, the absence of high-resolution linkage maps, QTL data, and functional gene markers continues to constrain genomic selection strategies (Li et al., 2020).

Propagation through seeds not only perpetuates genetic variability but also results in poor uniformity in key traits such as fruit size, oil content, and secondary metabolite composition. Furthermore, the presence of thorns, small berry size, and poor fruit detachment pose physical limitations for large-scale mechanized harvesting and processing, thus requiring targeted selection of less thorny and high-yielding genotypes (Moskalets et al., 2019). Although clonal propagation via root suckers is possible, it is inefficient and unreliable for commercial-scale production, underscoring the need for standardized tissue culture protocols and vegetative propagation methods (Mei et al., 2023). Finally, the genetic improvement of sea buckthorn is further challenged by the need to maintain its environmental adaptability. The species thrives in harsh climates and degraded soils, showing tolerance to drought, salinity, and extreme temperatures; however, enhancing agronomic traits must not compromise its resilience and ecological services. Hence, breeding strategies must balance productivity, phytochemical enhancement, and adaptability, ideally through integrative approaches that combine conventional selection with advanced genomic and biotechnological tools (Bayır et al., 2024; Ahani & Attaran, 2022).

5.**Conclusion**

Hippophae rhamnoides L. stands out as a unique underutilized species with rich phytochemical composition and remarkable environmental adaptability. Its therapeutic potential, encompassing antioxidant, cardioprotective, hepatoprotective, and dermatological effects, has been validated by modern pharmacology. Yet, its commercial exploitation is constrained by reproductive challenges, genetic heterogeneity, and limited breeding infrastructure. Recent advances in molecular tools, in vitro propagation, and preliminary biotechnological interventions provide a strong platform for future improvement. Integrating genomics, tissue culture, and traditional breeding can accelerate the development of uniform, high-value cultivars suitable for large-scale cultivation. Equally, strengthening conservation strategies—especially those involving local communities—will ensure both genetic preservation and sustainable use. Future research should prioritize genome sequencing, sex-marker development, high-throughput metabolomics, and CRISPR-based trait modification. Through interdisciplinary collaboration and policy support, *H. rhamnoides* can transition from an underutilized resource to a key functional crop, supporting health, ecology, and rural economies in marginal environments.

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INVESTIGATION OF ANTIPROLIFERATIVE AND ENZYME INHIBITORY EFFECTS OF THE PEELS OF FOUR POTATO (*Solanum tuberosum* L.) GENOTYPES

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Abstract

Potato (*Solanum tuberosum* L.) is one of the most important crops in the world that contributes to food security on a global scale due to its high yield. This study focuses on the comparative antiproliferative and tyrosinase enzyme inhibitory effect of peels of 4 different potato genotypes (ATA 25, CAN, KAAN, METE). The antiproliferative activity was evaluated using the MTT assay against the cell lines L929, A549, Hep3B and PC3. According to the results of inhibition of the mushroom tyrosinase enzyme using L-tyrosine as a substrate, the highest effect was observed in the methanol extract of the KAAN genotype with a value of 48.80% at 200 µg/ml. For the ATA 25, CAN and METE genotypes, these values were determined to be 38.36%, 32.16% and 34.20% respectively. Enzyme inhibition was found to be very low in aqueous extracts. No cytotoxicity was observed in healthy L929 cells in the concentration range of 20-400 µg/ml. The methanol extract of the ATA 25 genotype showed the highest antiproliferative effect against PC3 (IC_{50}:306.24 µg/ml) and Hep3B (IC_{50}:284.26 µg/ml) cells. However, no significant cytotoxic effect against cancer cells was observed in the aqueous extracts. Potato peels, which are considered waste, can be a new, sustainable and safe natural resource for the food, pharmaceutical and cosmeceutical industries.

Key Words: *Solanum tuberosum* L., genotypes, tyrosinase, MTT, antiproliferative

1.Introduction

The potato (*Solanum tuberosum* L.) is one of the most important foods for around 1.3 billion people worldwide, is cultivated in many countries and is known in more than 5000 varieties. As different genotypes of the same species can have different levels of phytochemicals and different nutritional value, they are important for human nutrition and the food industry. The potato is one of the most widely produced products after wheat and rice. The use of potato varieties with low yield potential also has a negative impact on yield. It is therefore very important to evaluate genotypes with high yield potential that are suitable for the local environmental conditions. As this increases adaptation to environmental conditions and yield, regional protection of the products is also achieved, which attracts the attention of consumers (Samaniego et al., 2020; Sampaio et al., 2021; Storey, 2007).

