



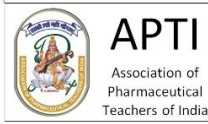
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ABSTRACTS & PROCEEDINGS BOOK  
17-19 October 2024, İzmir-Türkiye  
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# CMAPSEEC-2024

## PROCEEDINGS BOOK

### ABSTRACTS & FULL PAPERS





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**The 12<sup>th</sup> Conference on Medicinal and Aromatic Plants  
of Southeast European Countries**

**CMAPSEEC-2024**  
**PROCEEDINGS BOOK**  
**ABSTRACTS & FULL PAPERS**

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**October 17<sup>th</sup> – 19<sup>th</sup>, 2024**

**İzmir – Türkiye**

**ISBN: 978-625-98164-1-8 (PDF)**



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## PREFACE

### Dear participants and colleagues,

It is with great enthusiasm that we present the proceedings of the 12<sup>th</sup> International Congress on Medicinal and Aromatic Plants of Southeast European Countries (CMAPSEEC-2024), held on October 17-19, 2024, in the historic city of Izmir, Türkiye. Organized in cooperation with the Association of Medicinal and Aromatic Plants of Southeast European Countries (AMAPSEEC) and hosted by Dokuz Eylül University, this congress has once again brought together leading scientists, researchers, and industry experts. Besides, Gaziantep University and Lokman Hekim University partnered with the organization.

This year's conference offered a dynamic platform for the exchange of knowledge, innovation, and ideas among participants from Serbia, Bulgaria, Macedonia, Croatia, Portugal, Italy, China, Thailand, Moldova, Iran, Northern Cyprus, and Türkiye considering medicinal and aromatic plants in traditional and modern medicine, agriculture, and other fields. We were honored to see representation from both the public and private sectors, indicating the broad and interdisciplinary appeal of this field.

As we face global health, environmental, and sustainability challenges, the research and development of medicinal and aromatic plants become more significant than ever. This congress not only served as a forum for presenting the latest scientific findings but also facilitated collaborations that will inspire future advancements in the sector.

This congress has once again served as a cherished gathering where scientific knowledge flourishes amidst the warmth of friendship and mutual respect. For over the years, CMAPSEEC has been more than just a conference; it has become an annual reunion where colleagues become friends, and friends become family. Each year, as we come together to explore the latest advances in medicinal and aromatic plants, we are reminded of the deep bonds that connect our diverse community.

On behalf of the organizing committee, we extend our heartfelt thanks to all participants for making CMAPSEEC not only a leading scientific congress but also a gathering that feels like coming home. We are grateful for your dedication, your enthusiasm, and the warmth that you bring to this event.

We look forward to seeing the innovations, friendships, and collaborations that will continue to grow from the seeds planted at CMAPSEEC-2024.

Sincerely,

Prof. Dr. İlkey Erdoğan Orhan  
Prof. Dr. Nazım Şekeroğlu  
Prof. Dr. İpek Süntar  
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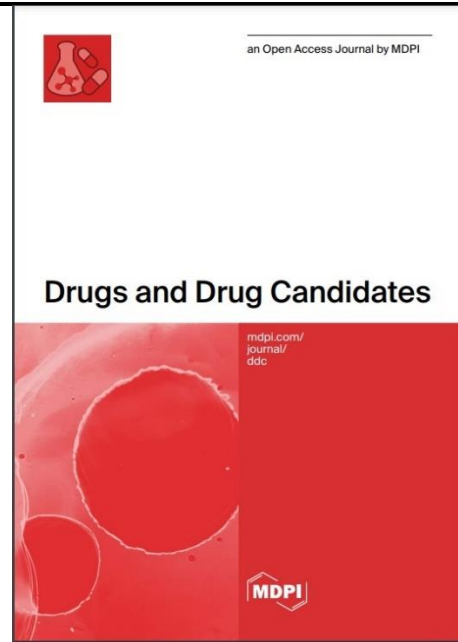
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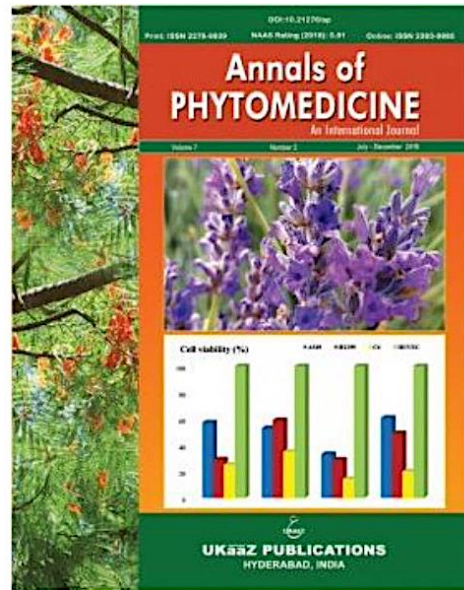
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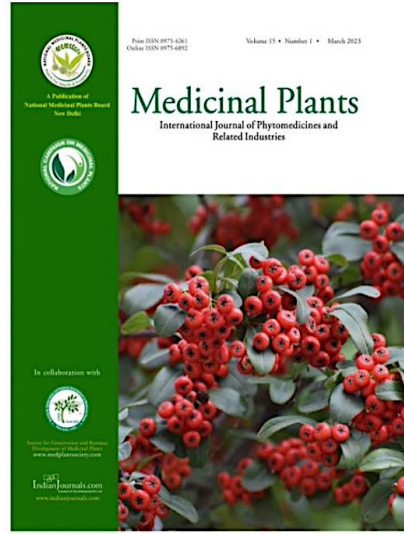
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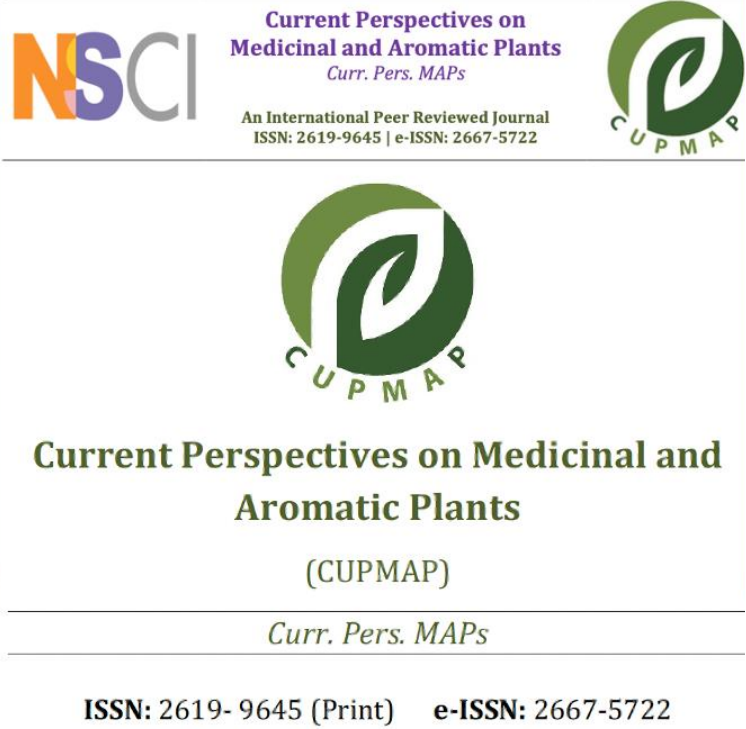
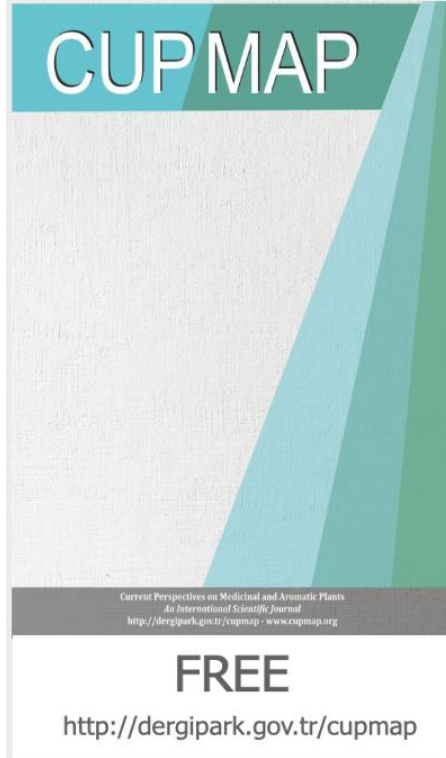
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CUPMAP is an open access, peer-reviewed, and refereed international journal published by MESMAP scientific group. The main objective of the CUPMAP is to provide an intellectual outlook on the scientific researches on Medicinal and Aromatic Plants. 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, applied studies, and review articles 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.

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**Last date of submission: December 13, 2024.**



CMAPSEEC-2024 Konferansında toplam 65 bildiri sunulmuştur, bunlardan 38'i sözlü, 27 tanesi ise poster sunum şeklinde olup; sunulan **sözlü bildirilerin %60'lık kısmı yabancı katılımcılar** tarafından sunulmuştur. Kongreye 10 ülkeden bilim insanları katılım sağlamıştır. **Katılım Sağlayan Ülkeler:** Tayland, Portekiz, Bulgaristan, Sırbistan, İtalya, Hırvatistan, Makedonya, İran, Kuzey Kıbrıs Türk Cumhuriyeti ve Türkiye. **Kongre katılımcıları ve sunum başlıkları "CMAPSEEC-2024 Abstracts & Proceedings Book" kitabının içindekiler kısmında sunulmuştur. CMAPSEEC-2024 Konferansı 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 Cumhurbaşkanlığı Kararı uyarınca:

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# **ABSTRACTS (INVITED SPEECHES)**



## **TÜRKİYE AS A SOURCE FOR MEDICINAL AND AROMATIC PLANTS**

**K. Hüsnü Can Başer**

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The rich and diverse flora of Türkiye is well documented in 11 volumes [1-3]. Thanks to its geographical location with land in Asia and Europe at the junction of three phytogeographic regions (Mediterranean, Euro-Siberian, Irano-Turanian), under the influence of three different climates (Mediterranean, Continental and Oceanic) and its natural diversity blended with cultural richness throughout centuries Türkiye enjoys a rich inventory of plants used in medicine, agriculture, food, cosmetics, flavours and fragrances and other related sectors. Important cultivated and wildcrafted plants like opium poppy, oregano, thyme, sage, sideritis, mint, rose, laurel, anis, licorice, gypsophila, salep, etc. which have been used and traded since time immemorial, and essential oils obtained from aromatic plants will be covered in this lecture.

**Key Words:** Türkiye, Medicinal, Aromatic

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## EXPLORING AROMATIC PLANT POTENTIAL: THERAPEUTIC INNOVATIONS FROM THE *PLECTRANTHUS* GENUS AND BEYOND

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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. *Plectranthus* spp., long valued in traditional medicine, have emerged as a rich source of bioactive abietane diterpenoids with a wide range of biological activities, increasingly validated by scientific research [1]. Several bioactive lead compounds from this genus have been explored for therapeutic applications. Notable examples include Parvifloron D (ParvD) from *P. ecklonii* and 7 $\alpha$ -acetoxy-6 $\beta$ -hydroxyroyleanone (Roy) from *P. grandidentatus*. 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. Remarkably, the anticancer efficacy of RoyBz, specifically involving PKC $\delta$ , was confirmed *in vivo* using xenograft mouse models of both control and PKC $\delta$ -knockdown human colon cancer cells [2].

Further research has revealed insights into RoyBz's molecular mechanisms, particularly its ability to interfere with glucose metabolism in colon cancer cells. This study highlights the crucial role of PKC in regulating tumor cell metabolism and demonstrates RoyBz's potential to target ATP-generating pathways, making it a strong candidate for anticancer therapies [3]. These findings reinforce the therapeutic promise of *Plectranthus*-derived compounds and underscore their potential in the development of novel anticancer treatments [1].

**Key Words:** *Plectranthus*, Lamiaceae, diterpenoids, cancer, PKC

### Acknowledgements

This work was supported by FCT (Portugal) through the projects with reference DOI 10.54499/UIBP/04567/2020 and DOI 10.54499/UIDB/04567/2020.

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## OREGANO: BREEDING OF NEW VARIETIES, REGULATION OF METABOLISM AND BIOLOGICAL FUNCTION STUDY OF CARVACROL

**Shi Lei<sup>1,2,\*</sup>, Li Hui<sup>1,2</sup>, Bai Hongtong<sup>1,2</sup>, Sun Meiyu<sup>1,2</sup>, Hao Yuanpeng<sup>1,2,3</sup>, Guo Xiaoqi<sup>1,2,3</sup>, Xia Fei<sup>1,2</sup>, Dong Yanmei<sup>1,2</sup>, Wang Di<sup>1,2</sup>**

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Oregano is an important medicinal and aromatic plant, which has broad application prospects in food preservation and feed additives instead of antibiotics. This paper introduces the research progress of the molecular breeding and activity function of oregano by Professor Shi Lei's team.

1. Collected and preserved 120 taxa of Oregano germplasm resources.
2. Through radiation breeding, polyploid breeding, cross breeding and molecular assisted breeding, 15 new germplasm were created, among which 'Zhiniv' oregano was authorized as a new variety.
3. Assembly and annotation of high-quality oregano genome at chromosome level; The synthesis pathway of carvacrol was analyzed by transcriptome sequencing.
4. The group effect relationship between complex essential oil components of oregano and antibacterial activity was analyzed by correlation network analysis. The bacteriostatic mechanism of oregano essential oil was analyzed by metabolomics and proteomics. A smart antibacterial cling film with oregano essential oil displaying color changing was developed.
5. Oregano essential oil enhances the production performance and egg quality of late-phase laying hens by modulating the gut microbiota composition and maintaining microbial homeostasis.

**Key Words:** Oregano, breeding, genome, carvacrol, bacteriostatic mechanism, antibiotics

### Acknowledgements

This research was funded and supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA26040306) and the International Partnership Program of the Chinese Academy of Science (Grant No. 063GJHZ2022038GC).

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## NURTURING LONGEVITY THROUGH NATURAL COMPOUNDS

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Aging is commonly referred to as a natural process accompanying the lifespan of a living being. The revolution in ageing research through the past decade has driven the progress in interventions that promote longevity. Nowadays, we live in a rapidly aging world, with an expectancy of more than a 2 billion people aged 65 years or older by 2050, alongside a rising proportion of age-related diseases. Thus, ageing represents a significant healthcare burden on our society [1]. The development of strategies targeting aging now becomes a challenge for science. Central to this pursuit is the recognition that metabolic health serves as a cornerstone for both healthy weight maintenance and prolonged lifespan. Additionally, key molecular pathways attributed to nutrient signaling that are implicated in obesity progression, intersect with those fundamental to longevity, suggesting potential shared targets for intervention [1].

Utilizing the model organism *Caenorhabditis elegans*, along with molecular pharmacology approaches, our research focuses on the discovery of natural products that target healthy aging and longevity [1]. Recent examples from authors lab include the evaluation of the longevity promotion potential of *Punica granatum* L. (pomegranate) leaf extract [2] and pure icariin [3]. Consequently, model mechanisms of action are provided and discussed further.

### **Acknowledgments**

This research received funding from the Bulgarian National Science Fund (contract number KII-06-H79/6).

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**TO BEE OR NOT TO BEE:  
CAN NATURAL PRODUCTS BE A SOLUTION AGAINST GLOBAL  
WARMING AND ENVIRONMENTAL RISKS?**

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In the last decade, global warming has become a major problem with potentially far-reaching consequences for life on Earth. It is predicted that global climate change will have critical implications in terms of agriculture and forestry, water resources, energy, human health, and biodiversity, triggering socioeconomic crises.

In the livestock sector, the use of feed supplements containing natural antioxidants, herbal secondary metabolites, vitamins, and probiotics has emerged as a remarkable strategy in recent years to make animals more resilient, healthy, and productive by preventing antioxidant defense and immune system disorders and growth/reproduction/production performance decreases due to global warming.

Unfortunately, the lives of honey bees are threatened by climate change and accompanying environmental pollution. Globally, honey bee colonies face numerous threats, such as habitat loss, diseases, chemical exposure, and high ambient temperatures. A drastic decrease in honey bee colonies has been recorded in recent years due to a combination of these stressors. Thus, their key role in the pollination of a wide variety of agricultural and wild plant species, and their contributions to the world economy, and human health by honey bee products are at risk. However, there is no comprehensive strategy for supporting honey bee health and adequately coping with climate change and malnutrition stress. There is a need for alternative dietary approaches that will make honeybees more resilient to environmental stresses, especially heat stress. Recently, numerous studies indicate that supplementation of honey bees' diet with various natural products including some medicinal and aromatic plant extracts and phytochemicals enhances their health and longevity.

In this presentation, the significance of developing natural feed and food additives to mitigate the deleterious effects of heat stress induced by global warming will be reviewed and discussed from different points of view, focusing on honey bees, medicinal plants, and bioindicators of molecular pathways triggered.

**Key Words:** honey bee, heat stress, heat shock proteins, dietary supplements, medicinal plant extracts, phytochemicals



## **MEDICINAL USES AND BIOCHEMICAL PROPERTIES OF MOSSES**

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The use of plants by humans is as old as human history itself, and plants have been utilized to seek cures for diseases from ancient times to the present. Bryophytes hold an important place among the plants used in disease treatment. While approximately 150 ethnobotanical bryophyte species are known globally, one of the most widespread members of this group is mosses. Mosses can grow in a wide range of geographical areas and climatic zones, in both humid and arid environments, on rocks, soil, and tree trunks. The potential of mosses to heal wounds and reduce infections was first discovered centuries ago by the Chinese and Native Americans. Since then, these plants have been used in various fields. Mosses are commonly included in the lists of Traditional Chinese Medicine and Native North American remedies. Additionally, it has been reported that mosses are used in traditional medicine in Latin America and India as well [1]. When the chemical structure of mosses is examined, it is seen that their main components are carbohydrates. So far, studies on the chemical composition of mosses have been limited, mostly focusing on specific groups of substances such as fatty acids, lipids, and volatile oils. The investigation of secondary metabolites in mosses is important for understanding their metabolism and their responses to environmental stresses (e.g., oxidative stress, heavy metal exposure, and UV radiation). The secondary metabolites found in mosses differ from those in higher plants, making them more worthy of research [2]. Although mosses are composed of the same basic organic components as higher plants, the proportions of these substances differ. Most of the mosses consist of hemicellulose and pectin (30-60%), followed by cellulose (15-25%), proteins (5-10%), polyphenols (5-10%), and inorganic substances (3-10%). Additionally, the predominant compounds include low-molecular-weight aliphatics, phenol, 4-ethenylphenol, and 1,6-anhydro- $\beta$ -d-glucopyranose, likely derived from carbohydrates and polyphenolic compounds. These metabolites tend to exhibit significant biological activity, further enhancing the research interest in bryophytes [3].

**Key Words:** moss, biochemical composition, medicinal

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## **MULTI-TARGET EFFECTS OF CYCLOASTRAGENOL AND ITS DERIVATIVES ON AGE-RELATED PATHWAYS**

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Aging is a multifaceted and complex process influenced by numerous cellular and systemic mechanisms, including telomere shortening, oxidative stress, and disrupted proteostasis [1-3]. These processes are recognized as key hallmarks of aging and contribute significantly to the development of age-related disorders, such as neurodegenerative diseases [1-3]. Cycloastragenol (CA), a triterpenoid saponin derived from *Astragalus membranaceus*, has demonstrated potential as a telomerase activator, presenting a therapeutic strategy for conditions like metabolic syndrome and neurodegenerative diseases [4-5]. Our research group focuses on the modification of cycloartane-type triterpenoids using fungal biotransformation to generate new metabolites with enhanced efficacy for promoting healthy aging and combatting degenerative diseases. In prior studies, we identified novel CA derivatives with superior telomerase activation at lower concentrations compared to the parent compound [6, 7]. In the present study, we examined the effects of these derivatives on major aging-related pathways, including NRF2/proteasome/telomerase regulation and replicative senescence, in human primary epidermal keratinocytes (HEKn). Our results indicate that CA derivatives not only activate NRF2 nuclear translocation, leading to the upregulation of phase-II enzymes such as HO-1 (heme oxygenase 1), GR (glutathione reductase), and GCLC (glutamate-cysteine ligase catalytic subunit), but also enhance proteasome activity, particularly in caspase-like ( $\beta 1$ ) and chymotrypsin-like ( $\beta 5$ ) subunits, even at low concentrations. Additionally, continuous treatment with these derivatives extends cellular lifespan by maintaining elevated NRF2, proteasome, and hTERT protein levels, even in senescent cells. Moreover, our investigation into their impact on the p53/p21 pathway revealed that these derivatives downregulate the aging-associated increase in p53 and p21 proteins, alleviating cell cycle arrest and promoting cellular proliferation [8]. Thus, our findings provide compelling evidence that CA derivatives enhance NRF2 activity, support proteostasis, and modulate telomerase and the p53/p21 pathways more effectively than CA, highlighting their potential as multi-targeted anti-aging agents by delaying replicative senescence and promoting cellular longevity.