In this study, the comparative antiproliferative and tyrosinase enzyme inhibitory effects of methanol and aqueous extracts obtained from the peels of 4 different potato genotypes (ATA 25, CAN, KAAN, METE) considered as waste were investigated. The cytotoxic effect was examined on the healthy cell line L929 cells and the concentration range that would not effect the cell viability was determined. Then, the antiproliferative effect on A549, Hep 3B, PC3 cancer cell lines was examined. The inhibitory effect against tyrosinase enzyme, one of the enzymes that has a key role in the regulation of melanogenesis, was investigated using L-tyrosine substrate.

2.Material and Methods

2.1. Plant Material

ATA 25, CAN, KAAN and METE varieties used in the study were obtained from Erzurum Eastern Anatolia Agricultural Research Institute in 2024.

2.2. Preparation of Peel Extracts of 4 Different Potato Genotypes

The potato tubers were thinly peeled and dried. Then, they were crushed and separated into 2 parts (50 g). The first part was extracted with methanol (250 mL), the second part with water (250 mL) at 40 °C for 6 hours. Then, the solvents were evaporated under vacuum to obtain methanol and aqueous main extracts. They were lyophilized and made ready for *in vitro* studies.

2.3. Evaluation of Antiproliferative Effect

Antiproliferative effect determination of methanol and aqueous extracts was performed according to MTT method (Mosmann, 1983). To evaluate the effect on the viability of L929, A549, Hep 3B, PC3 cell lines, cells were distributed as $5x10^4$ per well and cultured for 24 hours in an incubator under appropriate conditions. Then, cells were incubated with extracts in the concentration range of 0-400 µg/ml for 48 hours and after adding MTT solution, absorbance values of formazan crystals formed after 4 hours were measured at 570 nm and percentage viability was calculated.

2.4. Mushroom Tyrosinase Enzyme Inhibition

Inhibition of tyrosinase enzyme by methanol and aqueous extracts of potato peels was performed as in our previous studies by modifying some aspects of the method developed by Kim et al.(Güven et al., 2022; Kim et al., 2017). Kojic acid, a natural antihyperpigment compound, was determined as a standard compound (positive control) and L-tyrosine as a substrate. The absorbance values were measured spectrophotometrically at a wavelength of 475 nm and compared with the control groups to calculate the percentage inhibition.

2.5. Statistical analysis

All experiments were performed in triplicate and data are presented as mean ± SD. Statistical analysis of the results was performed using one-way ANOVA (analysis of variance) followed by Duncan's test using SPSS-22 software, and $p < 0.001$ was considered significant

3.Results and Discussion

3.1. Antiproliferative Effect of Extracts

The antiproliferative activity was evaluated using the MTT assay against the cell lines L929, A549, Hep3B and PC3. No significant cytotoxicity was observed in healthy L929 cells in the concentration range of 20-400 µg/ml in methanol and aqueous extracts (Fig. 1.). While the methanol extracts showed a very low antiproliferative effect on A549 cells at concentrations of 200 and 400 µg/ml, the viability of the cells remained unchanged even at high concentrations of the aqueous extracts (Fig. 2.). The methanol extract of ATA 25 genotype showed the highest effect against PC3 prostate cancer cells with an IC_{50} value of 306.24 µg/ml (Table 1). Then the highest effect was seen in METE, CAN and KAAN respectively. However, the anti-proliferative effect was found to be quite low in the aqueous extracts. Similarly, methanol extract of ATA 25 genotype showed the highest activity against Hep 3B cancer cells with an IC_{50} value of 284.26 µg/ml (Table 2). Then the highest activity was seen in CAN, KAAN and METE respectively. However, no significant cytotoxic effect against cancer cells was observed in the aqueous extracts.

Table 1. Antiproliferative effect of the extracts against PC3

Genotypes	Methanol Extract (IC_{50} µg/ml)	Water Extract (IC_{50} µg/ml)
ATA 25	306.24 ±2.4	>400
CAN	324.36 ±2.8	>400
KAAN	332.42 ±3.2	>400
METE	322.40 ±1.8	>400

*Data are are means±S.D. of three parallel measurements (p <0.05)

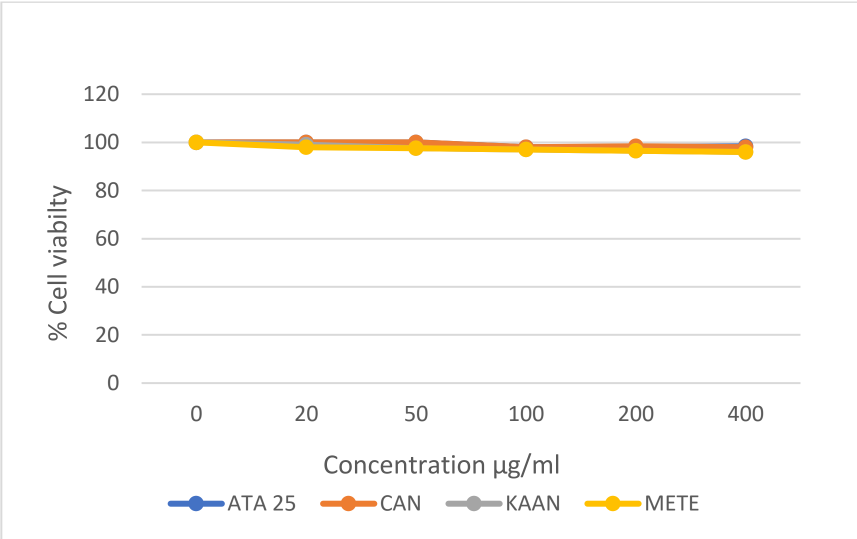

Fig. 1. Antiproliferative effect of the methanol extracts against L929

Table 2. Antiproliferative effect of the extracts against Hep3B

Genotypes	Methanol Extract (IC_{50} µg/ml)	Water Extract (IC_{50} µg/ml)
ATA 25	284.26 ±2.8	>400
CAN	302.18 ±3.4	>400
KAAN	306.42 ±2.2	>400
METE	322.10 ±3.2	>400

*Data are are means±S.D. of three parallel measurements (p <0.05)

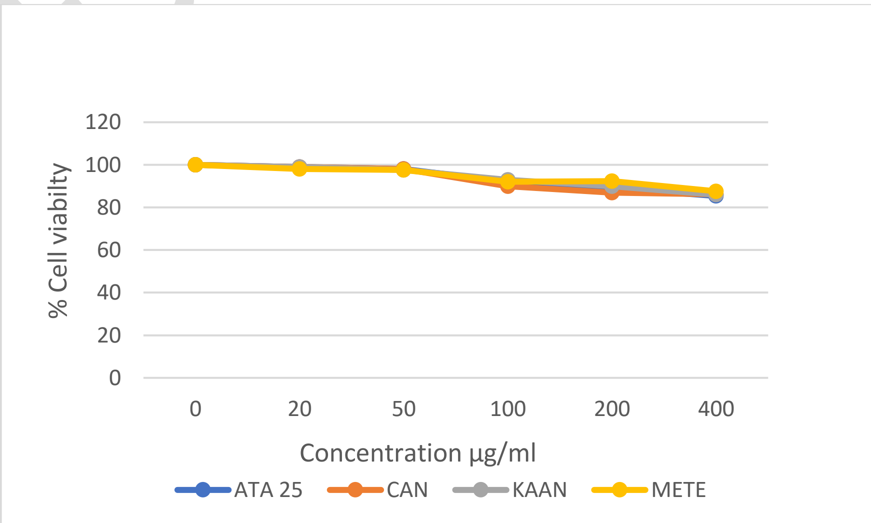

Fig. 2. Antiproliferative effect of the methanol extracts against A549

In a study by Xu et al., the cytotoxic effect of the water extract from *S. tuberosum* peel was investigated against human skin fibroblasts (HDF), glioblastoma (U118), colorectal adenocarcinoma (CaCo-2) and human ovarian cells (Skov-3) using the MTT test. The highest cytotoxic effect was observed against the Skov-3 cell line with an IC_{50} value of 4.31 µg/ml (Xu et al., 2023). It was found that microencapsulated anthocyanins from *S. tuberosum* have a strong cytotoxic effect on HepG2 and THJ29T cells (Barba-Ostria et al., 2024). The cytotoxic effect of 70% ethanolic extracts of potato peel (*S. tuberosum*) against osteosarcoma cell line (HOS) was investigated using the MTT method, and the IC_{50} was found to be 34.2 ± 0.20 µg/mL (El-Sawi, Ibrahim, Bassuiny, & Merghany, 2023).