**Key Words:** *Astragalus*, saponin, cycloastragenol, cellular longevity, antiaging agents

### **Acknowledgements**

This study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK, Grant number 119Z086). We thank the Pharmaceutical Sciences Research Centre (FABAL, Ege University, Faculty of Pharmacy) for equipment support and Bionorm Natural Products for donating CA. We would like to thank Dr. Melis Küçüksolak for her support to isolate and characterize CA derivatives.

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## **TAILOR-MADE GASTRORETENTIVE CARRIER WITH ENCAPSULATED GENTIAN ROOT EXTRACT**

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Gentian root (*Gentiana lutea* L., Gentianaceae) is used as traditional herbal medicinal product for temporary loss of appetite and mild gastrointestinal complaints. Systemic and local gastrointestinal (GI) effects of gentian root extracts are mainly associated with the presence of secoiridoid compounds, xanthenes and iridoids. Gentiopicroside (GP), the dominant bioactive compound in gentian root extract, has low bioavailability, short elimination half-life and inadequate stability during storage and digestion. Therefore, the aim was to optimize extraction and to develop gastroretentive carriers with encapsulated gentian root extract.

In the first phase of the research, it was shown that the high content of GP, isogentisine and phenolic compounds from gentian roots was extracted with water-ethanol solution (49 %, v/v) at a temperature of 65 °C when the drug/solvent ratio was 1: 40, and extraction time was 129 minutes. In the second phase of the research, a suitable gastroretentive carrier (solid lipid microparticles) with encapsulated gentian root extract was obtained by an innovative method involving a lyophilization of a double (water/oil/water) emulsion. The formulated gastroretentive carriers were characterized by high yield (> 92 %) and encapsulation efficiency (> 95 %) of GP, as well as suitable mechanical properties. The high gentiopicroside content (during 18 months) and antioxidant activity (during one year) were maintained, indicating that the stability of the selected carrier was high. The tablets with the optimal gastroretentive carrier showed mucoadhesive properties, and thus a gastroretention of the tablets was based on a dual mechanism. The results of the *in vitro* release study showed that the dissolution of GP from the selected tablet formulation was biphasic (29.04 % in 45 minutes; 67.95 % in 6 hours).

The results indicate that the extraction of bioactive compounds from gentian root was optimized. In addition, a tailor-made gastroretentive carrier with encapsulated gentian root extract was developed.

**Key Words:** gastroretentive carrier, double emulsion, solid lipid microparticles, quality by design, response surface methodology, artificial neural networks



**ABSTRACTS**  
**(ORAL**  
**PRESENTATIONS)**



## GREEN TEA (*CAMELLIA SINENSIS*) PRESSURIZED HOT WATER EXTRACTION INHIBITED LIPID PEROXIDATION IN HIGH-FAT DIET MICE AND OX-LDL-MEDIATED RAW246.7 MACROPHAGES APOPTOSIS

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Hyperlipidemia and obesity are recognized as risk factors that contribute significantly to the development of atherosclerosis and cardiovascular diseases. The oxidative modification of low-density lipoprotein (LDL) and inflammation are pivotal in the pathogenesis of atherosclerosis. Thus, the potent diet of medicinal plants is receiving attention to reverse such pathological states.

The present study aims to assess the effects of green tea (*Camellia sinensis*) pressurized hot water extraction (GPHWE) in high-fat diet mice. Additionally, we examined its impact on the attenuation of LDL oxidation and apoptosis in RAW 264.7 macrophage cells.

The findings revealed that GPHWE administration led to a significant reduction in body weight, low-density lipoprotein cholesterol (LDL-Chol), total cholesterol, triglycerides, atherosclerosis index (AI), coronary arteries index (CAI), and glucose levels in the bloodstream, comparable to Simvastatin-treated mice. Moreover, treatment with GPHWE effectively reduced lipid peroxidation, as evidenced by reduced levels of malondialdehyde (MDA) in both high-fat diet mice and RAW264.7 macrophage cells. Furthermore, GPHWE exhibited a protective effect on cell viability in RAW 264.7 macrophage cells exposed to oxidized low-density lipoprotein (Ox-LDL), thereby preventing nuclear condensation and apoptosis induction.

These outcomes suggest that the observed inhibition of lipid peroxidation and Ox-LDL-induced apoptosis by GPHWE underscores its potential as a therapeutic agent against atherosclerosis. Consequently, it may serve as a promising functional food supplement or alternative medicinal approach in managing cardiovascular health.

**Keywords:** atherosclerosis, oxidized LDL, Green tea (*Camellia sinensis*), pressurized hot water extraction (GPHWE), apoptosis

### Acknowledgments

The authors would like to thank the School of Allied Health Sciences, Walailak University, for all the facilities supporting this work and all authors for their help in the suggestion of the dataset and manuscript preparation. This research work was supported, in part, by Walailak University (WU601501).



## **ETHNOMEDICINAL STUDY OF *QUERCUS SP.* FROM THE FOLK AND TRADITIONAL MEDICINE OF REPUBLIC OF MACEDONIA**

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This study was carried out concerning ethnomedicine on preparations obtained from oak from the folk and traditional medicine of Republic of Macedonia, an area so far less frequently studied from the perspective of folk traditional medicinal, local food and handicraft and other uses. The oak bark act astringently, antimicrobial and anti-inflammatory. A water decoction is used against inflammation of intestine mucous membrane at diarrhea, dysentery and catarrh in intestine. Leafs and bark are used against diarrhea. 3% tea of oak bark is beneficial against poisoning with heavy-metal and alkaloids. Tea is administered as a medicine at bleeding in internal organs, uterus and hemorrhoids. 5% decoction is used for healing of wounds, wet eczema, ulcers, hemorrhoids, frostbite, sweating on the legs and mycoses. A water decoction is used at illnesses of mucous in mouth and throat, as gargle. Oak bark helps in blood in the urine, abnormal menstruation, and impotence in urine retention, varicose veins, bleeding gums etc. Oak leaves are used for preparing tea and pulvis from bark in combination with honey against bleeding. In the period of misery fried acorns is used as a substitution for cafe, and for making bread mixed with other bread flours. Also, acorns are used in the diet of livestock. Oak bark is used for tanning the skin in the industry. Oak tree is a holy tree where various religious practices take place. Its appearance, as well as his long-lived life and durability, can be regarded as a symbol of force. It is related to the cult of the dead, and also as a tree of the thunderbolt Perun. The district, from the ethnobotanical point of view, shows traces of the influences of the neighboring regions. Some medicinal uses are linked to beliefs or residual forms of magic prescriptions.

**Key Words:** oak, medicinal, food, handicraft, use

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**LC-HRMS PROFILING AND EVALUATION OF BIOLOGICAL ACTIVITIES  
OF ENDEMIC *STACHYS RUPESTRIS*  
MONTBRET ET AUCHER EX BENTH FROM TÜRKİYE**

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*Stachys* L. is among the largest genera in the Lamiaceae family, consisting of over 365 species distributed worldwide [1]. The genus *Stachys* comprises 96 species (123 taxa), with 67 taxa being endemic to Türkiye, demonstrating the country's profound impact on global biodiversity [2]. This extensive diversity of *Stachys* species leads to their widespread traditional use for medicinal purposes across various regions of Türkiye. They have been mostly applied to treat colds, coughs and stomachache [3]. *Stachys rupestris* Montbret et Aucher ex Benth. is one of the endemic *Stachys* species distributed throughout southeastern Türkiye. In this study, plant samples were collected from Andırın – Kahramanmaraş and an ethanol extract was prepared using aerial parts of the plant. Secondary metabolites of the extract were determined by an LC-HRMS analysis. Moreover, total phenolic and flavonoid contents were calculated, and *in vitro* antioxidant, anticholinesterase, also antimicrobial activity studies were conducted. Accordingly, 36 different compounds were identified, and among them, verbascoside ( $640.528 \pm 18.77 \mu\text{g}$  analyte/g extract) was found as the major constituent. The extract, characterized by its high total phenolic content ( $66.80 \pm 2.60 \mu\text{g PEs/mg}$  extract) and total flavonoid content ( $32.85 \pm 1.66 \mu\text{g QEs/mg}$  extract), exhibited significant antioxidant activity, as shown by three different assays: DPPH free radical scavenging ( $\text{IC}_{50}$ :  $26.92 \pm 0.89 \mu\text{g/mL}$ ), ABTS cation radical scavenging ( $\text{IC}_{50}$ :  $18.53 \pm 0.10 \mu\text{g/mL}$ , and CUPRAC activity methods ( $A_{0.5}$ :  $27.12 \pm 0.92 \mu\text{g/mL}$ ). The cholinesterase inhibitory activity results indicated  $37.98 \pm 0.27\%$  inhibition of acetylcholinesterase and  $74.84 \pm 0.88\%$  inhibition of butyrylcholinesterase at a concentration of  $200 \mu\text{g/mL}$ . Furthermore, the extract exerted moderate antibacterial activity against *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, and moderate antifungal activity against *Candida tropicalis*, and *C. parapsilosis* strains with MIC values ranging from 312.5 to 625  $\mu\text{g/mL}$ . It is aimed that this study will contribute to the advancement of research on *Stachys* species in Türkiye and support their development for medicinal applications.

**Key Words:** *Stachys rupestris*, LC-HRMS, antioxidant, anticholinesterase, antimicrobial

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## **CYTOTOXIC POTENTIAL OF *INFUNDIBULICYBE GEOTROPA* ACETONE EXTRACT IN PROSTATE CANCER (LNCAP) CELL LINES**

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Many species of mushrooms contain bioactive compounds such as polysaccharides,  $\beta$ -glucans, lectins, terpenoids, phenolic compounds, and proteins, which have been studied for their anticancer activities. Compounds with cytotoxic activity found in mushrooms like *Infundibulicybe geotropa* may serve as lead compounds for the development of new pharmaceutical drugs. Researchers often isolate and study these compounds to understand their mechanisms of action and evaluate their potential as drug candidates for various medical conditions. Some cytotoxic compounds from mushrooms have been studied for their potential anticancer properties. These compounds may inhibit the growth of cancer cells or induce apoptosis (programmed cell death) in cancer cells, potentially serving as therapeutic agents in cancer treatment. This research explores the cytotoxic potential and apoptosis mechanisms of the acetone extract of edible *Infundibulicybe geotropa* (Bull.) Harmaja mushroom against prostate cancer (LnCap) cell lines [1]. The MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was employed to assess the cytotoxic activity to find the effective concentration (EC<sub>50</sub>) value. The apoptotic effect was evaluated through three methodologies: image cytometry using the Annexin V-FITC/PI apoptosis detection kit, Western Blot analysis targeting apoptosis-associated proteins, and real-time PCR (qPCR) to determine mRNA levels of apoptosis-related genes.

The extract from *I. geotropa* exhibited a dose-dependent suppression of LnCap cell line proliferation, with an EC<sub>50</sub> value of 61.75  $\pm$  3.01  $\mu$ g/mL. The image cytometry results showed that *Infundibulicybe geotropa* acetone extract stimulated 2.5-fold apoptotic cells compared to the control group. According to real-time PCR analysis, acetone extract increased the expression of the BAX gene by 1.22-fold compared to the control group. At the same time, no significant change was detected in the expression of the BCL-2 gene. Moreover, the extract also notably increased mRNA expressions of Caspase 3, 8, and 9 (1.21, 1.93, and 1.22-fold, respectively). Results were normalized using GAPDH (glyceraldehyde 3-phosphate dehydrogenase). However, BAX, caspase 3, 8, and 9 protein levels were significantly induced due to extract treatment. Apoptosis involves both intrinsic and extrinsic pathways regulated by various genes. Since the acetone extract appears to induce apoptosis by affecting both pathways, isolation of bioactive compounds should be performed as further investigations.

**Key Words:** *Infundibulicybe geotropa*, apoptosis, cytotoxicity, prostate (LnCap) cancer cell lines

### **Acknowledgments**

This study was supported by TUBITAK (The Scientific and Technological Research Council of Türkiye) with Project number TUBITAK1001-121Z551. Cansel ÇAKIR would like to thank the Turkish Higher Education Commission (YÖK) for supporting the YÖK 100/2000 PhD and TUBITAK-BIDEP, 2211 - National PhD Scholarship Programs.

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## AROMATHERAPY APPLICATIONS USED IN CANCER PAIN

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Pain is one of the most common problems that occurs due to the progression or treatment of the disease that cancer patients fear the most. It significantly affects the quality of life and integrity of patients. The International Association for the Study of Pain defines pain as an unpleasant emotional behavior pattern that includes the person's past experiences and progresses with possible tissue damage originating from any part of the body. Cancer pain is a frightening and unbearable condition that occurs with the invasive spread of tumor cells that can be acute or chronic.

70% of cancer patients experience pain at some stage of the disease. Despite effective guidelines developed for the management of cancer pain, 80-90% are inadequately treated. Uncontrolled pain is the reason why cancer patients apply to the hospital more frequently and are admitted to the hospital. Due to the difficulties in the evaluation and control of pain treatment, interest in complementary treatments used in the management of cancer pain is also increasing. Complementary methods used in pain treatment management include meditation, yoga, relaxation techniques, hypnosis, massage, acupuncture, music therapy and aromatherapy.

Depending on the methods applied in the treatment of cancer patients, pain and anxiety can reduce the patient's compliance with the treatment and cause difficulties in the treatment procedure. In a study conducted in this field, pain and anxiety levels in cancer patients were investigated in a semi-randomized controlled trial with the placement of a needle into an implantable central venous port catheter using inhalation aromatherapy; patients were divided into 3 groups as lavender (n=41), eucalyptus (n=41) and control (n=41). Patients in the intervention group were inhaled with 3 drops of essential oil for 3 minutes just before the placement of the needle into the implantable venous port catheter. As a result of the study, a significant decrease in VAS scores was observed in the intervention groups compared to the control group, but no decrease was observed in the eucalyptus group. As a result, it was stated that lavender oil is effective in reducing pain levels during medical procedures due to its antinociceptive and analgesic properties and that lavender inhalation aromatherapy can be used to reduce pain during medical procedures. A study conducted on patients with invasive pain also suggested the use of inhalation aromatherapy in addition to pharmacological treatments during catheterization. It has been reported that aromatherapy massage with peppermint, chamomile and rosemary essential oils may be beneficial for neuropathic pain and fatigue due to chemotherapy.

Aromatherapy is one of the complementary methods used in addition to primary treatments to reduce physical symptoms, reduce treatment side effects, control treatment side effects and improve psychological health. Aromatherapy is used to improve symptoms such as depression, anxiety, pain, sleep disorders, nausea and vomiting, which are commonly seen in cancer patients, and to increase quality of life.

**Key Words:** pain, cancer, aromatherapy



## **ANTIOXIDANT, ANTI-APOPTOTIC AND ANTI-INFLAMMATION PROPERTIES OF *THUNBERGIA LAURIFOLIA* LINDL. EXTRACT**

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*Thunbergia laurifolia* Lindl., a Thai medicinal herb, is well-known for its antioxidant, anti-inflammatory, and antidote properties. However, its role in inhibiting apoptosis remains unclear. This study investigates the protective effects of *T. laurifolia* leaf extract against lipopolysaccharide (LPS)-induced apoptosis in RAW 264.7 macrophages. Additionally, the total phenolic and flavonoid contents and antioxidant capacity of the extract were evaluated. The cytotoxicity and anti-inflammatory potential were also assessed by measuring nitric oxide (NO) inhibition in LPS-stimulated macrophages. The results showed that the phenolic and flavonoid contents, as well as the antioxidant capacity of the extract, were comparable to those in previous studies. At low concentrations, the extract exhibited minimal cytotoxicity and significantly reduced NO production, an inflammatory mediator. Furthermore, pre-treatment with *T. laurifolia* leaf extract reduced LPS-induced apoptosis in the macrophages. These findings suggest that *T. laurifolia* leaf extract can inhibit LPS-induced apoptosis in RAW 264.7 macrophages, highlighting its potential therapeutic applications for inflammatory conditions.

**Keywords:** *Thunbergia laurifolia*, anti-apoptotic effect, anti-inflammation, RAW 264.7 macrophages

### **Acknowledgements**

This research was supported by a grant from the Individual Research Grant, Research Institute for Health Sciences, Walailak University (grant number WU-IRG-65-022).



## **ISOLATION AND STRUCTURE DETERMINATION OF A FLAVONOL GLYCOSIDE FROM *EUPHORBIA AMYGDALOIDES***

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*Euphorbia amygdaloides*, commonly referred to as wood spurge, is a bushy, evergreen shrub that typically reaches a height of up to 50 cm and is predominantly found in woodland margins across Europe, western Asia, and the Mediterranean region. It has significant importance in traditional medicine, where it was historically used for treating wounds, bruises, tumors, and burned skin, with records also indicating its use to combat typhoid and for animal medicine [1]. Main secondary metabolites of this plant species are flavonoids (quercetin- and camphorol-3-glycosides, rhamnetin and its glycosides), podophyllin and a cyclit. In this study *E. amygdaloides* specimens were collected from Zlatibor in 2023. The whole plant material was extracted with methanol and dichloromethane. Obtained extract was further subjected to a series of standard chromatographic techniques for extraction and purification, such as dry-flash column chromatography and RP-HPLC. Our study successfully achieved the first isolation of a known flavonol glycoside from *E. amygdaloides* [2]. The structure of the isolated quercetin-3-*O*- $\alpha$ -D-arabinopyranoside was elucidated using 1D and 2D NMR methods. Further research will focus on isolating other metabolites of this plant species and elucidating its biological activities. Isolation of secondary metabolites yields valuable insights into the chemical diversity within this plant genus and creates new opportunities for the potential drug discovery.

**Key Words:** *Euphorbia amygdaloides*, quercetin-3-*O*- $\alpha$ -D-arabinopyranoside, flavonol glycoside.

### **Acknowledgements**

This work was financially supported by the Serbian Academy of Sciences and Arts (Strategic Project MilkIng, No. 01-2022), and by Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. 451-03-47/2023-01/200026 and 451-03-47/2023-01/200168).

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## **RAMAN SPECTROSCOPY AS AN EMERGING TOOL IN CHARACTERIZATION OF NATURAL PRODUCTS AND FOODS: THE CAROTENOIDS AS A MODEL NUTRIENTS**

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Raman technique is a vibrational spectroscopy technique based on the interaction of high-energy light radiation with molecular vibrations. Raman spectra and images are used for evaluation of the physical, chemical, and biological properties of natural products and foods, in addition to application in studies of range of semisynthetic and synthetic materials of different complexity. Raman spectroscopy (RS) utilizes scattered light to gain signal about molecular vibration providing the information about the structure, symmetry, electronic environment, and bonding of a target molecules. RS allows performing of 2D and 3D imaging for studies focusing on identification, localization and spatial distribution of different components within complex observed structures. The high spatial resolution reveals molecular and chemical heterogeneity within cellular dimensions. The non-destructive nature of Raman analyses along with none to minimal requirement for sample preparation makes it exceptionally useful for various *in situ* analyses and molecular fingerprinting. RS-associated chemometrics is obligatory for proper interpretation of Raman spectra, discrimination and cluster analyses, as well as for quantification of studied molecules. RS is especially valuable for identification, localization, and quantification of molecules containing the double and/or triple bond in its structure. Therefore, the carotenoids are particularly suitable objects for Raman investigation, due to their polyene backbone containing conjugated double bonds. Even the carotenoids might be minor components in a sample, they are able to perform specific excitation in the visible wavelength (*e.g.* 532 nm). Our research includes the identification of different carotene (*e.g.*  $\alpha$ -carotene,  $\beta$ -carotene, lycopene) and xanthophyll molecules (*e.g.* lutein,  $\beta$ -cryptoxanthin, capsanthin, zeaxanthin) in range of foods and products, including carotenoid-rich nanoemulsions and encapsulates. Furthermore, we succeeded to identify the different maturity phases and different varieties of carotenoid-rich foods using RS associated with advanced chemometric modeling. Finally, results of quantification of carotenoids by RS verified through performed HPLC and HPTLC analytics will be presented.