3.2. Tyrosinase Enzyme Inhibition of Extracts

According to the results of inhibition of the mushroom tyrosinase enzyme using L-tyrosine as a substrate, the highest effect was observed in the methanol extract of the KAAN genotype with a value of 48.80% at 200 µg/ml. For the ATA, CAN and METE genotypes, these values were determined to be 38.36%, 32.16% and 34.20% respectively. Enzyme inhibition was found to be very low in aqueous extracts (Fig. 3).

In a study, peptides obtained from *S. tuberosum* peels were found to exhibit potent tyrosinase enzyme inhibition (12.07+0.0µg KE/µg peptides) (El-Sayed, Al-Azzouny, & Ali, 2019).

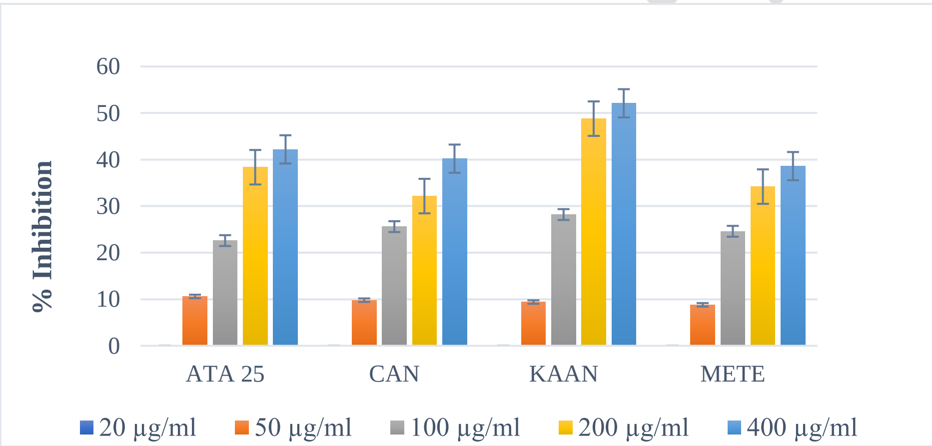

Figure 3. Tyrosinase enzyme inhibition of potato genotypes peels

4.Conclusion

In this study, the enzyme inhibitory and antiproliferative effects of methanol and aqueous extracts from peels of 4 different potato genotypes were analysed comparatively. The results show that potato peels, which are considered food waste, can be a promising potential natural resource for the food, pharmaceutical and cosmetic industries that can be safely utilised.

Acknowledgements

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Conflict of Interest

There is no conflict of interest.

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MEDICINAL AND AROMATIC PLANTS AND TERRITORIAL DEVELOPMENT MODELS

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Abstract

In Basilicata, the cultivation of Medicinal and Aromatic Plants (MAPs) remains a niche sector. However, in some areas, structured production systems have developed within micro-supply chains. Despite successful examples of cooperation among enterprises, the local MAP supply chain remains poorly structured and highly fragmented (De Luca, Ricciardi, 2022). The main challenges include sector dispersion, limited aggregation among small enterprises, and difficulties in implementing integrated and efficient organizational models. Against this backdrop, the transnational cooperation project ME.PLA.SU.S. (MEdicinal PLAnts in a SUstainable Supply chain. Experience of land-use practices) has been launched, promoted by the Council for Agricultural Research and Economics, Research Center for Policies and Bioeconomy (CREA-PB). This study presents the project's analysis of sector dynamics within specific work packages, exploring how cooperative models and integrated strategies can enhance the overall production framework, strengthen economies of scale, and facilitating the adoption of more sustainable and innovative organizational models (D'Oronzio, Costantini, 2021). Research on the MAP sector in Basilicata has revealed, early on, significant data fragmentation, necessitating an integrated methodological approach. This approach combines quantitative and qualitative analyses, structuring the investigation into multiple phases for a multidimensional assessment. Specifically, an initial desk analysis of the sector was conducted, followed by the administration of a structured questionnaire to a representative sample of enterprises. Subsequently, three in-depth Focus Groups identified key challenges, business needs, and barriers to collaborative network formation. The results contributed to **developing a model** aimed at strengthening supply chain dynamics in line with local producers needs. In Basilicata's MAP sector, characterized by both established relationships and weak connections between operators, the **Network Contract** could serve as a strategic solution to enhance competitiveness, foster innovation, and integratelocal resources. Establishing a structured network could unlock new development opportunities, expand international market access, promote knowledge exchange, and optimize shared infrastructure and equipment use. Moreover, a network-based organization would facilitate access to funding through regional and national Rural Development Programs, boosting the sector's sustainability and competitiveness.

Key Words: Sustainable supply chain, competitiveness, medicinal and aromatic plants.

1.Introduction

The evolution of consumer lifestyles has sparked renewed interest in the valorization of traditional knowledge, ancestral practices, and flavors, including those associated with Medicinal and Aromatic Plants (MAPs). This trend is also evident in Basilicata, a region in southern Italy renowned for its rich plant biodiversity, which include over 400 native medicinal plant species, both cultivated and wild (Sansanelli et al., 2017). MAPs encompass a vast and diverse range of species, each distinguished by unique botanical traits, biological properties, habitat preferences, and practical applications. A fundamental commonality among these plants is the presence of bioactive compounds, which, despite their diversity, exhibit significant efficacy across multiple fields of application (Primavera, 2021). These compounds can be used directly or undergo processing and extraction. According to Assoerbe, 45% of MAPs produced in Italy are destined for pharmaceutical use, 40% for food production, and 15% for the cosmetics industry (Martinasso, 2022).

In Italy, the MAP sector exhibits significant dynamism and increasing entrepreneurial activity, though it remains a niche industry compared to both large-scale crops and minor crops. In Basilicata, this sector has mirrored the national growth trend, with steady development over the years. Across various areas of the region, MAPs serve as an essential productive resource, with well-established micro-supply chains that have been active for over a decade (Tab.1). These supply chains respond to an increasingly demanding market, that continuously seeks products with high nutritional value and medicinal properties (Sica et al., 2021; D’Oronzio et al., 2023).

Table 1. Lucanian's MP micro supply chains

PRODUCER	**NOTE**	**PROCESSING COMPANY**	**PRODUCT**
Sud-Officinale Coop. in Irsina (MT)	Over 16 hectares of biological agriculture	**Bioplanta Company**	ESSENZIAL OILS
F.L.E.O. partnership (n. 53 partners, 3 public)	Produces 17 MPs for “Amaro Lucano”	**Amaro Lucano** in Pisticci (MT)	LIQUOR
	Plants for small and medium distribution	**SpeSì (brand)**	SEASONING PLANTS
Lucana Officinali Society Cooperative in Lauria (PZ)	Over 70 hectares	**EVRA Italia srl** in Lauria (PZ)	INTEGRATORI
Lucanian Regional Association of MPs and Saffron Producers in Chiaromonte (PZ)	N. 40 producers		SAFFRON
ORTI LUCANI (O.L.P.O.) Piante officinali	Business network		

Source: CREA PB

The development of Basilicata’s entrepreneurial landscape in medicinal plantcultivation and primary processing began to take shape in the 1970s, driven by young agricultural entrepreneurs who formed associations. Over time, some of these associations have played a pivotal role in establishing certified local medicinal plant value chains. Among the key players are the Lucana Officinali micro-supply chain, led by the secondary processing company EVRA (Extracts of Vegetal Research and Applications), and the Filiera Lucana Erbe Officinali (F.L.E.O.), coordinated by Lucano 1894 S.R.L. These stakeholders have been instrumental in fostering sectoral innovation and reinforcing integrated supply chain models. EVRA, a specialized processing company, is recognized for producing high-quality botanical extracts, partially sourced from cultivated or wild species within the Pollino National Park. In 2010, with support from the Lucanian Agency for Agricultural Development and Innovation (ALSIA), EVRA launched the pilot project *"Italian Micro Supply Chain of Medicinal Plants"*, engaging around twenty organic farms operating within the Pollino National Park. Lucano 1894 S.R.L. is a key player in the international medicinal plant sector renowned for producing *Amaro Lucano*, a bittersweet herbal liqueur with distinctive citrus and floral notes.

Today, it stands as one of the flagship products of Made in Italy, officially recognized in 2021 as a “Historic Brand of National Interest”. In recent years, the Rural Development Programs (RDPs) of the Basilicata Region have played a pivotal role in fostering stakeholder aggregation within the sector. These initatives have driven innovative processes aimed at enhancing the competitiveness and sustainability of agri-food value chains, increasing production volumes, and strengthening the processing and marketing of Lucanian agri-food products. In addition to supporting traditional value chains, attention has been directed toward “minor supply chains”, which also contribute to the socio-economic development of specific areas within the region. Despite the presence of best practices and succesful cooperation among certain primary producers, the local medicinal plant supply chain continues to faces structural challenges. The most critical issues include a high level of fragmentation, limited aggregation capacity among small enterprises, and difficultiesy in implementing integrated organizational models. These factors hinder the optimization of economies of scale, standardization of product quality, and access to broader markets, ultimately constraining the sector’s competitive potential.