**Keywords:** vibrational spectroscopy, carotene, xanthophyll, chemometrics

### **Acknowledgment**

The authors are grateful for support from the Project R-SPECT supported by the Science Fund of the Republic of Serbia, grant No 7750160 and Project EthnoHERBS funded by European Union's H2020-MSCA-RISE-2018 under grant agreement No 823973.



## PHYTOCHEMICAL STUDY OF *DAPHNE BLAGAYANA* FREYER

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*Daphne blagayana* Freyer is a species of flowering plant in the genus *Daphne*. Plants of this genus are used for medicinal purposes because they have been found to have antioxidant properties, they can be used in the treatment of skin diseases, toothache and malaria, as well as a natural laxative or anticoagulant. *D. blagayana* is an endemic species of the Balkan Peninsula and its typical habitat is mountain pastures, partially karst terrains and light coniferous forests. It can be found on mountains up to 1700 m above sea level. The aim of this phytochemical study was the investigation of previously unexamined plant species *D. blagayana*. The plant material was collected in February, 2024 in Divčibare (Serbia). For the purposes of the experiment, the stem and flower of the plant were used. The plant material was extracted with methylene chloride and the resulting extract was suspended in *n*-hexane and the soluble part was separated from the insoluble residue. For further examination hexane part was fractionated by column chromatography using silica gel as the stationary phase. Selected fractions were further fractionated using RP-HPLC. In this way, coumarin derivatives, 7-methoxy-8-*O*- $\beta$ -D-glucopyranosyl coumarin, 8-hydroxy-7-methoxycoumarin, and syringin were isolated as secondary metabolites of *D. blagayana*.

**Key Words:** *Daphne blagayana*, coumarine, glycosides

### **Acknowledgements**

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract number: 451-03-66/2024-03/200168 and 451-03-66/2024-03/200026).



## OPTIMIZING EXTRACTION STRATEGIES FOR ISOLATING 3'-8'' BIFLAVONES FROM GINKGO (*Ginkgo biloba* L.)

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Dimeric flavonoids, known as biflavonoids, have been significantly less studied compared to their monomeric counterparts. To date, around 600 natural biflavonoids have been identified. Among these, biflavonoids linked by a 3'-8''-C bond include notable compounds such as ginkgetin, the first isolated biflavonoid, along with amentoflavone derivatives and others [1]. Recently, 3'-8''-biflavones have gained increasing attention due to their promising biological activities, including antiviral, antimicrobial, neuroprotective, and anticancer properties, as well as their potential in treating neurodegenerative and metabolic diseases [2]. One of the plants known to accumulate 3'-8''-biflavones is *Ginkgo biloba* L. In this study, we aimed to optimize the extraction parameters for the isolation of these compounds from ginkgo leaves. We developed an HPLC-DAD method [3] for the simultaneous quantification of five biflavonoids—amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin—and investigated various factors affecting extraction yield, such as leaf drying methods [4], solvent selection, and experimental conditions. Furthermore, we investigated green extraction techniques, including deep eutectic solvents (DESs) [5], along with assisted extraction methods [6] such as enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), mechanically-assisted extraction (MAE), and chemically-assisted extraction (CAE). While green extraction methods offer many advantages, the selection of an appropriate methodology depends on the intended use of the 3'-8''-biflavones and the desired extraction yield.

**Key Words:** ginkgo, biflavonoids, 3'-8''-biflavones, extraction.

### Acknowledgements

This research was funded by the Croatian Science Foundation project "Biflavonoids role in plants: *Ginkgo biloba* L. as a model system" under Project No. UIP-2019-04-1018.

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## **PROTECTIVE POTENTIAL OF *CICERBITA ALPINA* LEAVES EXTRACT ON METABOLIC DISORDERS AND OXIDATIVE STRESS IN MODEL ANIMALS**

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Metabolic disorders (MD) include disease states such as diabetes mellitus, obesity, dyslipidemia, hyperuricemia, etc., affecting about 30% of the planet's population. The global prevalence of metabolic diseases has risen over the past two decades, demanding new approaches and strategies that are not only effective, but also locally acceptable and feasible. In this study we aimed at investigating the protective potential of *Cicerbita alpina* leaves extract (ECA) against nicotinamide/streptozotocin (NA/STZ)-induced Type 2 diabetes in Wistar rats. Additionally, some biochemical parameters related to oxidative stress in the blood serum and liver, and histopathological investigation were also performed. Quantitative determination of the main compounds in the tested extract was performed by Ultrahigh performance liquid chromatography-diode array detection (UHPLC-DAD) analyses using the external standard method. *C. alpina* extract revealed a beneficial effect on MD, lowering blood sugar levels and malondialdehyde quantity in the liver, and increased reduced glutathione level and antioxidant enzyme activity. Cichoric acid (CA) (91.93 mg/g dry extract (de)±4.64 mg/g de) was found to be the dominant compound in the extract, followed by caftaric (11.36 ± 2.10 mg/g de), and chlorogenic acid (CGA) (9.25 ± 0.05 mg/g de). The ECA, rich in caffeoyltartaric and caffeoylquinic acids provided beneficial effect on the serum biochemical parameters (cholesterol, triglycerides, ASAT, and ALAT). Cholesterol levels decreased by about 25% ( $p < 0.05$ ) and triglycerides by about 55% ( $p < 0.05$ ), while transaminase activity declined by around 20 % ( $p < 0.05$ ) compared to untreated diabetic rats. Our findings are a testament to the health-promoting effects of edible *Cichoriae* species, cichoric, caftaric, and chlorogenic acids in particular which can be considered as a viable approach in managing the diverse components of the metabolic disorders and for their applications in the alternative or conventional therapy.

**Key Words:** metabolic disorders, diabetes mellitus type 2, *Cicerbita alpina*, antioxidant enzymes

### **Acknowledgements**

This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project BG-RRP-2.004-0004-C01 "Strategic research and innovation program for development of Medical university - Sofia".



## LAVENDER: GENOMICS RESEARCH, BIOSYNTHESIS AND REGULATION OF TERPENOIDS AND THEIR ECOLOGICAL FUNCTIONS

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*Lavandula* plants, especially *L. angustifolia*(LA), *L. latifolia*(LL) and their natural hybrid *L. ×intermedia*(LX), are abundance sources of essential oils (EOs), possesses significant economic and ecological value. The terpenoids in EOs give lavender its unique aroma and play an important role in mediating ecological interactions. This paper introduces the research progress on genome, the biosynthesis and regulation of terpenoids, and ecological functions of lavender by Professor Lei Shi's team.

1. Presented a high-quality reference genome using a Chinese *L. angustifolia* cultivar 'Jingxun 2', analyzed the evolution of Lamiaceae evolution and the terpenoid-gene networks of lavender.
2. Assembled the first allele-aware chromosome-level genome with fully represented subgenomes (LX-LA and LX-LL) using a lavandin cultivar 'Super', unveiled the evolutionary history of lavandin and the genetic components responsible for the distinct EO composition of *Lavandula* species through genome phasing.
3. Three budding phases highly expressed genes LaTPS7, LaTPS8, and LaCYP71D582 were cloned and identified from *L. angustifolia* cultivar 'Jingxun 2', their functions in terpenoid synthesis were evaluated in vitro and in vivo experiments, the effects of the identified volatile limonene and carveol of repel aphids while attracting ladybugs was assessed in authentic standard tests.
4. 166 *LaBAHD* genes were identified from the lavender genome, five crucial candidate *LaBAHD* genes and five major TFs closely associated with the biosynthesis and regulation of linalyl acetate and lavandulyl acetate were determined, of them *LaBAHD57* shown to catalyze the formation of linalyl acetate from linalool in both tobacco and lavender systems. Both linalyl acetate and lavandulyl acetate were confirmed to attracting pollinators (honeybee) through odor selection tests.

**Key Words:** *Lavandula angustifolia*, *Lavandula ×intermedia*, genome, terpenoids, ecological interactions

### Acknowledgements

This work was supported by grants from the projects funded by the National Natural Science Foundation of China (Grant No. 31701956 and 32270411) and the International Partnership Program of the Chinese Academy of Science (Grant No. 063GJHZ2022038GC)

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## **BIOLOGICAL ACTIVITIES OF *CENTAUREA URVILLEI* SUBSP. *URVILLEI* AND ITS GREEN SYNTHESIZED SILVER NANOPARTICLES**

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*Centaurea* is an important, widespread genus in Asteraceae. There are 194 species in Türkiye of which 118 are endemic. The genus contains commonly phenolic structures and flavonoids. Nanoparticles, especially silver nanoparticles, have been the focus of attention in recent years due to their effects. It is known that nanoparticles are synthesized by the binding of secondary metabolites, especially phenolic structures in the plant. The objectives of the study were to obtain silver nanoparticles using *Centaurea urvillei* DC subsp. *urvillei* in an environmentally friendly, economical, and most effective way with green synthesis. Additionally, silver nanoparticles are intended to have higher activity than the water extract. Nanoparticles were characterized using various methods such as, UV-visible spectroscopy, FT-IR (Fourier Transform Infrared Spectroscopy) spectroscopy, polydispersity index, particle size and zeta potential. Transformation from a light yellow, almost transparent color to brown due to surface plasmon resonance represented the synthesis of silver nanoparticles. According to the results, a peak in UV spectrum, characteristic to silver nanoparticles was observed at 440 nm. In addition, changes in peak intensities and shifts in values that occur at the end of nanoparticle formation were determined by FT-IR. Polydispersity index was under 0,3 and zeta potential was between -30-(+30) which showed the stability of nanoparticles. Antioxidant activity was evaluated by superoxide, ABTS<sup>+</sup> and DPPH radical scavenging activity, CUPRAC reducing power and metal chelating properties. Tyrosinase inhibitory activities were also investigated and compared. According to the results, it is remarkable that silver nanoparticles show higher biological activity. The highest activity with an IC<sub>50</sub> value of 55.78 ± 0.02 µg/mL was seen in DPPH radical scavenging activity. Also, tyrosinase inhibitory activity of silver nanoparticles was more active than the water extract with an IC<sub>50</sub> value of 145.41 ± 0.21 µg/mL. It was concluded that *C. urvillei* subsp. *urvillei* can be presented as an effective, stable reducing agent with certain biological activities.

**Key Words:** Green synthesis, silver nanoparticles, *Centaurea*, antioxidant, tyrosinase inhibitor



## **CALENDULA OFFICINALIS L.: FROM SUSTAINABLE AGRICULTURE TO COSMETIC FORMULATIONS-CULTIVATION IN MEDITERRANEAN CLIMATES**

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*Calendula officinalis*, commonly referred to as pot marigold, is extensively acknowledged for its diverse medicinal utilities, attributed to its abundant phytochemical profile, which comprises flavonoids, terpenoids, and carotenoids. These bioactive constituents contribute to its anti-inflammatory, antimicrobial, and anticancer effects, thereby rendering *C. officinalis* significant in the management of dermatological injuries, infections, as well as gastrointestinal and gynecological health concerns. To optimize its therapeutic efficacy, the implementation of sustainable agricultural methodologies, including organic farming and crop rotation, is imperative, as these practices not only enhance the yield and quality of bioactive substances but also foster soil health and biodiversity. The effective cultivation of *Calendula officinalis* necessitates meticulous evaluation of variables such as soil composition, fertilization strategies, and plant density, all of which exert substantial influence on growth and productivity. Organic substrates, such as vermicompost and cocopeat, have been demonstrated to enhance root development and floral diameter, while the integration of crop residues, particularly from maize, contributes positively to plant height and flower yield. Specific fertilization parameters, notably a ratio of 120:45:45 N:P kg/ha, in conjunction with reduced plant spacing (30 x 20 cm), have been evidenced to optimize flower production, although broader spacing (40 x 30 cm) may facilitate the development of larger flowers. The adaptability of various *Calendula* cultivars to regional conditions also significantly influences the determination of optimal agricultural practices. This study sought to examine the interrelations among soil composition, fertilization, plant spacing, and environmental variables in order to elucidate strategies for enhancing the yield, quality, and sustainability of *C. officinalis* cultivation in İzmir.

**Key Words:** *Calendula officinalis*, pot marigold, phytochemical profile, flavonoids, sustainable agriculture



## **EVALUATION OF OLIVE LEAVES IN OLIVE PRUNING WASTE: SOME PHARMACOLOGICAL ANALYSES OF 4 DIFFERENT SELECTED OLIVE CULTIVARS (CV. HALHALI, KARAMANI, GEMLIK AND ARBEQUINA)**

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The olive tree, *Olea europaea* L., is an ancient and widespread cultivated plant that originally comes from the Mediterranean region. The family Oleaceae is distributed in both temperate and tropical parts of the world and includes about 600 species from 25 genera. In the present study, leaf samples were collected from four different olive cultivars including Halhalı (Türkiye), Karamani (Syria), Gemlik (Türkiye), and Arbequina (Spain) growing under the same conditions and at the same age. The leaf shoot ratio was calculated for all cultivars. Ethanol water extracts of phenolic compounds, anticancer activities, and antioxidant capacities were determined. The extracts showed large differences in phenolic compounds; kaempferol, rutin and kaempferol-3-glucoside were the most abundant compounds. The oleuropein content varied between 732.59 and 1556.32 mg/kg, the highest content was found in cv Halhalı. The tested biological activities of the leaves changed based on their chemical profiles. The leaves can be considered as sources of bioactive compounds for designing health promoting applications in the pharmaceutical and nutraceutical applications.

**Key Words:** rutin, kaempferol, oleuropein, anticancer



## **DETERMINATION OF ANTIMICROBIAL ACTIVITY OF *LIQUIDAMBAR ORIENTALIS* BALSAM OIL**

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*Liquidambar orientalis* Mill. is a species endemic to Türkiye and the island of Rhodes, belonging to Altingiaceae family and *Liquidambar* genus. It is common in Southwestern Anatolia in Türkiye. Balsam oil, a resinous exudate derived from the wounded trunk of *L. orientalis* Mill., has been traditionally used for the treatment of various ailments. This study aimed to evaluate the antimicrobial activity of *L. orientalis* balsam oil against selected Gram-positive, Gram-negative bacteria, and fungi using different methodological approaches. Specifically, its effects on *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Salmonella* spp., *Proteus mirabilis*, *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* were assessed through well diffusion and broth microdilution methods. Additionally, time-dependent killing assays were conducted to further explore its antimicrobial efficacy. Balsam oil was either added directly or dissolved in DMSO at a 50% w/v ratio, then introduced into wells created in the agar medium, followed by overnight incubation. As a control, DMSO was added to one well. The oil showed a measurable inhibition zone diameter against *S. epidermidis* and *C. albicans* compared to DMSO alone. In broth microdilution analysis, the minimum inhibitory concentration (MIC) values for *S. epidermidis* and *C. albicans* were determined to be 3.13% and 0.78%, respectively. No significant effects were observed against the other tested microorganisms. Time-kill studies demonstrated a bacteriostatic effect, inhibiting the growth of *S. epidermidis* and *C. albicans* within 24 hours. In conclusion, *L. orientalis* balsam oil shows promising potential in treating skin infections predominantly caused by *S. epidermidis* and *C. albicans*.

**Key Words:** *Liquidambar orientalis*, antimicrobial activity, *Staphylococcus aureus*, *Candida albicans*



## EVALUATION OF COMMERCIAL THYME ESSENTIAL OIL SAMPLES ACCORDING TO EUROPEAN PHARMACOPOEIA 10.0 CRITERIA

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Essential oils are complex mixtures of volatile plant extracts. Each essential oil has a unique chemical composition that determines its distinctive aroma and potential therapeutic effects [1]. Thyme essential oil, obtained from the fresh or dried leaves and flowers of *Thymus* spp. through steam distillation, is widely used in industries such as medicine, pharmacy, perfumery-cosmetics and food [2]. Additionally, it exhibits a variety of biological properties such as antimicrobial activity, antitussive, antispasmodic and antioxidative [3]. Ensuring the quality and safety of essential oils is of great importance for public health due to potential deviations from the established standards pose a risk to consumers. This study aimed to evaluate the quality of 12 different Thyme essential oil samples from pharmacies and non-pharmacy markets (various sources) via focusing on their adherence to the standards set by the European Pharmacopoeia (EP). The EP serves as the official reference for national and international regulations, providing both qualitative and quantitative methods for analyzing active ingredients and excipients used in pharmaceutical production. Initially, appearance, solubility and resinified essential oils tests were done to primarily evaluate possible adulterations. Then, High-Performance Thin layer chromatography (HPTLC) and gas chromatography-mass spectrometry (GC-MS) techniques were applied to all Thyme essential oil samples to provide comprehensive information about their phytochemical composition. Additionally, relative density and refractive index tests were employed for each sample. Results revealed that none of the samples on the Turkish market meet the standards of EP 10.0 criteria. The proportion of samples meeting the criteria is 43.75% for those sourced from pharmacies, compared to 39.38% for non-pharmacy samples. The inability of the thyme essential oil samples to meet the EP standards highlights the need for greater efforts and regulatory oversight to enhance the quality of products available in the market.

**Key Words:** Thyme oil, European Pharmacopoeia, GC-MS, HPTLC, essential oil

### Acknowledgements

This study has been financially supported by Acibadem University, Scientific Research Projects Commission (ABAPKO).

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## **INFLUENCE OF MACERATION PERIOD ON THE COLOR CHANGE OF *HYPERICUM PERFORATUM* L. OLIVE OIL MACERATE**

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*Hypericum perforatum* L. (Hypericaceae) is a historically significant medicinal herb utilized in pharmaceuticals. It is a popular herb frequently used in the management of anxiety and depression. Furthermore, its oil macerate (Oleum Hyperici) derived from its flowering aerial parts is extensively utilized in traditional medicine throughout the Balkans and Türkiye as a topical remedy for wounds and ulcers. The red color of Oleum Hyperici is a significant quality attribute of this pharmaceutical formulation. Hence, this study aimed to investigate the color changes in *H. perforatum* olive oil macerate with fresh and dried plant material during a twelve-weeks maceration period under sun and shade conditions. Weekly, overall appearance was determined applying the International Commission of Illumination (CIE L\*a\*b\*) color system. There were significant differences among the produced *H. perforatum* L. olive oil macerate (HPOO) by different maceration techniques, with respect to L\*, a\*, b\*, and  $\Delta E$  ( $p < 0.05$ ). The L\* values varied from 22 to 59 at the end of 12 weeks, and the lowest values were observed in macerate obtained under sunlight by the fresh plant. The lower L\* value may result from the degradation of pigments or other chemical alterations induced by exposure to light and heat. The a\* value representing redness is one of the most important color parameters for HPOO. The highest a\* values (34.02) were found in macerate obtained under sunlight by the fresh plant and showed an increasing weekly trend. The characteristic red color can be attributed to lipophilic compounds resulting from the breakdown of hypericin upon exposure to sunlight. The most significant color change ( $\Delta E = 62.75$ ) was observed in the fresh plant macerates kept in the sun. Consequently, if brilliant red colors of the macerates are desired, it could be suggested to employ fresh plant material and sunlight conditions for extraction.

**Keywords:** *Hypericum perforatum* L, olive oil maceration, total color change, light conditions





## **HEAVY METAL COMPOSITION OF SOME *VERBASCUM* SPECIES GROWN IN GAZIANTEP FLORA\***

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*Verbascum* genus, a member of Scrophulariaceae, has 3000 species on the world and is represented by 466 species in different parts, mostly in Iran-Turan phytogeographical region, in Türkiye [1]. All the plant parts are rich in different phytochemicals such as saponins, monoterpenes, phenylethanoid and neolignanes glycosides, iridoids and flavonoids. Because of their useful photochemical compositions, these plants are commonly used in the Traditional Anatolian Medicine in order to cure of asthma, bronchitis, cough, tuberculosis, rheumatism and hemorrhoid and other respiratory diseases. Topsoil parts of the plant have many pharmacological effects like antispasmodic, sedative, antiseptic, hemostatic, wound healing, analgesic, emollient, antihistamine, antibacterial, antifungal, anticancer, antioxidant, antiviral and antidiabetic [2, 3]. Besides its health benefits, these plants are though as a bio-indicator species in the monitoring of increased Cd<sup>2+</sup>, Cr<sup>3+</sup>, Pb<sup>2+</sup>, and Zn<sup>2+</sup> in the environment. Thus, in the present study, heavy metal compositions of three different taxa including *Verbascum lasianthum* Boiss. Ex Bentham (woolly mullein), *Verbascum tenue* Murb. (puny mullein) and *Verbascum sinuatum* subsp. *sinuatum* L. (scallop-leaved mullein) grown in Gaziantep flora were investigated. The results showed that heavy metal concentrations changed by investigated species and different plant parts.

**Keywords:** heavy metal, bio-monitor, *Verbascum*, Gaziantep

**Acknowledgment:** This research was financially supported by Scientific Research Projects Coordination Unit of Gaziantep University, Gaziantep-Türkiye (Project number: FEF.YLT.24.01).

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## **A NEW ANTIOXIDANT-RICH CROP FOR TÜRKİYE: ARONIA**

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For our country, Aronia is a plant that has the status of a new crop and its production, marketing and processing have not yet become widespread. It takes time for each new cultivated plant to be adopted by the producer and to start production activities. In addition, the sources of information on where to obtain the plant production material, how to grow it, or how to harvest and process it are very limited and there are concerns about its reliability.

Aronia (*Aronia melanocarpa* (Michx) Elliot) is a branching and shrub-like perennial plant belonging to the Rosaceae family, which is native to the eastern North America. It is a cold-resistant berry fruit that can grow between 1 and 2.5 meters tall, sheds its leaves in winter and is cold-resistant. Since Aronia plant is represented by 4 genera, Photinia, Pyrus, Sorbus, there are difficulties in its taxonomy. The genus *Aronia* is classified as black chokeberry (*Aronia melanocarpa*) and red chokeberry (*Aronia arbutifolia*). Where the two species overlap geographically, a third separate species, the purple chokeberry *Aronia prunifolia* (*Aronia x floribunda* hybrid), is recognized.

The USDA reports that aronia berries are 177% higher in flavonol, 406% higher in anthocyanins, 429% higher in proanthocyanidins and 344% higher in total antioxidants than blueberry berries.

Aronia fruits are tasteless, when consumed unprocessed; however, since it has more antioxidant effect than vitamins C and E, its use as food and nutraceutical is quite common in Europe. It is especially used in the production of fruit juice, jelly, alcoholic beverages, energy drinks and jams.

**Key words:** chokeberry, *Aronia melanocarpa*, berry



## **GENERAL PHYTOCHEMICAL PROFILE AND ANTIOXIDANT ACTIVITY OF *HYPERICUM PERFORATUM* TEA FROM THE RTANJ MOUNTAIN REGION: INSIGHTS INTO TRADITIONAL MEDICINAL USE**

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In Serbia, *Hypericum perforatum* L. holds a significant place in traditional medicine, known for its versatile healing properties. Found abundantly in the country's natural landscapes, this herb has been used for generations to treat ailments ranging from mood disorders to skin conditions. Renowned for its rich content of bioactive compounds such as phenolics, which contribute to its anti-inflammatory and antidepressant effects, *Hyperici flos* remains a foundation of Serbian herbal practices. For this research, a monocomponent tea was sourced from the wild flora of the Rtanj mountain region. The extraction was performed using hot water, simulating traditional preparation methods, to evaluate its phytochemical composition and antioxidant activity. Spectrophotometric analysis was conducted to determine the total phenolic content (TPC), total flavonoid content (TFC), total tannin content, and total hydroxycinnamic derivatives (HCAs), while antioxidant properties were evaluated using five assays: ABTS<sup>•+</sup>, DPPH<sup>•</sup>, *in vitro* phosphomolybdenum total antioxidant capacity (TAC), ferric reducing power (FRP), and cupric reducing antioxidant capacity (CUPRAC), with results expressed on a dry weight (DW) basis. The TPC was determined to be 35.40 mg/g GAE, while the TFC was measured at 151.30 mg/g RE. The analysis of HCAs yielded a value of 3.28 mg/g CGAE, and the total tannin content was recorded at 12.83 mg/g TAE. Antioxidant capacities were assessed through quencher assays, with the ABTS<sup>•+</sup> and DPPH<sup>•</sup> tests demonstrating values of 176.48 and 132.96  $\mu\text{mol trolox/g}$ , respectively. Additionally, the TAC assay indicated a value of 20.73 mg/g AAE, underscoring the extract's significant antioxidant potential. The FRAP evaluation further illustrated the sample's reducing ability, measuring 21.08 mg/g AAE, while the CUPRAC assay confirmed its antioxidant activity, yielding a result of 27.50 mg/g AAE. The findings underscore the therapeutic potential of *Hyperici flos* tea, revealing its rich bioactive profile and significant antioxidant properties that support its long-standing role in traditional medicine.

**Key Words:** *Hypericum perforatum* L., tea, phytochemical profile, antioxidant capacity

### **Acknowledgements**

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant numbers 451-03-47/2023-01/200116 and 451-03-47/2023-01/200054. Grateful acknowledgment to *Adonis d.o.o.* for providing the tea material used in this research



## INVESTIGATION INTO ESSENTIAL OIL COMPOUNDS OF SOME CONIFEROUS SPECIES GROWING IN TÜRKİYE

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Coniferous grow almost everywhere in the world, because they are resistant to different climatic conditions. Although there are many species in the world, there are seven different species and their subspecies that grow naturally in Türkiye. Due to its durable, hard, strong woods, it is used in the furniture, construction, shipbuilding, paper and packaging sectors both in the world and in Türkiye. A few species, such as some juniper species, are used in industry as medicinal and aromatic. On the other hand, their cones and needles are used in the treatment of various diseases among the people [1,2].

In this study, cones and needles samples of six different coniferous species and some of their subspecies (*Juniperus foetidissima*, *Juniperus excelsa*, *Juniperus oxycedrus*, *Cupressus sempervirens* var. *pyramidalis*, *Cedrus libani*, *Pinus brutia*, *Pinus nigra*, and *Pinus pinea*) collected from different regions of Türkiye were used. Subsequently, essential oils of the samples were obtained by hydrodistillation method and volatile component analysis was performed in the GC-MS device

When the volatile compounds results were examined, it was detected that main compound as  $\alpha$ -thujone in *Juniperus foetidissima*,  $\alpha$ -pinene in *Juniperus excelsa* and *Cupressus sempervirens* var. *pyramidalis*,  $\beta$ -myrcene in *Juniperus oxycedrus*,  $\beta$ -phellandrene in *Cedrus libani*,  $\delta$ -3-carene in *Pinus brutia*, Germacrene-D in *Pinus nigra* and limonene in *Pinus pinea*. In this study, the volatile components of cones and needles of coniferous species in Türkiye were compared. Moreover, the main components of the cones and needles of these species, which are used for folk medicine, have been determined and it is aimed to pioneer the use of these species for biological studies.

**Key Words:** Coniferous, essential oil, GC-MS, hydrodistillation

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## **CAPPARIS AND ITS APPLICATIONS IN ANIMAL NUTRITION AND TREATMENT**

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The genus *Capparis* (Capparidaceae), commonly known as caper, has long been recognized for its medicinal properties in human health. In recent years, interest has grown in its application within animal nutrition and therapy, due to its bioactive components and potential health benefits. This paper examines the nutritional value of *Capparis* and its therapeutic effects on animal health, focusing on its antioxidant, anti-inflammatory, and immunomodulatory properties. The bioactive compounds in *Capparis*, such as flavonoids, alkaloids, and glucosinolates, contribute to its health-promoting effects. These compounds may help reduce oxidative stress, leading to improved immune function in animals. The anti-inflammatory properties of *Capparis* could offer potential benefits in managing chronic inflammation-related conditions, such as arthritis or other inflammatory diseases in livestock or pets. Moreover, the immunomodulatory effects are noteworthy, as they could enhance the immune response in animals, improving resistance to diseases and supporting overall health. However, challenges remain in incorporating *Capparis* into animal diets, including variability in plant composition, determining optimal dosages, and assessing long-term safety. Despite these challenges, *Capparis* holds promise as a natural supplement in animal nutrition, offering an alternative to synthetic additives. The paper also discusses the opportunities for future research, particularly in optimizing its use as a functional ingredient to improve animal health and performance, paving the way for new advancements in animal care.

**Key Words:** *Capparis*, medicinal use, animal nutrition



## **ISOLATION, PURIFICATION AND STRUCTURE DETERMINATION OF SECONDARY METABOLITES FROM *DRACOCEPHALUM IMBERBE***

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*Dracocephalum* is a genus consisting of approximately 60 to 70 species in the world. It is a plant in the Lamiaceae family, known for its beautiful flowers and mostly found in the temperate regions of Asia and Europe. Generally, the plants called “dragonhead” due to the shape of their flowers are annual or perennial [1-4].

*Dracocephalum* species are medicinal aromatic plants known for their strong biological activities and pharmacological properties. It has been reported in the literature that *Dracocephalum* species have many different activity potentials such as antimicrobial, antioxidant, immunomodulatory, insecticidal, cytotoxic activities [3,5]. The source of the various biological activities they exhibit is secondary metabolites with many different structures such as phenolics, flavonoids, terpenes [2-5]. Recently, interest in the *Dracocephalum* genus and the species it contains has increased.

In this context, it was aimed to obtain new and/or biologically active natural compounds from the *Dracocephalum imberbe*, whose chemical content has not been determined before. The process involves solvent extractions (MeOH, BuOH (1:1), and *n*-hexane), followed by chromatographic techniques such as RP-VLC and column chromatography with different adsorbents and solvent systems. Structural determination studies were accomplished through advanced analytical techniques such as IR, UV, 1D- and 2D-nuclear magnetic resonance (NMR) spectroscopic methods.

**Key words:** Lamiaceae, *Dracocephalum imberbe*, isolation, purification, extraction.

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# **ABSTRACTS (POSTER PRESENTATIONS)**



## INFLUENCE OF ABIOTIC AND BIOTIC ELICITORS ON SESQUITERPENE LACTONE PRODUCTION IN *ARNICA MONTANA* L. *IN VITRO* SHOOT CULTURES

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*Arnica montana* L. (Asteraceae) is an endemic plant to Europe, used for centuries in ethnomedicine and is currently applied widely in pharmacy and cosmetics due to its antiseptic, anti-inflammatory, and antioxidant properties. The biological activity of this species is due to the presence of sesquiterpene lactones of helenalin and 11,13-dihydrohelenalin type. The plant is listed as threatened in many European countries due to habitat loss and extensive harvesting for commercial purposes. *In vitro* cultivation is an attractive and ecofriendly approach for large-scale plant production and elicitation is one of the most effective biotechnological tools for modulating, generating, and enhancing valuable plant secondary metabolites.

The aim of the current study was to assess the effect of abiotic (salicylic acid and methyl jasmonate) and biotic (yeast extract) elicitors applied at different concentrations on sesquiterpene lactone accumulation and lactone profile of *in vitro* micropropagated *A. montana* shoots.

The results showed that salicylic acid and yeast extract enhanced the production of sesquiterpene lactones in the shoot culture compared to the untreated *in vitro* shoots. In this study, the elicitor methyl jasmonate was ineffective. All samples revealed the same lactone profile assessed by HPLC analysis. The maximum sesquiterpene lactone content was measured in micropropagated shoots treated with 200  $\mu$ M salicylic acid. The treatment with 100  $\mu$ M salicylic acid and 100 mg/l yeast extract was also effective. Helenalins were dominant in all tested *in vitro* samples. The main sesquiterpene lactones in the studied samples are methacryloyl esters of helenalin and 11 $\alpha$ ,13-dihydrohelenalin.

This is the first report to demonstrate higher production of sesquiterpene lactones in shoot cultures of *A. montana* by elicitation with salicylic acid and yeast extract.

**Key Words:** *Arnica montana* L., *in vitro*, micropropagated shoots, abiotic and biotic elicitors, sesquiterpene lactones, HPLC

### Acknowledgements

This research was funded by National Science Fund, Bulgaria, grant number KII-06-H76/5 (05.12.2023).





## **ST. JOHN'S WORT: FROM AN ANCIENT PROTECTOR TO A MODERN ANTIDEPRESSANT**

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St. John's wort (*Hypericum perforatum* L., fam. Hypericaceae) is a perennial plant native to Eurasia, known for its traditional use in treating various ailments. Its medicinal uses have been documented by eminent herbalists such as Hippocrates, Pliny, Dioscorides, Galen and Paracelsus. Since antiquity, the plant has been commonly referred to as "Fuga daemonum" and it was believed to guard against evil spirits and helped those who suffered from mental illnesses. In today's modern society, depression is the most common mental disorder worldwide, and its increasing prevalence is alarming. There are various classes of conventional antidepressants that can be prescribed to patients, however due to their numerous side effects, patients often struggle to adhere to the treatment. *Hypericum* extracts have been extensively studied for the past four decades for the treatment of mild to moderate depression. Clinical evidence has shown that *Hypericum* extracts may be as effective as conventional antidepressants in the treatment of mild to moderate depression, with lower incidence of side effects and a superior tolerability profile, however more prospective studies are required to fully understand their long-term efficacy and safety. Additionally, *Hypericum* extracts interact with a large number of drugs due to the induction of isoenzymes of the cytochrome P450 system and P-glycoprotein, which can potentially lead to life-threatening conditions such as serotonin syndrome. Therefore, healthcare professionals play a critical role in managing the use of St. John's wort by carefully monitoring for any side effects or drug interactions, providing counselling, and collaborating with other professionals to optimize patient safety and therapeutic outcomes.

**Key Words:** St. John's wort, traditional use, treatment for depression, herb-drug interactions

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## **IN SILICO ANALYSIS ON SOME BIOACTIVE COMPOUNDS OF CITRUS SP.**

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In the present study, *in silico* analyzes were performed to make computer-based predictions about the bioactivity profiles of bioactive compounds of *Citrus* species namely isomeranzin, 3-methoxy nobiletin, nobiletin and tangeretin. For this purpose, the PASS (Prediction of Activity Spectra for Substances) program was used. Based on the biological activities that may play a role in the anticancer effect, the probability of being active (Pa) for the relevant biological activity was determined. The effect ranking was made by taking into account (Pa-Pi) values (Pi: probability of being inactive). Possible cytotoxic effect of the compounds on cancer cell lines was also predicted using CLC-Pred (cell line cytotoxicity predictor) software (v. 2.0). VEGA QSAR mutagenicity consensus model (v.1.0.4), ToxTree (SAR) mutagenicity model (v.3.1.0) and ToxRead (0.25 Beta) (Read-across) approaches were also used for the mutagenicity evaluation. Considering the prediction results for the anticancer activity of isomeranzin, it was calculated that it could be used for antineoplastic purposes with a probability of 0.691 at the highest. Among the four compounds investigated, isomeranzin was the least effective compound in terms of anticancer activity. The other three compounds gave similar results that anticancer activity was estimated between 0.7-0.89 probability value in terms of antineoplastic, apoptosis agonist and free radical scavenging effects. In the mutagenic activity evaluation, the compounds nobiletin, 3-methoxy nobiletin and tangeretin were predicted to be “non-mutagenic” but isomeranzin was found to be “mutagenic”. The anticancer activities of the major compounds of *Citrus* species evaluated by *in silico* methods are compatible with experimental studies. Moreover, it has been shown that the polymethoxyflavonoid-type compounds evaluated in the current study may be suitable candidates for preclinical and clinical studies in terms of anticancer activity.

**Key Words:** *Citrus*, *in silico*, nobiletin, 3-methoxy nobiletin, tangeretin, isomeranzin, PASS

**Acknowledgements:** We would like to thank The Scientific and Technological Research Council of Türkiye (TÜBİTAK, project number of 220S197) for the financial support.



## **ASSESSING THE EFFECTIVENESS OF CHAMOMILE ROOT ESSENTIAL OIL FOR CONTROLLING *ASPERGILLUS FLAVUS***

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German chamomile (*Chamomilla recutita* L.) is a medicinal plant species that is cultivated in large areas due to the beneficial effects of the flower heads. While the essential oil of chamomile flowers is widely used in cosmetics and medicinal preparations, little attention has so far been paid to the essential oil of the root. The use of this plant organ could contribute to a new added value of chamomile production in terms of waste utilization. In this study, we aimed to evaluate the potential yield of chamomile roots and essential oil and to investigate the effects of the essential oil on suppressing the growth of *Aspergillus flavus*, which causes postharvest decay of different crops, fruits, and vegetables, major health problems in the human diet and is a potential producer of the most potent mycotoxin, aflatoxin. To this end, we hydrodistilled chamomile roots that we excavated after harvesting the flowers, performed GC/GC-MS chemical analysis of the essential oil, and evaluated its biological activity in the *in vitro* growth inhibition of *A. flavus* by broth microdilution method. The estimated root yield at a plant density of 40 x 10 cm was 110.2 - 112.5 kg/ha, while the essential oil yield was 0.1 - 0.12 %. Chromatographic analysis of the essential oil showed that it consisted exclusively of sesquiterpene components, of which *trans*- $\beta$ -farnesene (43.9 %), *cis*-spiroether (23.4 %),  $\alpha$ -cadinol (7.4 %) and  $\beta$ -isocomene (4.1 %) were the most abundant. An antimicrobial test showed that chamomile root essential oil has significant potential, with a concentration of 0.1 mg/mL completely inhibiting the growth of *A. flavus*. Based on the results of our research, we can conclude that chamomile root is an easily accessible, underutilized biological waste that has a high potential to suppress the development of the mycelium of *A. flavus*, which can cause various diseases in humans and could be regarded as alternatives to chemical pesticides to reduce fungal species attacking different crops.

**Key Words:** chamomile, root, essential oil, antimicrobial, *Aspergillus flavus*

### **Acknowledgements**

The authors acknowledge their gratitude to the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract number 451-03-66/2024-03/ 200003/1).



## **EXTRACTION OF ANETHOLE FROM FENNEL LEAVE**

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In order to obtain the highest yield of anethole from fennel leaves three extraction methods were used. The extractions were done by hydrodistillation, Soxhlet apparatus and the supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) extraction at laboratory scale. The obtained extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). The yield obtained by hydrodistillation and the yield obtained by Soxhlet apparatus, were lower compared to the yield obtained by SC-CO<sub>2</sub> extraction. Using response surface methodology for the optimization of SC-CO<sub>2</sub> extraction the optimum extraction conditions were found at lower pressures and higher temperatures. The SC-CO<sub>2</sub> extraction is the method of choice for obtaining the highest anethole yield from fennel leaves.

**Key words:** fennel, hydrodistillation, Soxhlet apparatus, supercritical CO<sub>2</sub> extraction, GC-MS



## FERMENTED FOOD PRODUCTS FROM RASPBERRIES AND BLACKBERRIES - A NEW FORMULATION FOR FUNCTIONAL BEVERAGES

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The healing effect of wines and vinegars from fruits with high amount of natural antioxidants is well known and scientifically confirmed in treatments of anemia, diabetes, hypertension, arteriosclerosis and other cardio vascular and cancer diseases. However, the most powerful natural pigments such as anthocyanins, proanthocyanins and condensed tannins are presented in raspberry and blackberry fruits. In addition, anthocyanins and proanthocyanidins are the most stable antioxidants in an acidic environment (pH= 2-4) such as vinegars and wines. Production of healing wines and vinegars by technology of interrupted fermentation and a maximum of 5-6% alcohol has been developed as a new technology for production of healthy functional beverages. For production of healing wines and vinegars, raspberries and blackberries were collected and pressed, separately, in order to completely squeeze the liquid from the fleshy part of the fruit. The berry liquid was separated from pomace and subjected to fermentation by yeast Lalvin ICV D80 (Lallemant, France). Yeast nutrient was added, (FERMAID E, Lallemant, France), to prevent slow fermentation. During alcoholic fermentation, the temperature was controlled and maintained between 14-18 °C. After fermentation, sedimentation and filtration, the obtained wines and vinegars had high amounts of natural antioxidants, pleasant flavor and taste. The amount of D-glucose+D-fructose was below 0.5 g/L, while the amount of sucrose was less than 0.85 g/L. The level of total phenols was higher than 850 mg/L wine and vinegars, the amount of higher molecular proanthocyanidins was over 1115 mg/L, while the amount of lower proanthocyanidins was 800 mg/L. The level of total anthocyanins was over 950 mg/L expressed as cyanidin-3-O-glucoside. This new technology of interrupted fermentation is promising for production of high-valuable functional beverages due to the facts that 5% of alcohol was sufficient for extraction of all phenolic compounds and natural antioxidant presented in raspberries and blackberries.