To support the growth of the supply chain, the transnational cooperation project MEdicinal PLAnts in a SUstainable Supply chain – ME.PLA.SU.S. has been launched. Ppromoted by the Council for Agricultural Research and Economics – Research Centre for Agricultural Policies and Bioeconomy (CREA-PB), the project involves several Italian research institutions (CREA-PB, DiS-UNIBAS, ALSIA) and international partners such as the Hellenic Agricultural Organisation DEMETER, Research and Experimental Centre of Athens, and the Faculty of Agriculture at the University of Belgrade, alongside numerous stakeholders from the sector. A key objective of the project is to strengthen the medicinal plant supply chain in Mediterranean areas by fostering cooperative processes among agricultural producers, processing companies, research institutions, and advisory services. Through the establishment of a structured network, ME.PLA.SU.S. brings together the expertise and knowledge of all actors involved in various project activities, organized into work packages. This collaborative framework aims to define best practices and operational guidelines, promotinge new aggregation processes within the sector, enhance technological innovations in production, and maximize the sustainability of the supply chain through by-product recovery and valorization from medicinal plants processing.

This study seeks to illustrate how the project has analyzed the sector's dynamics in relation to aggregation processes, highlighting how cooperative models and integrated strategies can improve the overall production framework, reinforce economies of scale, and enable the adoption of more sustainable and innovative organizational models (D'Oronzio, Costantini, 2021).

2.Material and Methods

The initial analysis of the medicinal plant sector in Basilicata revealed significant fragmentation, necessitating an integrated methodological approach. This approach combined both quantitative and qualitative analyses, structuring the research process into multiple phases to ensure a comprehensive assessment of the sector.

- The first phase involved a desk analysis aimed at identifying cultivated species and mapping farms engaged in medicinal plant (MP) cultivation across the region. This activity was carried out by consulting official sources, including the Chamber of Commerce, Industry, Agriculture and Crafts (C.C.I.A.A.), the National Agricultural Information System (SIAN), the Farm Accountancy Data Network (RICA), the Basilicata Region, and the Lucanian Agency for Agricultural Development and Innovation (ALSIA). Additionally, direct engagement with local stakeholders further enriched the dataset.
  The integration of these sources enabled the development of a detailed and structured database on businesses operating in the sector, outlining their main production characteristics (Verrascina et al., 2023).
- The second phase involved two different types of qualitative investigative approaches within the frameworkof the "Filiera in Tour" initiative:
  Stakeholder Engagement: a series of local stakeholder meetings were conducted to administer a structured questionnaire to agricultural entrepreneurs.
  Focus Groups: were organized to explore, through a participatory approach, key issues that emerged from the questionnaire analysis.
  The direct engagement with industry operators was crucial in understanding production dynamics, identifying structural changes challenges, and recognizing emerging opportunities. This participatory process provided valuable insights for shaping future strategies aimed at developing the value chain.

From the initial database, 12 farms were selected using specific criteria to ensure a representative sample of the regional production landscape. Selection criteria included: **cultivation type, geographic distribution, level of integration across production stages, and representation of women and young farmers**.

The interviews focused on the following key industry aspects:

- **Production processes**: from cultivation and harvesting to primary processing (drying, essential oil extraction, etc.) packaging and marketing.
- **Target markets**: analysis of main sales channels and marketing strategies.
- **Types of production**: classification of product categories, such as dried products, processed goods (e.g. essential oils), and bioeconomy-based by-products.
- **Multifunctionality**: exploration of complementary business activities, including experiential tourism and medicinal plant applications in cosmetics and therapy.
- **Business relationships and value chain dynamics**: examination of existing collaborations, commercial partnerships, and socio-cultural connections among enterprises.