**Key Words:** healing wines and vinegars, raspberry and blackberry functional drinks, polyphenols, anthocyanins, natural antioxidants, fermentation.

### Acknowledgements

We are grateful to Mrs. Dragana Popovska, the manager of the company "Bioactive-Antioxidant" for supplying the high-quality samples of wines and vinegars from raspberries and blackberries from the region of North Macedonia and her support and help during the investigation and preparation of the study.

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## PHYTOCHEMICAL PROFILING OF SELECTED ASTERACEAE SPECIES: A RICH SOURCE OF SECONDARY METABOLITES WITH HEALTH BENEFITS

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Herein, the phytochemical profile of selected Asteraceae species as *Echinops ritro* L., *Prenanthes purpurea* L., *Geigeria alata* Benth. & Hook.f. ex Oliv., *Inula helenium* L., and *Telekia speciosa* (Schreb.) Baumg. was investigated. An ultra high-performance liquid chromatography-high-resolution mass spectrometry analysis (UHPLC-HRMS) was conducted on the plant extracts, delineating the secondary metabolites of the studied species. As a result, 95 secondary metabolites were reported for the first time for the profile of *E. ritro*, including acylquinic acids, flavonoids, and coumarins. Protocatechuic acid *O*-hexoside, quinic, chlorogenic and 3, 5-dicaffeoylquinic acid, apigenin, apigenin 7-*O*-glucoside, hyperoside, jaceosidene, and cirsiliol were the dominated compounds. *P. purpurea* profile was characterized by a series of phenolic acid-hexosides, acylquinic, acylhydroxyquinic and acyltartaric acids, and flavonoids. Overall, the main secondary metabolites were quinic acid, chlorogenic, 3,5-dicaffeoylquinic and 5-feruloylhydroxyquinic acid, caffeoyltartaric and cichoric acids, eryodictiol-*O*-hexuronide, and luteolin *O*-hexuronide. *G. alata*, *I. helenium* and *T. speciosa* analysis revealed 32 caffeoylhexaric acids. A variety of mono-, di-, tri- and terta-caffeoylhexaric acids, as well as their propionyl, isobutyryl, hydroxypropionyl, methylbutyryl/isovaleryl, hydroxybutyryl- and others derivatives were tentatively identified. The identification of leontopodic acid A and B highlighted *T. speciosa* as a new source of caffeoyl-D-glucaric acid derivatives. In conclusion, the results verified the studied species as a rich source of secondary metabolites with health benefits including antioxidant and hepatoprotective as well as cosmetic application.

**Key Words:** Asteraceae species, UHPLC-HRMS, acylquinic acids, hydroxybenzoic acids, hydroxycinnamic acids, caffeoylhexaric acids

### Acknowledgements

This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project BG-RRP-2.004-0004-C01 "Strategic research and innovation program for development of Medical University – Sofia".



## RAPID ANNOTATION OF CAFFEIC ACID CONJUGATES AND PRENYLATED PHLOROGLUCINOL- $\alpha$ -PYRONES IN *HELICHRYSUM ITALICUM* BY ORBITRAP MASS SPECTROMETRY

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*Helichrysum italicum* ssp. *italicum* (Roth) G. Don (Asteraceae) aerial parts represents an important herbal drug used as flavor, digestive and tonic in traditional medicine of Mediterranean areas of Europe. The aim of the study was to develop a state-of-art liquid chromatography - high resolution Orbitrap mass spectrometry (LC-HRMS) method for annotation of caffeoylquinic and caffeoylhexaric acids, and prenylated phloroglucinol- $\alpha$ -pyrones in *H. italicum* ssp. *italicum* aerial parts. Compounds were annotated/dereplicated on the base of diagnostic ions in Full MS and ddMS<sup>2</sup> modes, ions relative abundances, conformity with the simulated monoisotopic profiles and comparison with standard references. Based on the diagnostic ions, the fragmentation patterns for each class of the compounds were proposed. Overall, caffeoyl-, feruloyl- and *p*-coumaroylquinic, dicaffeoylquinic and feruloyl-caffeoylquinic acids were evidenced. For the first time, malonyl-dicaffeoylquinic and caffeoyl-hydroxydihydrocaffeoylquinic were established. Among the acylhexaric acids, isobutanyl-tricaffeoylhexaric, hydroxybutanyl-tricaffeoylhexaric, and isovaleryl/2-methylbutanyl/tricaffeoylhexaric acid are evidenced for the first time. A comprehensive characterization of phloroglucinol  $\alpha$ -pyrone heterodimers by LC-HRMS was achieved. The fragmentation patterns ethylpyrones, isopropylpyrones, 1-methyl-propylpyrones and methylpyrones were suggested. Among this group, 23 compounds are undescribed in the literature. Prenyl, (di)hydroxyprenyl, geranyl and hydroxygeranyl moieties were dedicated at the C-3 of the phloroglucinol residue in the heterodimers. The variety of phloroglucinol  $\alpha$ -pyrones was also related to the presence of oxoethyl, oxopropyl, 2-methyloxopropyl and 2-methyloxobutyl functional groups at C-1. Within the corresponding groups, 3,4- and 3,5-dicaffeoylquinic acid, and tricaffeoylhexaric acid dominated the profiling, being present at 19%, 18.9% and 22%, respectively. Among the phloroglucinol  $\alpha$ -pyrones, arzanol (19.28%), together with two ethylpyrone (11.34% and 10.72%) appeared to be preponderant in *H. italicum* extract. The detailed phytochemical investigation highlights immortelle as valuable source of caffeoyl conjugates and heterodimers.

**Key Words:** *Helichrysum*, LC-HRMS, acylquinic and acylhexaric acids, phloroglucinol- $\alpha$ -pyrones.

### Acknowledgements

This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria (project No. BG-RRP-2.004-0004-C01).



**A COMPREHENSIVE METABOLIC PROFILING AND BIOLOGICAL  
POTENTIAL ASSESSMENT OF *PELARGONIUM GRAVEOLENS* L'HÈR.  
(ROSE GERANIUM)**

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*Pelargonium graveolens* L'Hèr. (Geraniaceae) is renowned for its ethnomedicinal usage as aromatic plant and for the treatment of metabolic and gastrointestinal disorders. We aimed at investigating the specialized metabolites profiling and biological potential of methanol-aqueous extracts from the rose geranium leaves. An UHPLC-HRMS analysis revealed more than 100 specialized metabolites including 8 acyltartaric and 11 acylcitric/acylisocitric acids, 8 gallotannins, 36 flavonol and flavanone glycosides, aglycones and methoxylated derivatives together with 17 phenolic and aliphatic acids and 21 phenolic acids glycosides. For the first time, acylcitric acids accompanied with feruloyl- and coumaroyltartaric acids are reported in the rose geranium. Compound annotation/dereplication was on the base of the fragmentation patterns in mass spectrometry analyses; relative abundances of diagnostic fragment ions; elemental composition; rings plus double bonds equivalents and literature data. Fragmentation keys for acyltartaric and acylcitric/isocitric acids were proposed. The profiling was dominated by caffeoylcitric/isocitric acid, caftaric acid and its glycoside, methylgalate and gallic acid hexosides, syringic acid-hexoside, tartaric, malic and citric/isocitric acids. Myricetin-*O*-rutinoside and deoxyhexoside, and kaempferol-methyl and dimethyl ether appeared to be characteristic for rose geranium leaves. Antioxidant properties assessment revealed that the leaves extract actively scavenged DPPH (273.45 mgTE/g) and ABTS radicals (531.97 mgTE/g), and showed high reducing potential (431.32 and 292.21 mg TE/g, for CUPRAC and FRAP, respectively). In metal chelating and phosphomolibdenum assays, the results were 13.44 EDTAE/g and 2.71 mmol TE/g respectively. The rose geranium extract exhibited high inhibition against butyryl- and acetylcholinesterase (2.20 and 2.80 mg galantamine equivalent/g, respectively), and tyrosinase (75.49 mg kojic acid equivalent/g). It inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase (0.79 and 3.75 mmol acarbose equivalent/g, respectively) and lipase (28.91 mg orlistat 44equivalent). The study highlights rose geranium as valuable starting point for possible applications in pharmaceuticals and nutraceuticals products.

**Key Words:** *Pelargonium*, LC-HRMS, Phenolics, Antioxidants, Enzyme inhibition

**Acknowledgements**

This study is financed by the Contract AUF ECO 2024 DRECO 8591.





## **INTRASPECIFIC VARIATION IN BIFLAVONOID PROFILES DURING LEAF YELLOWING IN *GINKGO* LEAVES**

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The *Ginkgo* tree (*Ginkgo biloba* L.) is a globally recognized species, renowned for its unique botanical features, evolutionary significance, and numerous health benefits. Its medicinal properties are largely attributed to a diverse range of phytochemicals, with 3'-8'' biflavonoids being some of the most significant, though relatively underexplored, components [1]. A total of 13 biflavonoids have been identified in ginkgo leaves, with amentoflavone, ginkgetin, isoginkgetin, bilobetin, and sciadopitysin being the most prevalent [2].

In this study, we utilized a developed HPLC-DAD method [3] to track changes in the composition of these five biflavonoids during the leaf yellowing phase, from mid-October to the end of the growing season. The analysis was carried out on five biological replicates of ginkgo trees. While biflavonoid concentrations varied between individual trees, the overall trend in their changes remained consistent across the sampling period. Sciadopitysin was consistently the most abundant biflavonoid across all samples and collection dates.

**Key Words:** ginkgo, 3'-8'' biflavones, biflavonoids

### **Acknowledgements**

This research was funded by the Croatian Science Foundation project "Biflavonoids role in plants: *Ginkgo biloba* L. as a model system" under Project No. UIP-2019-04-1018.

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## BIOMETRIC CHARACTERISTICS OF SEEDS OF ENDANGERED HERBACEOUS PEONY SPECIES: INSIGHTS INTO MORPHOLOGICAL VARIATION AND CONSERVATION IMPLICATIONS

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Herbaceous peonies are endangered plant species valued for their ornamental beauty, medicinal properties, and edibility. Understanding the seed biology of wild plant species is essential for overcoming ecological and conservation challenges. *Paeonia* species, in particular, are known for their slow and low germination rate due to dormancy, which is partly influenced by the morphology of their seeds. The aim of this study was to evaluate the biometric traits of seeds of different herbaceous *Paeonia* species to increase a deeper understanding of their variations. The seeds of three herbaceous *Paeonia* species (*Paeonia daurica* Andrews, *Paeonia peregrina* Mill., and *Paeonia tenuifolia* L.) were collected during the summer of 2024. For morphological analysis, 60 fully developed seeds of each species were randomly selected. Seed size (width, length, thickness, hilum width) was measured using calipers, and seed/seed coat weight was determined on an analytical scale. The obtained results indicated significant morphological differences among the analyzed species. *P. daurica* had the lowest proportion of seed coats and a higher nutrient content in the endosperm, which could indicate a greater potential for survival under unfavourable conditions. In addition, the round shape of the seeds improves dispersal potential. On the other hand, *P. tenuifolia* had the smallest seeds, the highest percentage of seed coat and the widest hilum, which can may indicate a greater adaptation of this species to drought conditions. The heaviest seeds belong to the species *P. 46eregrine*, followed by *P. daurica*, while *P. tenuifolia* produced the lightest seeds. These results align with the findings from previous studies, providing further consistency. Remarkably, the seeds were approximately 15% lighter than in multi-year average, due to the unusually dry and warm conditions of 2024. These findings are important for understanding the ecological adaptations of herbaceous *Paeonia* species and for supporting conservation strategies.

**Keywords:** *Paeonia daurica* Andrews, *Paeonia 46eregrine* Mill., *Paeonia tenuifolia* L., seed coat, seed morphology

### Acknowledgements

This work was supported by the Ministry of Science and Technology of the Republic of Serbia (451-03-66/2024-03/200003/1 and 451-03-66/2024-03/200032).



## **SURVEY ON AGROFORESTRY UTILIZATION OF INDUSTRIAL HEMP**

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The aim of our research was to assess the potential of industrial hemp (*Cannabis sativa* L.) as a fatty oil-producing plant species in agroforestry systems. In recent years, hemp cultivation has increased significantly due to its environmental benefits, which contribute to the goals of the European Green Deal. In alignment with the Common Agricultural Policy (CAP), the EU aims to promote green solutions, including the establishment of agroforestry systems. In agroforestry experiments, beyond the general environmental benefits of the method, research must also focus on the interactions between trees and co-cultivated plants to successfully integrate different species into agroforestry systems. Based on this, we examined the effects of mild (30%) shading on biomass production, seed yield (containing fatty oil active ingredients), and fatty oil accumulation of hemp in open-field experiment. Soxhlet extraction was used to extract the fatty oils.

For hemp, shading did not cause significant differences in the measured quantitative (fresh biomass: treated –  $574.67 \pm 53.5$  g/6 plants, control –  $549.67 \pm 207.46$  g/6 plants; dry biomass: treated –  $275.67 \pm 26.09$  g/6 plants, control –  $245.5 \pm 95.35$  g/6 plants; dry biomass of generative shoots: treated –  $88.67 \pm 5.43$  g/6 plants, control –  $95.83 \pm 29.67$  g/6 plants; seed mass: treated –  $41.5 \pm 4.09$  g/6 plants, control –  $37.83 \pm 15.47$  g/6 plants) and qualitative (fatty oil content: treated –  $21.21 \pm 2.09$  g/100 g dry seeds, control –  $24.41 \pm 0.51$  g/100 g dry seeds) properties, thus indicating that hemp is a promising species for cultivation in agroforestry systems. This further enhances the environmental benefits of this versatile species.

**Key Words:** fatty oil, shade effect, climate smart crop production



## ALKALOID PROFILING AND ANTI-CHOLINESTERASE POTENTIAL OF *GALANTHUS FOSTERI* BAKER GROWN IN GAZIANTEP PROVINCE

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*Galanthus* L., represented by 16 taxa in Türkiye (Davis, 1999; Zubov, 2019) is a perennial plant well known for its ornamental value and alkaloidal metabolites called Amaryllidaceae alkaloids. Phytochemical investigations on *Galanthus* L. (Amaryllidaceae) species, revealed a diverse alkaloidal profile with interesting biological activities (Jin and Yao, 2019). Galantamine, an Amaryllidaceae alkaloid, is an acetylcholinesterase inhibitor used to slow the process of neurological degeneration in Alzheimer's disease (Heinrich, 2010). Previous investigations on *G. fosteri* samples collected from different regions of Türkiye indicated that this species is a potential source of bioactive metabolites (Emir et al., 2020; Korcan et al., 2024). In the present study, chemical characterization of a specimen of *Galanthus fosteri* Baker growing in Gaziantep Province, southeast of Türkiye, has been achieved by gas chromatography/mass spectrometry (GC/MS). Alkaloidal extract of the whole plant material was examined and twenty alkaloids were detected. Demethylhomolycorine (34.18 %), tazettine (20.06 %), galanthindole (13.63 %), galanthamine (7.56 %), hordenine (6.16 %) and lycorine (5.69 %) were identified with relatively higher percentage amounts. Cholinesterase inhibitory potential of the alkaloidal extract was determined by *in vitro* Ellman's method (Ellman, 1961) and the alkaloidal extract displayed acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory properties (AChE: IC<sub>50</sub>: 52.03 µg/ml and BuChE: IC<sub>50</sub>: 64.91 µg/ml). The present study highlighted that the alkaloidal composition in *G. fosteri* specimens shows significant variation with respect to geographic area.

**Key Words:** *Galanthus fosteri* Baker, Amaryllidaceae alkaloids, anticholinesterase activity, GC-MS.

### Acknowledgements

This study was partially supported by The Scientific and Technological Research Council of Türkiye (TÜBİTAK) (104T272).

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## **EVALUATION OF SOME HERBAL SLIMMING TEAS / SUPPLEMENTS SOLD ON THE MARKET IN TERMS OF ADULTERATION**

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Obesity is caused by environmental and genetic factors and is now among the common disorders. Excess weight has harmful effects on an individual's health and quality of life throughout their life. Reports from the World Health Organization have shown that obesity is increasing at an alarming rate, leading to diseases such as diabetes, neurological disorders, cardiovascular diseases, and cancer becoming more threatening. Considering this situation, preventing obesity and being overweight is becoming increasingly important. The demand for slimming formulations and dietary supplements made from natural ingredients is significantly rising due to the belief that these natural products are harmless and won't cause harmful side effects like synthetic chemicals. However, it has been found that synthetic compounds are mainly added to herbal slimming products, products meant to enhance sexual power, and products intended to increase muscle mass to improve their effectiveness.

Studies have determined that sibutramine, an anorectic chemical, is most commonly added to herbal slimming products/teas. Other compounds identified besides sibutramine include sibutramine analogues like *N*-desmethyl sibutramine, *N*-didesmethyl sibutramine, 11-desisobutyl-11-benzylsibutramine, some laxatives, antidepressants, diuretics, caffeine, ephedrine, theophylline, and their mixtures. These compounds have been reported in the literature to have adverse effects, particularly on the cardiovascular system, such as hallucinations, stroke, and heart attack, depending on the dose used. In our country, sibutramine has been detected in some herbal teas sold on the market due to inspections by the Ministry of Agriculture and Forestry, and the companies found to have adulterated were made available to the public. In this study, especially 14 herbal products/teas sold with the claim of having a slimming effect in the market were extensively examined with LC-QTOF-MS for adulteration with different chemical substances, and the amount of sibutramine was determined by HPLC in one product where it was detected.

**Key Words:** adulteration, herbal teas, herbal supplements, weight-loss



## **THE POTENTIAL USE OF EMULSION ADJUVANTS CONTAINING IMMUNOMODULATORY SAPONINS IN VACCINE FORMULATIONS AGAINST INFECTIOUS BRONCHITIS DISEASE**

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Infectious bronchitis (IB) is a highly contagious viral disease caused by the Infectious Bronchitis Virus (IBV) which leads to respiratory tract infections in poultry [1]. Vaccination programs play a crucial role in achieving herd immunity [2]. Live attenuated vaccines can be dangerous due to they carry the risk of reverting to a virulent state and potentially causing infection in a live organism. Inactivated vaccines can elicit a lower and shorter-lived immune response compared to replicable live attenuated vaccines [3]. Vaccines consist of two main components: antigens and adjuvants. Adjuvants are substances added to vaccine formulations to help enhance the immune response [4]. Therefore, in light of the inadequacy of existing vaccines, there is a need for the development of new vaccine adjuvants against IBV.

Saponins are natural steroid or triterpene glycosides and contain one or more sugar chains in their structure [5]. The potential of elmalienoside A saponin isolated from *Cephalaria elmalienensis* plant as an immunomodulator in the Coralvac RZ-528 adjuvant was investigated through an *in vivo* experimental study conducted on specific-pathogen-free (SPF) chickens. Water-in-oil (W/O) emulsion adjuvant system was used. Vaccination was performed as a single dose of 0.5 ml administered into the breast muscle of SPF chickens.

The results showed that in comparison with other vaccine groups, the stimulation rates of CD4+ and CD8+ cells in the saponin group were not significantly different from those with commercially used adjuvants. However, IgA titers were notably higher in the RZ-528 + saponin group, indicating that elmalienoside A saponin enhances mucosal immunity when combined with RZ-528. This indicates that the RZ-528 + saponin formulation is at least as effective as the commercial adjuvant formulations while establishing superiority by better stimulating mucosal immunity. With the need for further studies, the potential use of saponin as an immunomodulator against IBV may be possible.

**Key words:** saponin, immunomodulator, adjuvant, vaccine, IBV

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## **INVESTIGATION OF THE IMMUNOMODULATORY POTENTIAL OF SAPONINS AND IRIDOIDS ISOLATED FROM *SCABIOSA PSEUDOGRAMINIFOLIA* ON MACROPHAGE POLARIZATION**

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Macrophage polarization is one of the immunological treatment methods that has been intensively investigated by researchers in recent years<sup>[1]</sup>. Saponins and iridoids are generally compounds with plant-derived anti-inflammatory properties that can show various biological effects on the immune system. In this study, the immunomodulatory effect of saponin and iridoids isolated from *Scabiosa pseudograminifolia* plant on macrophage polarization using THP-1 cell line was investigated by flow cytometry. THP-1 cells were cultured in RPMI-1640 medium at 5% CO<sub>2</sub> and 37 °C and PMA was applied for their differentiation into M0-THP1 macrophages. Iridoids and saponins were applied at concentrations of 3 and 6 µg/ml and stained with anti-human CD11b, human CD163, human CD80 antibodies and the transformation of cells into M1 (pro-inflammatory) and M2 (anti-inflammatory) phenotypes was examined by flow cytometry. Of the saponins applied at 3 µg/ml, 7 transformed the cells to M1 and 5 to M2, while 6 µg/ml application transformed 6 to M1 and 5 to M2. Of the iridoids applied at 3 µg/ml, 4 transformed the cells to M1 and 5 to M2, while 6 µg/ml application transformed 4 to M1 and 3 to M2. In line with these results, it was determined that some iridoids and saponins used in this study have immunomodulatory effects on THP-1 derived macrophage cells. For this reason, it is thought that they have potential for use in immunological treatments.

**Key Words:** macrophage polarization, iridoid, saponin, THP-1 cells

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## REPRODUCTIVE POTENTIAL OF PEPPERMINT (*MENTHA PIPERITA*) CULTIVATED UNDER DIFFERENT MULCHES

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The study evaluates the reproductive potential of peppermint (*Mentha piperita*) when cultivated under various treatments (mulching and two controls). Mulching is a widely used agricultural practice that can influence weed suppression, soil properties, microclimate, and plant growth. In this study, different types of mulches, including organic and synthetic materials, were applied to peppermint to assess their impact on stolon yield.

The experimental field was in South-East Banat, near Pančevo city, Serbia (44°52'20.0"N, 20°42'04.7"E). The experiment was conducted under natural moisture conditions on the chernozem soil type with 2.3% humus, while the total content of nitrogen was 0.19%, pH in H<sub>2</sub>O was 6.4, while in KCl it was 5.4. Peppermint was established in autumn by hand planting stolons (1500 kg ha<sup>-1</sup>) in rows 0.7 m. In spring next year two organic (sawdust of acacia and dry pine needles) and two synthetic mulches (silver-brown and black agrotexil mulch films) were spread between rows, in the layer of 10 cm. Two controls were included in study, one weeded control (hand-moved weeds every two weeks), and one non-weeded control (peppermint stay in thought with weeds). After two harvests of peppermint and in the end of peppermint vegetation, the stolons were removed by hand from the surface layer of the soil (from 5 to 15 cm depth). The total mass of stolons yield was measured on a technical scale (KERN 573-46NM, D-72336 Balingen) after drying the samples in the oven (SANYO MOV-212) at a temperature of 105° for 48 hours. The absolute dry biomass weight was shown in the paper.

The research compared the stolon yields under mulch treatments with controls, providing insights into the effectiveness of mulching in enhancing peppermint's reproductive potential. The findings revealed that mulch type significantly affects the yield, with the best yields reached on silver-brown film (7.5 t ha<sup>-1</sup>) and dry pine needles (8.8 t ha<sup>-1</sup>), while in non-weeded control was 4.2 t ha<sup>-1</sup> and in weeded control was 9.2 t ha<sup>-1</sup>. These results underscore the importance of selecting appropriate mulching materials to optimize the reproductive performance of peppermint, ultimately contributing to improved cultivation practices and productivity.

**Key Words:** peppermint's stolon yield, stolon, mulching, organic mulch, synthetic mulch

### Acknowledgements

This study was conducted with financial support of Ministry of Science, Technological development and Innovation of the Republic of Serbia (grant numbers: 451-03-66/2024-03/200003 and 451-03-65/2024-03/200116).





## IN SILICO EVALUATION OF SEMI-SYNTHETIC DERIVATIVES OF 7 $\alpha$ -ACETOXY-6 $\beta$ -HYDROXYROYLEANONE FOR ANTICANCER POTENTIAL

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The abietane diterpenoid 7 $\alpha$ -acetoxy-6 $\beta$ -hydroxyroyleanone (Roy), isolated from *Plectranthus grandidentatus*, demonstrates notable cytotoxicity across numerous cancer cell lines, as well as promising antibacterial and anti-inflammatory properties [1]. To enhance its anticancer potential, a series of semi-synthetic Roy derivatives were synthesized and examined through comprehensive in silico analyses. These analyses included predictions of absorption, distribution, metabolism, excretion, and toxicity (ADMET) to evaluate drug-likeness and toxicity risks. The anticancer potential was quantified using PASS, while density functional theory (DFT) models assessed reactivity and stability. Molecular docking and molecular dynamics (MD) simulations determined the binding affinity and stability of the derivatives against cancer-related proteins.

The ADMET predictions indicated favourable attributes and acceptable toxicity profiles for all compounds. Quantum mechanical calculations and DFT models revealed modifications in HOMO-LUMO gaps (3.39–3.79 eV) and global reactivity indices. The derivatives exhibited strong anticancer potential with PASS scores ranging from 0.819 to 0.879. Molecular docking highlighted favourable binding against key cancer-related proteins such as cyclin-dependent kinases, BCL-2, caspases, receptor tyrosine kinases, and p53, with stable binding poses confirmed by MD simulations. These findings suggest that Roy and its derivatives are effective molecules with significant anticancer properties, supporting future experimental validation.

**Key Words:** 7 $\alpha$ -acetoxy-6 $\beta$ -hydroxyroyleanone, *Plectranthus grandidentatus*, anticancer activity, in silico analyses

### Acknowledgements

This work was supported by FCT (Portugal) through the projects with reference DOI 10.54499/UIBP/04567/2020 and DOI 10.54499/UIDB/04567/2020.

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## ACTIVITY OF THE HYDROLATE OF CHEMOTYPE OF *SATUREJA MONTANA* AGAINST *ALTERNARIA* SP.

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A perennial plant species of the Lamiaceae family, winter savory (*Satureja montana* L.) is indigenous to regions of Europe, the Mediterranean, and Africa. It is used as a tea in traditional medicine to treat disorders of the digestive, urinary, and respiratory systems. It can be applied externally to reduce irritation of the skin. Also, it is used as a spice during cooking. Hydrolates are co-products from the distillation or hydrodistillation of *S. montana* to produce essential oils. It can be used in perfumery, cosmetics, food flavoring, and as insect pest repellents. The aim of the study was to assay the activity of the hydrolate of chemotype of *S. montana* against *Alternaria* sp. isolated from leaf of *Paeonia peregrina* Mill. from Bogovo Guvno.

The hydrolate of the chemotype (main component thymol) was prepared by extraction methods, maceration (MAC) and microwave-assisted extraction (MAE). The tubes of hydrolates were kept at 4 °C until analyses. The microdilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of the hydrolates. Fresh overnight cultures of fungi were made in tubes containing the mycelia of the test organism (to) (*Alternaria* sp.) and 0.02 mL of Tryptic soy broth (TSB) medium, achieving a concentration of  $1.0 \times 10^6$  per well. The MIC and MFC values were determined by serially subculturing 0.08 mL of hydrolate into microtiter plates with 0.02 mL of TSB per well and 0.005 mL of to. The values were observed after addition of 0.04 mL of  $\rho$ -iodonitrotetrazolium violet and incubation at 37 °C for 30 min. In the control treatments (TSB + to; TSB + streptomycin sulfate, and TSB + streptomycin sulfate + to) were used 0.02 mL of TSB, 0.08 mL of streptomycin sulfate, and 0.005 mL of to.

The hydrolate obtained by MAE had inhibitory and antifungal activity against *Alternaria* sp. (0.1 and 0.2 mg/mL), while the hydrolate obtained by MAC showed antifungal activity (0.2 mg/mL). In the first control treatment, the inhibition of the growth of mycelia manifested (0.4 mg/mL), while other treatments had antifungal activity (obtained by MAE and MAC).

It can be concluded that the tested hydrolate (prepared by MAE) can be classified as an inhibitor of *Alternaria* sp., and has antifungal ability. The hydrolate (prepared by MAC) shows only antifungal activity.

**Key Words:** *Satureja montana* L., chemotype, hydrolate, activity, *Alternaria* sp.

### Acknowledgements

This manuscript was supported by the Ministry of Science and Technology of the Republic of Serbia (451-03-66/2024-03/200003/1) and the Chinese Academy of Agricultural Sciences, Institute of Vegetables and Flowers, Beijing, China (2021YFE0110700).



## TOXICITY OF INFUNDIBULICYBE GEOTROPA METHANOL EXTRACT ON HEK293 KIDNEY CELLS AND ITS EFFECTS ON ATOPIC DERMATITIS CELL CULTURE

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Medicinal mushrooms are sources of polysaccharides and triterpenoids with anti-cancer and immunomodulatory properties (Öztürk et al., 2015). Studies show that mushrooms used in food, pharmacy, and medicine have immunomodulatory and antitumor antiviral, antimicrobial, antimutagenic, antihypertensive, anti-inflammatory, and antiallergic activities. For this reason, mushrooms are generally regarded as natural products and dietary supplements and are produced in various formulations worldwide (Zhang et al., 2007). It is seen from the literature that there have been no studies on the effect of mushrooms on atopic dermatitis, which is a chronic, recurrent inflammatory skin disease that affects one in ten people throughout life. In atopic dermatitis, impaired immune regulation, epidermal gene mutations, and environmental factors disrupt the epidermis and cause intensely itchy skin lesions (Frazier et al., 2020). Atopic dermatitis, also known as eczema, is closely associated with itching, sleep disturbance, psychosocial symptoms, and impaired quality of life (Tan et al., 2017; Hon et al., 2013). Corticosteroids, antihistamines, and symptomatics are commonly used to treat atopic dermatitis. Still, these medications have some side effects, such as decreased bone density, dry skin, susceptibility to infections, or return of symptoms when discontinued. Therefore, alternative treatments are needed to eliminate the underlying factors (Yamada et al., 2018). This study aimed to investigate the methanol extract of *Infundibulicybe geotropa* mushroom, distributed in our country, against eczema (atopic dermatitis). For this purpose, methanol extract of dried edible mushroom species was obtained under room conditions. First, its toxicity was investigated against kidney (HEK293) healthy cell lines using the MTT method. Then, the effect of the extract on lymphocyte proliferation was analyzed on mononuclear cells isolated from peripheral blood samples of atopic dermatitis patients via flow cytometry.

According to the results, the methanol extract from the *I.e geotropa* reduced the proliferation of lymphocytes from 69.4% to 23.2%. According to this activity result, further studies on methanol extract will be carried out by activity-guided fractionation to obtain bioactive compounds to understand which compounds are responsible for the activity. Then, their structures will be elucidated using spectroscopic techniques.

**Key Words:** atopic dermatitis, *Infundibulicybe geotropa*, Toxicity on HEK293 healthy cell lines

### Acknowledgments

This study was supported by The Scientific and Technological Research Council of Türkiye (TÜBİTAK) with project number TÜBİTAK1001-121Z551.

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## ***IN VITRO* BIOACTIVITIES OF *SILENE SEDOIDES***

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The aerial parts of *Silene sedoides* were extracted using petroleum ether (A), dichloromethane (B), ethyl acetate (C), methanol (D), and water for infusion (E) and methanol for maceration (F). These extracts were tested for antimicrobial, antioxidant, and enzyme-inhibitory activities, including acetylcholinesterase, butyrylcholinesterase, tyrosinase, and  $\alpha$ -glucosidase inhibition.

The antimicrobial activity was assessed against various pathogens, including *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, and *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750 using the micro broth dilution method [1, 2]. The methanol maceration extract (F) showed the strongest antimicrobial activity against *E. faecalis* (MIC = 19.53 mg/L). Extracts (B) and (C) exhibited significant antifungal activity against *C. tropicalis* (MIC = 156.2 mg/L).

Total phenolic content [3] and total flavonoid content analysis [4] revealed that the dichloromethane extract (B) had the highest phenolic content ( $26.26 \pm 0.28$   $\mu$ g PEs/mg extract), while the same extract had a high flavonoid content ( $32.61 \pm 0.48$   $\mu$ g QEs/mg extract). On the other hand, the antioxidant potential of the extracts was evaluated by DPPH and ABTS radical scavenging and CUPRAC activity methods [5-7]. methanol extract (D) was the most active regarding the DPPH, ABTS (IC<sub>50</sub>:  $141.83 \pm 2.06$   $\mu$ g/mL, IC<sub>50</sub>:  $81.65 \pm 0.48$   $\mu$ g/mL, respectively) and ethyl acetate extract (C) was the most active regarding the CUPRAC assays (A<sub>0.5</sub>:  $62.62 \pm 0.05$   $\mu$ g/mL).

Extracts were not active against acetylcholinesterase enzyme and butyrylcholinesterase compared with galantamine [8]. The ethyl acetate extract (C) was the most active against tyrosinase compared with kojic acid (IC<sub>50</sub>:  $329.07 \pm 2.2$   $\mu$ g/mL) [9]. The ethyl acetate extract (C) was also the most active against  $\alpha$ -glucosidase compared with acarbose (IC<sub>50</sub>:  $1218 \pm 100.4092$   $\mu$ g/mL) [10].

**Key Words:** *Silene sedoides*, enzyme inhibitory activities, antimicrobial activity, antioxidant activity

### **Acknowledgements**

This work was financially supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) with the Project number: 1919B012223591

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## APPLICATION OF MULTIVARIATE REGRESSION ANALYSIS FOR PREDICTION OF ESSENTIAL OIL COMPOSITION FROM 46 DIFFERENT SAGE POPULATIONS

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Three closely related *Salvia* species (*Salvia officinalis* L., *Salvia fruticosa* Mill., and *Salvia 57omifera*) from Lamiaceae are widely recognized for their economic value as medicinal and aromatic plants. These species are rich in essential oils (Eos), which vary in terms of quality, bioactivity, and sensory properties. Therefore we studied the chemical composition of Eos from 46 Sage populations, from 10 countries from Southeast Europa. The yield and chemical composition of the Eos of the analysed samples were previously published by Cvetkovikj et al. 2015<sup>a</sup> [1] and Cvetkovikj et al. 2015<sup>b</sup> [2].

Multivariate regression analysis was applied to assess the impact of independent variables (the climate/temperature where the plant material was grown or harvested, and the type of sage) upon the chemical composition of the EOs.

The resulting Partial Least Square (PLS) regression model consisted of four components. The first component explained 29.5% ( $R^2Y$ ) of variations with predictability ( $Q^2$ ) of 22.9%, the second component ( $R^2Y = 60.3\%$ ) of the variation with predictability of ( $Q^2$ ) of 36.8%, the third component explained 75.3% ( $R^2Y$ ) of variations with a predictability ( $Q^2$ ) of 49.7%, and with the inclusion of fourth component, 81.0% ( $R^2Y$ ) of the variations was explained, with a predictability ( $Q^2$ ) of 53.0%. PLS indicated that climate, as a quantitative variable related to the origin of the raw material was positively correlated with the content of  $\beta$ -pinene and 1,8-cineole in the essential oils, while it was negatively correlated with bornyl acetate and  $\alpha$ -humulene. The statistical analysis confirmed that the content of 1,8-cineole was positively correlated with *S. fruticosa*, and negatively correlated with *S. officinalis*, whereas the bornyl acetate showed the opposite trend. The content of camphene, camphor, bornyl acetate and  $\alpha$ -humulene were positively correlated with *S. officinalis*.

**Key Words:** sage, essential oil, composition, statistical analysis

### Acknowledgements

SEEDNet project and SEE-ERA.NET PLUS Joint Call project: ERA 64/01

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## INSIGHTS FROM UHPLC-ORBITRAP MS ANALYSIS OF *PLANTAGO MAJOR* AS A POTENTIAL HERBAL DRUG IN WOUND HEALING

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*Plantago major* has been used in wound healing since ancient times, with historical references approaching from early medical texts [1]. Exploring lesser-known or unidentified compounds may uncover new bioactive substances with therapeutic benefits and expand this plant species' pharmacological potential.

UHPLC-ORBITRAP MS analysis of the 50% ethanol extract of *Plantago major* identified eighty-three compounds, presenting a diverse array of secondary metabolites. Among these, flavonoid O-glycosides were the most numerous class with 31 compounds. This group was followed by flavonoid aglycones (15), hydroxycinnamic acid derivatives (11), phenylethanoid glycosides (10), hydroxybenzoic acid derivatives (9), iridoid glycosides (2), flavonoid C-glycosides (2) and other metabolites (3). Despite the dominance of flavonoid O-glycosides, the most prominent compounds identified in the extract were phenylethanoid glycosides, particularly plantamajoside, along with the metabolite quinic acid. Another significant phenylethanoid glycoside was verbascoside, which has a chemical structure almost identical to plantamajoside, differing only by one oxygen atom. The most dominant flavonoid O-glycosides included hispidulin 7-O-hexuronide, quercetin 3-O-hexuronide, isorhamnetin 3-O-hexuronide, luteolin 7-O-hexuronide, and the biflavonoid 3',8''-biapigenin, followed by apigenin 7-O-hexuronide, isorhamnetin 3-O-hexoside, patuletin 3-O-hexoside, quercetin 3-O-(2''-acetyl)-rhamnoside, isorhamnetin 3-O-(6''-rhamnosyl)-hexoside, and luteolin 7-O-hexoside. Hispidulin and chrysoeriol stood out as the most dominant compounds in the flavonoid aglycones group.

The presence of these bioactive compounds, underlines the therapeutic potential of *Plantago major*, particularly in the context of wound healing. Their antibacterial, antioxidant and anti-inflammatory activity [1] makes them attractive candidates for further pharmacological studies aimed at developing new wound care treatments.

**Key Words:** *Plantago major*, UHPLC/HRMS, flavonoids, phenolic acids, iridoids

### Acknowledgements

The research was financed by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, Grant No. 451-03-66/2024-03/200007.

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# FULL PAPERS



## **CAPPARIS AND ITS APPLICATIONS IN ANIMAL NUTRITION AND TREATMENT**

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### **Abstract**

The genus *Capparis* (Capparidaceae), commonly known as caper, has long been recognized for its medicinal properties in human health. In recent years, interest has grown in its application within animal nutrition and therapy, due to its bioactive components and potential health benefits. This paper examines the nutritional value of *Capparis* and its therapeutic effects on animal health, focusing on its antioxidant, anti-inflammatory, and immunomodulatory properties. The bioactive compounds in *Capparis*, such as flavonoids, alkaloids, and glucosinolates, contribute to its health-promoting effects. These compounds may help reduce oxidative stress, leading to improved immune function in animals. The anti-inflammatory properties of *Capparis* could offer potential benefits in managing chronic inflammation-related conditions, such as arthritis or other inflammatory diseases in livestock or pets. Moreover, the immunomodulatory effects are noteworthy, as they could enhance the immune response in animals, improving resistance to diseases and supporting overall health. However, challenges remain in incorporating *Capparis* into animal diets, including variability in plant composition, determining optimal dosages, and assessing long-term safety. Despite these challenges, *Capparis* holds promise as a natural supplement in animal nutrition, offering an alternative to synthetic additives. The paper also discusses the opportunities for future research, particularly in optimizing its use as a functional ingredient to improve animal health and performance, paving the way for new advancements in animal care.

**Key Words:** *Capparis*, medicinal use, animal nutrition

### **Introduction**

*Capparis*, a genus of plants that includes species like *Capparis spinosa* (the caper bush), is known for its culinary and medicinal uses (Tlili et al. 2011). The bioactive compounds found in *Capparis*, such as flavonoids, alkaloids, and polyphenols, have made it a candidate for research in animal nutrition. A large amount of polyphenols and flavonoids were reported in the leaves and flowers (Arrar et al. 2013). *C. spinosa* L. have been traditionally used for diet and in folk medicine. The important use of *C. spinosa* L. has been reported in many papers (Mollica et al. 2017, Adwan, 2023, Yildirim et al. 2018). The increasing interest in using natural plant-based supplements for animals has opened up new avenues for exploring how *Capparis* can contribute to animal health and welfare.

### **Nutritional Value of *Capparis***

*Capparis* contains a wide range of nutrients and bioactive compounds that make it valuable in both human and animal diets. The plant is rich in vitamins A, B, C, and E, minerals like calcium, magnesium, and potassium (Öğüt and Er, 2010). High levels of antioxidants such as rutin and quercetin were reported by Tlili et al. (2010). These antioxidants are particularly important in preventing oxidative stress, a common issue in both livestock and companion animals. Including *Capparis* in animal diets may enhance the nutritional profile of feed and support overall animal





health. (Pattanayak et al., 2013).