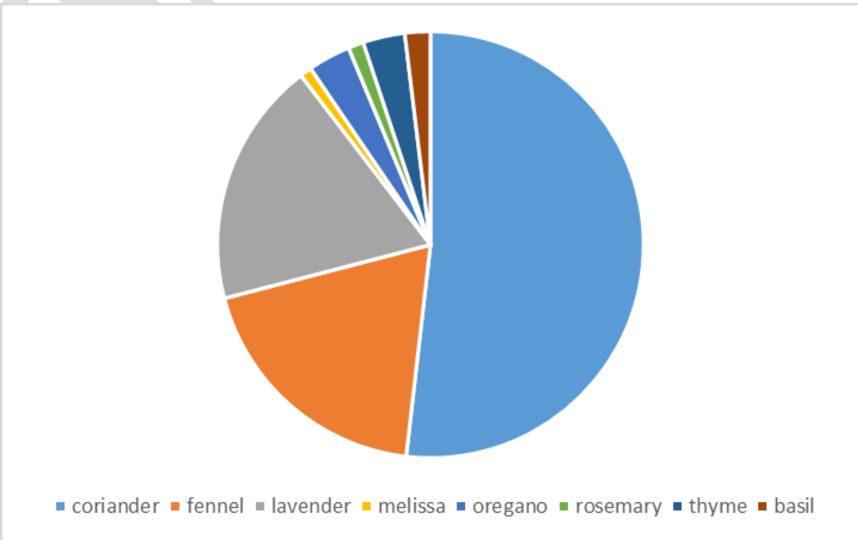
To gain deeper insights, three Focus Groups were organized involving primary producers, processing farms, institutional representatives (Regional Government), and stakeholders from the Lucanian agri-food sector, including producer organizations, the Agri-Food District, Local Action Groups (GALs), and the Regional Federation of Chefs.

- Focus Group 1: identification of sectoral weaknesses and stakeholders' needs.
- Focus Group 2: exploration of key barriers to network formation and local value chain development.
- Focus Group 3: evaluation of organizational models tailored to Lucanian producers, assessing their effectiveness within the territorial and market context.

The combined outcomes of the desk analysis, interviews, and participatory activities enabled the identification of strategic measures to support the development of Basilicata's medicinal plant value chain. These strategies are designed to promote sustainability and innovation and greater sector integration, ensuring long-term growth and competitiveness.

3.Results and Discussion

The **desk analysis** revealed a growing sector, consisting of approximately 100 farms spread accross 360 hectares, with an average farm size of 5.8 hectares. The farms included in the study cultivate a wide variety of medicinal and aromatic plant species native to the Basilicata region. These range from commonly grown crops such as coriander and fennel, to more niche species like thyme, rosemary, lavender, and lemon balm (Melissa officinalis).



Source: CREA PB

Figure 1. Main crops cultivated by Lucanian farms

The analysis of the Lucanian production sector revealed a partially consolidated organizational structure, with a predominance of small-scale, family-run farms. These characteristics were confirmed during the **second phase of the investigation**, relying onon direct interviews. The analyzed sample , primarily composed of farms managed by young entrepreneurs and women, shows the following characteristics: 57% of farm managers are between 30 and 45 years old; 36% of the farms are managed by women; 64% of respondents hold a university degree, making it the most common level of education within the sample. The interviews also revealed that:

- Post-harvest processing is widespread among farms, with only a small portion of the products being sold fresh and in bulk. Fresh sales mainly apply to culinary herbs such as basil, rosemary, thyme, etc.
- Harvest operations typically involve selection, cleaning, and preparation for drying. Some species such as lavender, rosemary, and rosehip, undergo essential oil extraction.
- Due to the high perishability of medicinal plants, farms supply fresh material directly to local distillers. However, two farms have invested in their own distillation units, positioning themselves as reference points for other producers. Despite this, drying remains the predominant post-harvest process.
- All farms are equipped with manual or controlled dryers, using solar exposure outdoors or indoor facilities with self-built drying systems. Many integrate both methods.
- Some dried products are entrusted to local professionals for packaging and labeling, while others undergoe further processing, enabling product diversification. Value-added goods include: food products (honey, candies, chocolate, flavorings); baked goods (panettone, pastries, pasta, and saffron-based cookies); beverages (liqueurs, herbal teas, craft beers); cosmetics; dietary supplements; animal feed; and household hygiene products.
- Each farm manages its own sales channels independently. On-farm direct sales are common, alongside distribution to regional and national retail points, especially for branded herbal teas, supplements, food products, liqueurs, and beers. Only five farms rely on intermediaries (buyers, wholesalers, distributors) to access out-of-region markets. Most farmers actively seek out new clients, particularly in the hospitality and restaurant sectors. Online sales via dedicated websites and e-commerce platforms are becoming increasingly effective.
- Some farms employ multifunctionality strategies to diversify their activities, integrating agritourism, cultural events, and recreational experiences. A notable example is lavender farms, which, in addition to producing essential oils and derivatives, offer immersive tourism experiences, capitalizing on the environmental and scenic beauty of their landscapes.
- In terms of business relationships and cooperation, several barriers have hindered the formation of structured networks, collaborations, and supply chains. These challenges negatively affect production valorization and weaken the bargaining power of individual farms.
- However, the analysis also highlights success stories where small farms in distinctive locations (e.g., within natural parks) have integrated into organized supply chains, restructuring their operations around collaborative models. This approach has enabled them to access new markets and achieve a better balance between profitability, innovation, and experimentation