### **Therapeutic Applications in Animal Health**

#### **Antioxidant Properties**

Oxidative stress occurs when there is an imbalance between free radicals and antioxidants in the body. Animals, especially those under stress from poor diet, environmental factors, or disease, often suffer from oxidative damage, which can impact growth, immune function, and overall vitality (Puppel et al., 2015; Ponnampalam et al., 2022). Studies have demonstrated that the flavonoids and polyphenols in *Capparis* can act as powerful antioxidants, reducing oxidative stress in animals (Turgut et al., 2015; Yu et al., 2017). This makes *Capparis* a potential natural supplement for enhancing health and performance in livestock and pets.

#### **Anti-Inflammatory and Immunomodulatory Effects**

Inflammation is a common response to infections, injuries, or stress. Chronic inflammation can lead to long-term health problems in animals, including reduced productivity in livestock. *Capparis* has been shown to possess anti-inflammatory properties that can help mitigate these effects (Cheniti et al., 2022). The plant's ability to modulate the immune system also suggests potential in improving disease resistance in animals, leading to healthier herds and flocks.

#### **Effects Gut Health and Digestive Benefits**

The digestive system of animals plays a critical role in nutrient absorption and overall health. *Capparis* has been linked to improvements in gut health due to its fiber content and natural bioactive compounds that promote healthy gut microbiota. By maintaining a balanced gut flora, *Capparis* could help in improving digestion and nutrient absorption in animals, contributing to better growth rates and feed efficiency in livestock (Areqi et al., 2021).

#### **Potential for Disease Prevention**

The pharmacological effects of *Capparis* may extend to disease prevention in animals. Some studies have suggested that *Capparis* extracts have antimicrobial and antifungal properties, which could be useful in preventing infections in farm animals (Tagnaout et al., 2016; Adwan et al., 2023). Additionally, its antioxidant and anti-inflammatory properties may contribute to the prevention of chronic diseases in both livestock and pets.

#### **Challenges and Opportunities**

Despite the promising benefits of *Capparis* in animal nutrition and therapy, there are challenges to its widespread adoption. Limited research on dosage, long-term effects, and species-specific responses makes it difficult to create standardized guidelines for its use in animal feed. Furthermore, variability in the concentration of active compounds depending on the plant's origin and processing can affect its efficacy.

However, the growing trend towards natural and plant-based supplements in animal feed presents a significant opportunity for further research and development. *Capparis*, with its diverse range of bioactive compounds, has the potential to be a valuable addition to animal nutrition strategies, particularly in sustainable and organic farming systems.

#### **Conclusion**

*Capparis* holds promise as a natural supplement in animal nutrition and therapy. Its antioxidant, anti-inflammatory, and immune-boosting properties make it a valuable candidate for improving animal health and performance. Further research is needed to establish appropriate dosages and to explore the full potential of *Capparis* in various animal species. With growing interest in plant-based solutions, *Capparis* could play a key role in the future of animal nutrition and therapeutic approaches.



### Acknowledgements

The authors are thankful to the Director of the Medicinal Plants Research Center, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran for providing all the research facilities during this study.

### Conflict of Interest

The authors announce that they have no conflict of interest.

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## **ETHNOMEDICINAL STUDY OF *QUERCUS SP.* FROM THE FOLK AND TRADITIONAL MEDICINE OF REPUBLIC OF MACEDONIA**

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### **Abstract**

This study was carried out concerning ethnomedicine on preparations obtained from oak from the folk and traditional medicine of Republic of Macedonia, an area so far less frequently studied from the perspective of folk traditional medicinal, local food and handicraft and other uses.

The oak bark act astringently, antimicrobial and anti-inflammatory. A water decoction is used against inflammation of intestine mucous membrane at diarrhea, dysentery and catarrh in intestine. Leaves and bark are used against diarrhea. 3% tea of oak bark is beneficial against poisoning with heavy-metal and alkaloids. Tea is administered as a medicine at bleeding in internal organs, uterus and hemorrhoids. 5% decoction is used for healing of wounds, wet eczema, ulcers, hemorrhoids, frostbite, sweating on the legs and mycoses. A water decoction is used at illnesses of mucous in mouth and throat, as gargle. Oak bark helps in blood in the urine, abnormal menstruation, and impotence in urine retention, varicose veins, bleeding gums etc. Oak leaves are used for preparing tea and pulvis from bark in combination with honey against bleeding.

In the period of misery fried acorns is used as a substitution for cafe, and for making bread mixed with other bread flours. Also, acorns are used in the diet of livestock.

Oak bark is used for tanning the skin in the industry. Oak tree is a holy tree where various religious practices take place. Its appearance, as well as his long-lived life and durability, can be regarded as a symbol of force. It is related to the cult of the dead, and also as a tree of the thunderbolt Perun. The district, from the ethnobotanical point of view, shows traces of the influences of the neighboring regions. Some medicinal uses are linked to beliefs or residual forms of magic prescriptions.

**Key Words:** oak, medicinal, food, handicraft, use

### **Introduction**

During the past decade, the Balkans have been the focus of a number of ethnobotanical studies that have revealed the complex relationships of humans and plants at the location of a geographical cross roads where the complex features of their history and the structure of their ethnical and religion meet. Most of the previous studies were mainly aimed at medicinal and edible plants and traditional handicrafts in the countries on the Balkans [1-8] and shed new light on existing ethnological and anthropological data.

Republic of Macedonia has an old ethnomedicine traditions, consisting of many recipes with herbal, animal, and mineral original ingredients. The folk and traditional medicine of Republic of Macedonia pays special attention to disease prevention. This study was carried out concerning ethnomedicine on preparations obtained from *Quercus sp.* from the folk and traditional medicine of Republic of Macedonia, an area so far less frequently studied from the perspective of plant folk traditions. In this study local medicinal uses, local food uses and local handicraft and other uses of oak are described.



### Local names

**Republic of Macedonia:** *Quercus robur* L.= *Q. pedunculata* Ehrh. даб, стежов, стежај, благун, *Quercus petrea* (Matt.) Liebl.= *Q. sessiliflora* Salisb. црн даб, јаглед, црнок; [9][10].

Yörüks: dab [11].

**English:** oak [10].

### Botany and ecology

Oaks are perennial up to 30 meters tall trees with branched and thick crown. *Quercus robur* L. is high above 20 m. The bark of the young stems and branches is bright, plane and gray. Leaves are reversed egg-shaped long up to 20 cm in, wide up to 10 cm, improperly cut with small leaf petiole, lengths up to 1 cm. The fruit is called acorn is green with a thin pedunculate and with bright lines along the length. Acorns are usually alone on the pedunculates or could be found two acorns together. *Quercus petrea* (Matt.) Liebl. is tree high high more than 20 m. Bark of the young stems and branches being smooth naked with gray brownish color. How much more the tree is older, and the bark is more cracked and the color is darker. Leaves are reversed egg-shaped long up to 2.5 cm, improperly cut with small leaf petiole long up to 2.5 cm. Acorns of this oak are smaller than *Quercus robur* L., without pedunculates, and in one place could be found 2-3, rarely alone. *Quercus robur* L. grows in the lowlands, by the river and on the humid places, and *Quercus petrea* (Matt.) Liebl. in the mountainous places at an altitude of up to 1500 m. Parts that are collected is bark (*Quercus cortex*) from the both oaks. Time of gathering is in spring when the primordia starts to open. In Macedonia *Quercus robur* L. grows in the lowlands, by the river and on the humid places, and *Quercus petrea* (Matt.) Liebl. in the mountainous places at an altitude of up to 1500 m [12]. It is not indicating the exact degree of danger of the oaks in Republic of Macedonia. [13].

### Results and discussion

#### Local medicinal uses

The oak bark act astringently, antimicrobial and anti-inflammatory. A water decoction is used against inflammation of intestine mucous membrane at diarrhea, dysentery and catarrh in intestine. Leafs and bark are used against diarrhea [14]. 3% tea of oak bark is beneficial against poisoning with heavy-metal and alkaloids. Tea is administered as a medicine at bleeding in internal organs, uterus and hemorrhoids. 5% decoction is used for healing of wounds, wet eczema, ulcers, hemorrhoids, frostbite, sweating on the legs and mycoses. A water decoction is used at illnesses of mucous in mouth and throat, as gargle [9]. Oak bark helps in blood in the urine, abnormal menstruation, and impotence in urine retention, varicose veins, bleeding gums etc [15]. Oak leaves are be used for preparing tea and pulvis from bark in combination with honey against bleeding [16].

#### Local food uses

In the period of misery in Republic of Macedonia fried acorns are used as a substitution for cafe [17], and for making bread mixed with other bread flours. Also, acorns are used in the diet of livestock [18].

#### Local handicraft and other uses

Oak bark is used for tanning the skin in the industry of Republic of Macedonia [19]. Manufacture of stick (коледашки) for Christmas Eve (коледе, бадник) in Vrazinovski texts are mentioned [20]. Oak twig is used as a badnik (Бадник), the main ritual object by which one of the main customs on Christmas Eve (Бадник) is performed [19]. Oak twig is used on Badnik for stirring the fire as a sign for beginning of the dinner [21,22]. Oak twig with the thickness of a human hand from *Quercus cerris* is used as badnik in the Shopsko-bregalnic ethnographic entity. This badnik was burned after the dinner on Badnik, and the ash is left in a field or vineyard to give



more crops. In the village Pcinja the second part of badnik is kept for the summer as defend against storm clouds [23]. In the village Galicnik the second part of badnik is burned little every day to call the God and the rest is burned on Vodici (19.01). There is a medicinal oak cer [церов даб] in the village of Cumovo and in its rots there is water named as cerium water [церова вода]. People washed themselves with this water before the sun rise to be health [22]. Surova or Vasilica is a holiday that marks the beginning of the Old New Year according to the Julian calendar in Shopsko-bregalnica ethnographic entity. The stick named *surovica* [суровица] or *surovaska* [суроваска] made of oak branch, or of cornel or hazelnut, served as a ritual requisite through which the magical power of the tree was transferred with the hit [23]. In the village Dulica *surovacka* [суровачка] is made from *Quercus cerris* [22]. In Bregalnica's area, on the most important commemoration to the deceased after 40 days from the death, in the places where there is no active church, oak twig with leaves is carried on the grave. After that this oak twig is carried to the closest church to pass the ceremony with the priest [24]. Ritual acts with oak twigs named as *dubica*, *dabica* are carried out with the assurance that they will bring health to the participants. Such practices are connected with some holidays, mainly with Gjurgjovden (Ѓурѓовден) Saint George's Day because oak give birth to acorns [22].

### **Folk symbol**

Oak tree is a holy tree where various religious practices take place. Its appearance, as well as its long-lived life and durability, can be regarded as a symbol of force. It is related to the cult of the dead, and also as a tree of the thunderbolt Perun. Under it, villagers pray for rain, leaving various gifts. On St Georges day (Ѓурѓовден) in the area of Delchevo under the oak trees' rituals connected with butchering the oxen and rams were performed [25]. On St Georges day (Ѓурѓовден) under the oak tree named as "Gjurgjov" oak Ѓурѓов даб in the village Virce, lambs are butchered for kurban, candles are burned, and icons are put on. Oak is presented with soaks, shirts etc and after that the presents are taken to the church. In the village Sasa on the oak tree cross is made and people light candles on it [22]. Many places where the oak grows are known as cult places, and the oaks are sacred. These oaks have a medicinal power and are in a position to induce a rain with the aid of various rituals [25].

### **Folk belief (folk story)**

It is believed that oak trees in "vakafski" reon вакафски реон must not be cut. Those who are trying to do so can hardly get sick and even die [25]. Also, the fallen oaks of old age are not touch by anyone. Oak trees are also fairy trees. Such is the oak tree in the village Brostica, which poses healing properties [20]. People put three cookies under this fairy oak and depending from the cookie eaten by fairy, people have to go in church or mosque to be healed [22]. It is believed that on some oak trees, so-called cross eagles fight with dragons which are carrying storm [20]. Beliefs still exist about fairy oaks [25]. There are holy oaks in the villages Rusa, where people go to crack branches for healing; than saying how the branch grows up like that, my health to races. In the "vakafski" reon вакафски реон behind the village of Veselchani nobody is allowed to cut the "vakavski" oaks вакавски дабје. Also, holy oak exists in the village Radozda named as "krstaten" oak крстатен даб. People belief that if they put food under the holy oak fairy will come to eat and they will be healed if they are mentally ill. If someone cuts it or takes a branch from it he will get sick [22].

### **Conclusion**

The usage of preparations obtained from *Quercus sp.* from the folk and traditional medicine of Republic of Macedonia, from the ethnobotanical point of view, shows traces of the influences of the previous nations who lived in this area. Some medicinal uses are linked to beliefs or residual forms of magic prescriptions. The use of oaks remains well alive nowadays.



### Conflict of Interest

The authors declare no conflict of interest.

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## **ST. JOHN'S WORT: FROM AN ANCIENT PROTECTOR TO A MODERN ANTIDEPRESSANT**

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### **Abstract**

St. John's wort (*Hypericum perforatum* L., fam. Hypericaceae) is a perennial plant native to Eurasia, known for its traditional use in treating various ailments. Its medicinal uses have been documented by eminent herbalists such as Hippocrates, Pliny, Dioscorides, Galen, and Paracelsus. Since antiquity, the plant has been commonly referred to as "Fuga daemonum" and it was believed to guard against evil spirits and helped those who suffered from mental illnesses. In today's modern society, depression is the most common mental disorder worldwide, and its increasing prevalence is alarming. There are various classes of conventional antidepressants that can be prescribed to patients, however due to their numerous side effects, patients often struggle to adhere to the treatment. *Hypericum* extracts have been extensively studied for the past four decades for the treatment of mild to moderate depression. Clinical evidence has shown that *Hypericum* extracts may be as effective as conventional antidepressants in the treatment of mild to moderate depression, with lower incidence of side effects and a superior tolerability profile, however more prospective studies are required to fully understand their long-term efficacy and safety. Additionally, *Hypericum* extracts interact with a large number of drugs due to the induction of isoenzymes of the cytochrome P450 system and P-glycoprotein, which can potentially lead to life-threatening conditions such as serotonin syndrome. Therefore, healthcare professionals play a critical role in managing the use of St. John's wort by carefully monitoring for any side effects or drug interactions, providing counselling, and collaborating with other professionals to optimize patient safety and therapeutic outcomes.

**Keywords:** St. John's wort, traditional use, treatment for depression, herb-drug interactions

### **Introduction**

St. John's wort (*Hypericum perforatum* L., fam. Hypericaceae) is a perennial plant that grows along roadsides and in meadows up to 100 cm in height, and it is originally native to the temperate regions of Eurasia. It is characterized by golden yellow flowers with a characteristic balsamic fragrance, stems that are often reddish, and yellow-green leaves with translucent dots that create the perforated appearance when held up to the light (hence the species name- "perforatum") (Kenda et al., 2022). The therapeutic properties are mainly attributed to the aerial parts of St. John's wort. The plant contains numerous bioactive compounds of which the following are significant - phloroglucinol derivatives (e.g., hyperforin), flavonoids (e.g., quercetin), procyanidins, naphthodianthrons (e.g., hypericin), xanthenes, phenolic acids (e.g., ferulic acid), essential oils (Patočka, 2003).

St. John's wort is one of the most studied plants until today and its medicinal properties have been documented since ancient times by eminent medical herbalists such as Hippocrates, Pliny, Dioscorides and Galen. They noted its effectiveness in healing wounds, treating snakebites, expelling intestinal parasites, soothing menstrual cramps and documented its antimalarial and diuretic effects. Paracelsus also referred to St. John's wort as the "arnica of the nerves" and recommended its use for melancholy and depression (Klemow et al., 2011; Butterweck, 2003;



Bilia et al., 2002).

Throughout history, mental disorders were often explained as manifestations of evil spirits or supernatural forces. Since antiquity, the plant has been commonly referred to as “Fuga daemonum” and it was believed to guard against evil spirits and helped those who suffered from mental illnesses. (Istikoglou et al., 2010; Mierzwińska-Hajnos, 2017). Today, in our modern society, depression is the most common mental disorder and it is very concerning that the number of people affected by this disorder is constantly increasing. Symptoms of depression can vary from mild to severe and may include intense sadness, feelings of hopelessness, loss of interest in activities that once brought joy, fatigue, digestive problems and appetite changes, low libido, insomnia, irritability, reduced ability to concentrate, and even suicidal ideation (Benitez et al., 2022, National Institute of Mental Health, 2021). Various classes of conventional antidepressants are available for the treatment of depression, including selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), noradrenaline and dopamine reuptake inhibitors (NDRIs), serotonin modulators, and atypical antidepressants (Sheffler & Abdijadid, 2021). However, patients often struggle to adhere to conventional antidepressants due to the risk of numerous side effects (Kenda et al., 2022). Since the early 1980s, St. John’s wort extracts have been studied extensively for their antidepressant effects. Clinical evidence has shown that Hypericum extracts may be as effective as conventional antidepressants in the treatment of mild to moderate depression, with lower incidence of side effects and a superior tolerability profile (Patočka, 2003). However, individuals should avoid self-treatment, as using Hypericum extracts without medical guidance increases the risk of potentially dangerous drug interactions (Henderson et al., 2002).

### **The historical journey of *Hypericum perforatum* in treating mental disorders**

In traditional Chinese medicine (TCM) depression itself is not defined as a disease, but it is manifested through five diseases (hysteria, lily disease, dementia, amnesia and insomnia) with similar clinical symptoms that cause Qi stagnation. The Chinese Pharmacopoeia documents nine herbal medicines, including *Hypericum perforatum*, with “dispel melancholy functions” that aim to restore and balance the Qi energy in depressed patients (Hu et al., 2021).

Apart from its use in traditional Chinese medicine, in ancient Greek times and through the Middle Ages, St. John’s wort was considered to possess magical powers. It was commonly referred to as “Fuga daemonum”, because it was believed to protect against evil spirits and illnesses. In fact, “Yperikon” was the Latin name for the plant, deriving from the words *hyper* (above) and *ikon* (image) and it was placed over religious icons to guard against evil spirits and to tame hallucinations (Hobbs, 1997). This belief led to practices like bringing its flowers into the home for protection and good luck. The term “Fuga daemonum” was also found in myths and legends concerning treatments of mental disorders, which in fact aligns with the modern use of St. John’s wort today in the treatment of depression and other mental disorders (Hobbs, 1990; Mierzwińska-Hajnos, 2017). In the early 1500s Paracelsus stated “Nothing chases away disease like strength. Therefore, we should seek medicines with power and strength to overcome whatever illnesses they are used against. From this it follows that God has given to Peforatum (St. John’s wort) the strength to chase (away) the ghosts of nature... and all downheartedness” (Chevallier, 1999, p.33).

In the 19<sup>th</sup> and 20<sup>th</sup> centuries, St. John’s wort was widely prescribed for nervous disorders like depression, mania, anxiety and fatigue. In Germany, many doctors preferred St. John’s wort over conventional antidepressants, leading to further investigation of the plant’s effects by the German Commission E, which was formed in 1978. A few years later, the German Commission E confirmed





St. John's wort effectiveness in the treatment of mild to moderate depression, resulting in widespread use with almost three million doses prescribed in 1994 in Germany (Lee, 1999). Hypericum extracts have been licensed in Germany for depression treatment in adolescents since 1984 and today they still remain a popular treatment option, while in the USA they are not FDA approved and are considered as a dietary supplement. Despite being classified as a dietary supplement, there are studies that prove the effectiveness of St. John's wort in treating mild to moderate depression with a significantly lower frequency of side effects compared to conventional antidepressants. However, further research is necessary to understand its long-term use and safety (Benitez et al., 2022).