Despite the presence of micro-supply chains across the region (Table 1), the medicinal plant sector remains poorly structured (De Luca, Ricciardi, 2022). Persistent sectoral challenges continue to hinder full integration, limiting growth and market expansion.

Table 2. Focus group results

1st **Critical Issues**	2nd **Obstacles to Aggregation**	3rd **Organizational Model**
Poor road network accessibility	Aging of agricultural entrepreneurs	Flexibility in defining objectives, action boundaries, and the level of partner involvement
Lack of collective storage and processing facilities	Lack of information on the benefits of association	Preserving business autonomy
Absence of irrigation systems specifically for MAPs	Low interest in cooperation	Sharing experiences
Low level of mechanization	Limited awareness MAPs culture in Basilicata	Economies of scale
Labour shortages	Shortage of specialized training	Encouraging continuous training
Poor alignment of crops with market demands	Absence of consulting services	Non-binding from a legal and economic standpoint
Lack of a brand and/or quality certification	Small-sized enterprises with limited bargaining power	Exchange of industrial, commercial, technical, or technological services
Insufficient product promotion	Restricted generational turnover	Innovation, promotion, and marketing common goals

Source: CREA PB

Agricultural entrepreneurs have identified several key challenges, primarily related to deficiencies in infrastructure and structural aspects, as well as limitations in marketing and promotion activities, particularly within the restaurant sector. Additionaly, they face high production costs and a lack of specialized consultants in the agricultural industry. Meanwhile, obstacles to network creation are largely associated with human and social capital. During the third Focus Group, participants outlined the essential characteristics of a coherent and effective aggregation model tailored to the needs of local businesses. The analysis of these characteristics revealed that the Network Contract is the most appropriate tool to facilitate cooperation among enterprises. This enables companies to collaborate in enhancing innovation and market competitiveness by pooling certain activities while maintaining managerial autonomy (Licciardo, Tarangioli, 2021).

4.Conclusion

The medicinal plant sector in Basilicata is characterized by a heterogeneous production landscape, where established supply chains, such as Lucana Officinali and F.L.E.O., coexist alongside less connected operators with weaker industry ties. However, thanks to engagement and participation activities promoted by the ME.PLA.SU.S. project, there is a growing interest in cooperative dynamics within the sector. In this evolving scenario, the Network Contract stands out as a promising solution to meet demands for competitiveness, innovation, and resource integration. This agile organizational model allows for the effective integration of technology, processes, and strategies, responding promptly to the demands of local production needs. Although the medicinal plant sector remains a niche market, it is experiencing significant expansion due to the increasing demand for high-quality products and Made in Italy goods. The multifunctionality and broad diversification of production have enhanced the sector's potential, supported by consumers' need for natural substances for therapeutic, cosmetic, health, and food applications. This demand has also led to increased spontaneous collection of known species, which have become a focus of widespread interest (D'Oronzio et al., 2023).

The establishment of a business network would create new development opportunities, promoting the expansion of markets atnational and international level, while facilitating knowledge exchange among companies. Furthermore, this organizational model would improve planning and investment management capabilities, easing access to funding within the framework of Rural Development Programs (PSR) at both regional and national levels. Structuring the supply chain through Network Contract would also boost the competitiveness of participating companies, encouraging new products development and optimizing existing product quality. Beyond these advantages, network-based cooperation would allow for the development of synergies in key strategic areas including marketing, training, research, technological innovation, and experiential tourism, ultimately strengthening the sustainability and resilience of the sector. A key success factor for this model is the active involvement of the research sector, which plays a crucial role in stimulating collaborative project initiatives, encouraging the aggregation of operators, and providing specialized consultancy. By integrating these elements, the medicinal plant sector in Basilicata can achieve greater cohesion, innovation, and long-term growth, ensuring its continued development within an increasingly competitive market.

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Conflict of Interest
No conflict of interest between authors.

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