### **Antidepressant mechanism of St. John's wort**

Hypericin was the first naphthodianthrone isolated from St. John's wort in 1830 by the German chemist Buchner. However, more than a century later, in the 1980s scientists began to focus on discovering the compounds and mechanisms of action that were responsible for the antidepressant effect (Chevallier, 1999). The inhibition of the enzyme monoamine oxidase (MAO) by hypericin was initially considered as the basic mechanism of the antidepressant action of St. John's wort, which was reported by Suzuki *et al.* back in 1984, leading to the standardization of Hypericum extracts to this compound (Nathan, 2001). However, it was later found that Hypericum extract has high potency for reuptake inhibition of monoamine neurotransmitters- serotonin, dopamine, norepinephrine, like the conventional antidepressants, but it also inhibits the reuptake of amino-acid neurotransmitters gamma-aminobutyric acid (GABA) and L-glutamate. This effect was shown to be primarily due to the phloroglucinol derivative hyperforin (Zanoli, 2004). While conventional antidepressants inhibit neurotransmitter transporters, hyperforin activates the non-selective cation channel Transient Receptor Potential Cation Channel Subfamily C Member 6 (TRPC6). This activation increases intracellular sodium [Na<sup>+</sup>] concentration, resulting in reduced activity of the neurotransmitter transport system (Leuner et al., 2007). While hyperforin has been thought to be the main component responsible for this effect, extracts that were low in hyperforin, as well as those without hyperforin, still had some capacity to inhibit the reuptake of neurotransmitters. It was found that other compounds in the extract, such as procyanidins, may also play a role in this mechanism.

Additionally, chronic treatment with St. John's wort extracts leads to the upregulation of serotonin receptors (5-HT<sub>1</sub> and 5-HT<sub>2</sub>) and reduced expression of beta-1 adrenergic receptors (Nathan, 2001). Elevated levels of corticotropin-releasing hormone (CRH) and cortisol have also been found in depressed patients. *Hypericum* extracts have been shown to inhibit cytokine production (IL-1, IL-6, and TNF- $\alpha$ ), thus resulting in decreased levels of corticotropin-releasing hormone (CRH) and reduced cortisol production (Chevallier, 1999).

### **Clinical efficacy, safety and considerations**

The first trials evaluating St. John's wort antidepressant effects in patient volunteers were conducted between 1979 and 1989, when it was combined with other calming herbs like valerian. The results showed that the combination was superior compared to placebo (Hobbs, 1997). In August 1996, a meta-analysis of 23 randomized trials conducted by Linde et al., which used Hypericum extract in patients with mild to moderate depression, was published in the renowned British Medical Journal and gained global scientific attention. The meta-analysis showed that *Hypericum* extracts were significantly superior to placebo and similarly effective when compared to conventional antidepressants (Linde et al., 1996). A meta-analysis by Rahimi et al. in 2009, which included 13 randomized double-blind clinical studies in relation to SSRIs (6 studies in relation to placebo), showed that *Hypericum* extracts have comparable efficacy to SSRIs (Rahimi et al., 2009). Another meta-analysis concluded that St. John's wort not only matches SSRIs in effectiveness, but also has less side effects than SSRIs, indicating superior safety compared to



conventional antidepressants (Cui & Zheng, 2016). Similar conclusions were presented in a more recent meta-analysis published in 2023 by Xin Zhao et al. (Zhao et al., 2023). Additionally, St. John's wort has also been shown to be effective in managing depression in postmenopausal women and improving emotional and behavioral complications in children (Eatemadnia et al., 2019; Peterson & Nguyen, 2023).

Although *Hypericum* extracts are generally considered effective and have a lower incidence of side effects compared to conventional antidepressants, more prospective studies are required to fully understand their long-term efficacy and safety for treating mild to moderate depression in comparison to other antidepressant treatments. Additionally, further research is needed to compare different extracts and doses with conventional antidepressants in well-defined patient groups (Benitez et al., 2022; Linde et al., 1996).

Side effects of St. John's wort are generally mild and temporary and may include gastrointestinal complaints (constipation, flatulence, diarrhea, stomach pains), allergic reactions such as pruritus, fatigue, agitation, dry mouth or photosensitization (hypericium) (Ernst et al., 1998).

While St. John's wort has a generally positive safety profile when used as a monotherapy, it can interact with many drugs because it induces the activity of several CYP450 enzymes (CYP3A4, CYP2D6 and CYP2C19) and the P-glycoprotein. These include drugs such as immunosuppressants, antidepressants, anticonvulsants, HIV protease inhibitors, HIV non-nucleoside reverse transcriptase inhibitors, warfarin, digoxin, oral contraceptives, muscle relaxants, and others. Some of the interactions can be dangerous including serotonin syndrome, a potentially life-threatening drug reaction that can occur when St. John's wort is used along with serotonin reuptake inhibitors or with 5-HT receptor agonists. Healthcare professionals must be aware of these interactions to prevent potential complications and to ensure safe use of St. John's wort (Borrelli & Izzo, 2009).

### **Conclusion**

St. John's wort is a fascinating example of how traditional wisdom can evolve into a scientifically proven antidepressant in today's modern society. What was once considered as an ancient herbal protector against mental illnesses, is now recognized for its therapeutic efficacy in the treatment of mild to moderate depression, with fewer side effects and better tolerability profile compared to conventional antidepressants. However, further research is essential to establish the long-term efficacy and safety of *Hypericum* extracts.

### **Conflict of Interest**

The authors declare no conflict of interest.

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## INVESTIGATION INTO ESSENTIAL OIL COMPOUNDS OF SOME CONIFEROUS SPECIES GROWING IN TÜRKİYE

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### **Abstract**

Coniferous grow almost everywhere in the world because they are resistant to different climatic conditions. Although there are many species in the world, there are seven different species and their subspecies that grow naturally in Türkiye. Due to its durable, hard, strong wood, it is used in the furniture, construction, shipbuilding, paper and packaging sectors both in the world and in Türkiye. A few species, such as some juniper species, are used in industry as medicinal and aromatic. On the other hand, cones and needles are used in the treatment of various diseases among people. In this study, cones and needles samples of six different coniferous species and some of their subspecies (*Juniperus excelsa*, *Juniperus oxycedrus*, *Cupressus semp. Pyramidalis*, *Cedrus libani*, *Pinus brutia*, *Pinus nigra*, and *Pinus pinea*) collected from different regions of Türkiye were used. Subsequently, essential oils of the samples were obtained by hydrodistillation method and volatile component analysis was performed in the GC-MS device. When the volatile compounds results were examined, it was detected that main compound  $\alpha$ -pinene in *Juniperus excelsa* and *Cupressus semp. pyramidalis*,  $\beta$ -myrcene in *Juniperus oxycedrus*,  $\beta$ -phellandrene in *Cedrus libani*,  $\delta$ -3-carene in *Pinus brutia*, Germacrene-D in *Pinus nigra* and limonene in *Pinus pinea*. In this study, the volatile components of cones and needles of coniferous species in Türkiye were compared. Moreover, the main components of the cones and needles of these species, which are used for folk medicine, have been determined and it is aimed to pioneer the use of these species for biological studies.

**Key Words:** Coniferous, essential oil, GC-MS, hydrodistillation.

### **Introduction**

Coniferous, sometimes called evergreen trees, have a woody and most species are tree-shaped [1]. They have cones with bract scales and their leaves are needle-shaped veined [2]. These gymnosperms contain 7 families, 70 genera and more than 600 species [3]. Although they spread widely northern hemisphere, some conifers grow in the southern hemisphere because of resistant to different climatic conditions [4]. Also in Türkiye, 7 species and their subspecies grow naturally.

It is known that in folk medicine different parts of conifers have been utilized in the treatment of many diseases, although they are mostly used in furniture, lumber, paper and construction. For example; some parts of *Cupressus* spp. as leaves, cones and bark are used in the treatment of diseases such as asthma, common cold, hemorrhoids, diarrhea, and rheumatism. Also, it has been determined that the extracts of some *Juniperus* species cure diseases such as eczema, tuberculosis and bronchitis [5].

Essential oils are important herbal products with economic value used in the food, cosmetic and pharmaceutical industries. On the other hand, they have protective properties of plants against fungi and insects. The volatile oils of conifers have an important place compared to other tree species. They have a unique essential oil composition and are not affected by the environment [6]. It is known that the essential oils of different parts of them are used as ointments, inhalers, and medicinal oils in the treatment of some diseases. Moreover, in the literature, there are a lot of studies on antioxidant, antimicrobial, antibacterial, larvicidal, anti-fungal, herbicidal, anti-inflammatory, and free radical scavenging activity of these oils [7-14]. The aim of this study is to collectively identify the components of these coniferous volatile oils which are medicinal and cosmetic importance and to compare the variation between species.

## Material and Methods

### Plant Materials

In this study needles and cones of *Juniperus excelsa*, *Juniperus oxycedrus*, *Cupressus semp. pyramidalis*, *Cedrus libani*, *Pinus brutia*, *Pinus nigra*, and *Pinus pinea* are used as samples (Table 1).

**Table 1.** Sampling site of tree species

Species	Sampling site
<i>Juniperus excelsa</i>	Silifke, Mersin
<i>Juniperus oxycedrus</i>	Karabük
<i>Cupressus semp. Pyramidalis</i>	Antalya
<i>Cedrus libani</i>	Adana
<i>Pinus brutia</i>	Bartın
<i>Pinus nigra</i>	Bartın
<i>Pinus pinea</i>	Bartın

### Hydrodistillation

The essential oils of the needles and cones of samples were obtained by hydrodistillation using a Clevenger apparatus (ILDAM CAM Ltd. Ankara-Türkiye). 1000 g each of fresh samples were used and the oils were collected 3-4 h. The samples were dried with anhydrous sodium sulphate in a sealed vial until analyses.

### GC-MS Analysis

Analysis of the EO sample was performed on a Shimadzu GCMS-QP2010 instrument equipped with a Teknokroma 5MS (30 m × 0.25 mm, film thickness 0.25 µm). Helium was used as the carrier gas at 1.0 mL/min flow rate. The column oven temperature was programmed starting from 60 °C and after 5 min of hold time the temperature program was continued at 3 °C/min to 280 °C. The split-injector and MS-transfer line were 260 °C and 280 °C, respectively. The MSD was operated in electron impact ionization mode at 70 eV electron energy. Samples were injected by splitting, and the split ratio was 1:10 [15]. Compound identifications were based on mass spectra, referring to NIST147, WILEY and FFNSC mass spectral libraries, and also comparing measured retention index (RI) values of components with literature data [16]. The quantitative area-percent measurements were based on peak-areas from the GC-MS data.

### Results and Discussion

The essential oil compounds of needle of samples are shown in Table 2.



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**Table 2.** The composition of the essential oils of samples' needle (%)

Nr	RRI	r.t.	Compound	J.E.	J.O.	C.L.	C.S.P.	P.P.	P.N.	P.B.
1	917	8.843	Tricyclene	0,45	0,44	0,26	0,27	-	0,25	0,04
2	924	9.111	$\alpha$ -Thujene	-	-	0,12	0,36	-	0,09	-
3	933	9.331	a-Pinene	13,41	10,17	8,01	11,52	6,84	12,97	12,58
4	942	9.842	a-Fenchene	-	0,33	-	-	-	-	-
5	944	9.864	Camphene	1,36	0,77	1,83	-	0,16	1,82	0,66
6	950	10.107	Verbenene	0,14	0,14	0,04	-	-	0,07	-
7	970	10.923	Sabinene	-	-	-	0,26	-	-	-
8	972	10.989	$\beta$ -pinene	2,45	2,21	10,15	3,12	2,01	11,58	12,61
9	990	11.694	$\beta$ -Myrcene	4,65	4,07	12,75	4,75	3,62	2,50	2,14
10	1001	12.138	a-Phellandrene	0,21	0,27	0,44	0,08	1,19	0,15	0,05
12	1007	12.391	d-3-Carene	3,12	1,96	0,77	18,53	0,26	0,11	1,83
13	1014	12.633	a-Terpinene	0,26	0,38	0,38	0,34	0,45	0,14	0,11
14	1021	12.945	<i>p</i> -Cymene	0,67	0,72	0,76	0,09	-	0,10	0,04
15	1026	13.134	Limonene	5,60	5,15	24,83	4,18	53,78	5,13	6,29
16	1028	13.217	Eucalyptol (1,8-cineole)	-	-	-	-	-	-	0,04
17	1037	13.580	cis-Ocimene	-	-	-	-	-	-	0,16
18	1048	13.984	$\beta$ -Ocimene <(E)>	-	-	-	0,07	0,13	1,11	0,44
19	1056	14.340	$\gamma$ -Terpinene	1,68	1,84	0,55	0,61	0,28	0,19	0,17
20	1072	14.958	Capryl alcohol	-	-	0,20	-	-	-	-
21	1085	15.513	a-Terpinolene	1,96	1,74	3,08	9,64	1,15	0,64	1,21
22	1098	16.029	Linalool	-	-	-	-	0,07	0,25	0,15
23	1103	16.240	Pelargonaldehyde	-	-	0,11	-	0,10	-	-
24	1107	16.438	1,3,8- <i>p</i> -Menthatriene	-	-	-	-	0,09	-	-
25	1110	16.480	D-Fenchyl alcohol	0,09	0,09	-	-	-	-	0,19
36	1118	16.830	trans-Sabinene hydrate	-	0,10	-	-	0,09	-	0,06
27	1123	17.002	a-Campholene aldehyde	0,13	0,15	-	-	-	-	-
28	1135	17.449	Trans Pinocarveol	0,11	0,17	-	-	0,06	0,08	-
29	1138	17.583	Verbenol	0,10	0,10	-	-	-	-	-
30	1140	17.668	Camphor	0,19	0,25	-	-	-	-	-
31	1142	17.733	Z-thujenol	-	0,27	-	-	-	-	-
32	1143	17.741	cis- $\beta$ - Terpineol	-	-	-	-	0,13	-	-
33	1162	18.517	Borneol	0,07	0,06	-	-	-	0,13	0,13
34	1164	18.578	<i>p</i> -Mentha-1,5-dien-8-ol	0,19	0,18	-	-	-	0,17	-
35	1171	18.824	cis Pinocamphone	0,15	0,19	-	-	-	-	-
36	1174	18.966	Terpinen-4-ol	0,10	0,23	0,51	0,58	0,33	-	0,20
37	1183	19.277	<i>p</i> -Cymen-8-ol	-	0,06	0,05	-	-	-	-
38	1185	19.400	trans- <i>p</i> -Mentha-1(7),8-dien-2-ol	-	-	-	-	0,09	-	-
39	1188	19.490	$\alpha$ -terpineol	0,41	0,40	0,58	1,23	1,33	1,67	4,12
40	1192	19.678	cis-dihydro-Carveol	-	-	-	-	0,15	-	-
41	1193	19.704	Myrtenol	0,14	0,10	0,08	-	-	0,12	-



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42	1194	19.736	cis-dihydrocarvone	-	-	-	-	0,11	-	-
43	1206	20.204	Verbenone	0,20	0,16	-	-	-	-	-
44	1216	20.558	trans-Carveol	-	-	-	-	0,48	-	-
45	1219	20.643	Endo-Fenchyl acetate	0,12	0,12	-	-	-	-	-
46	1228	20.992	cis-Carveol	-	-	-	-	0,22	-	-
47	1241	21.471	Carvone	-	-	-	-	0,56	-	-
48	1242	21.507	Carvacrol methyl ether	0,08	0,09	0,12	0,34	-	-	-
49	1252	21.872	Piperitone	-	-	-	-	0,12	-	-
50	1255	21.975	Linalyl acetate	-	-	-	-	-	-	0,15
51	1272	22.592	Decyl alcohol	-	-	0,09	-	-	-	-
52	1284	23.041	Bornyl acetate	0,85	0,83	0,14	0,16	0,29	0,70	0,30
53	1296	23.496	$\alpha$ -Terpinyl formate	-	-	-	0,43	-	-	-
54	1298	23.545	trans Pino-carveylacetate,	-	-	-	-	-	0,11	-
55	1312	24.051	2,4-Decadien-1-ol	0,18	-	-	-	-	-	-
56	1337	24.904	$\delta$ -Elemene	-	-	-	-	0,16	-	-
57	1349	25.286	$\alpha$ -Terpinyl acetate	-	-	-	4,57	-	4,98	3,32
58	1349	25.330	$\alpha$ -Longipinene	-	-	0,21	-	0,44	-	-
59	1371	26.059	$\alpha$ -Ylangene	-	-	-	-	-	0,14	-
60	1375	26.208	$\alpha$ -Copaene	-	0,09	0,15	-	-	0,31	0,10
61	1383	26.487	Geranyl acetate	-	-	0,16	-	0,12	-	-
62	1384	26.508	$\beta$ -Bourbonene	-	-	-	-	-	0,29	0,56
63	1385	26.538	$\alpha$ -Duprezianene	0,08	0,11	-	-	-	-	-
64	1387	26.700	$\beta$ -Cubebene	-	-	-	-	-	-	0,08
65	1389	26.727	7-epi-Sesquithujene	0,09	0,14	-	-	-	-	-
66	1391	26.765	$\beta$ -Elemene	-	3,08	0,07	-	-	-	0,19
67	1402	27.155	Methyl Eugenol	-	-	-	-	-	0,11	-
68	1404	27.205	Junipene	-	-	0,42	-	1,37	-	0,41
69	1412	27.475	$\beta$ -Funebrene	2,12	-	-	0,17	-	-	-
70	1417	27.688	trans- $\beta$ -Caryophyllene	-	-	3,82	1,02	3,93	14,60	11,92
71	1419	27.714	$\beta$ -Cedrene	0,69	1,04	-	-	-	-	-
72	1430	28.062	Cis Thujopsene	0,52	0,78	-	-	-	-	-
73	1439	28.338	$\alpha$ -Guaiene	-	-	-	-	0,08	-	-
74	1449	28.674	Alloaromadendrene	-	-	-	-	0,19	-	-
75	1453	28.805	$\alpha$ -Humulene	0,21	0,36	0,90	1,84	0,90	2,35	2,38
76	1456	28.903	$\beta$ -Farnesene (E)	0,17	0,18	-	-	-	0,11	-
77	1472	29.468	Lauryl alcohol	-	-	0,48	-	0,07	-	-
78	1473	29.486	10 $\beta$ -Cadina-1(6),4- diene	-	0,61	-	0,34	-	0,09	-
79	1476	29.608	$\alpha$ -Amorphene	0,09	0,18	0,36	0,47	0,15	2,74	0,92
80	1481	29.708	Germacrene-D	1,56	1,75	4,28	1,88	3,42	21,15	27,77
81	1486	29.898	$\beta$ -Selinene	-	-	-	-	0,64	0,12	-
82	1490	30.006	Phenethyl Isovalerate	-	-	-	-	-	0,32	0,69
83	1495	30.182	$\alpha$ -Chamigrene	0,25	0,46	-	-	-	-	-
84	1499	30.328	$\alpha$ -Muurolene	-	0,50	0,33	-	0,10	0,82	0,52



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85	1508	30.601	$\beta$ -Bisabolene	0,13	0,18	-	-	-	-	-
86	1523	30.764	$\gamma$ -Cadinene	-	-	0,26	-	0,21	1,87	0,72
87	1516	30.850	Bornyl isovalerate	-	-	-	-	-	-	-
88	1524	31.060	$\delta$ -Cadinene	0,68	1,35	0,85	1,27	0,41	4,92	2,15
89	1531	31.304	12-Hydroxy-dodecanoic acid, (lactone)	-	-	0,32	-	-	-	-
90	1532	31.333	Cadina-1,4-diene	-	-	-	-	-	0,17	0,07
91	1543	31.668	cis- $\alpha$ -bisabolene	-	-	1,76	-	-	-	-
92	1549	31.870	$\alpha$ -Elemol	-	-	-	-	0,06	-	-
93	1564	32.330	Dodecanoic acid	-	-	-	-	0,29	-	-
94	1584	32.915	Caryophyllene oxide	-	-	0,43	0,15	1,05	0,15	-
95	1590	33.153	Viridiflorol	3,08	4,16	-	-	-	-	-
96	1593	33.179	Diethyl Phthalate	1,36	1,01	0,59	0,85	1,06	1,03	0,96
97	1598	33.371	Guaiol	-	-	-	-	2,61	-	-
98	1605	33.480	$\alpha$ -Cedrol	38,72	40,48	-	7,38	-	-	-
99	1629	34.298	diepi- $\alpha$ -cedren	-	1,26	-	-	-	-	-
100	1631	34.342	$\alpha$ -Acorenol	-	-	-	0,12	-	-	-
101	1633	34.396	$\gamma$ -Eudesmol	-	-	-	-	0,49	-	-
102	1635	34.471	$\alpha$ -Acorenol	0,27	0,35	-	-	-	-	-
103	1642	34.675	$\alpha$ -Muurolol	0,26	-	1,15	1,87	-	-	-
104	1652	34.947	$\beta$ -Eudesmol	-	-	-	-	0,27	-	-
105	1656	35.070	$\alpha$ -Cadinol	-	-	1,72	-	-	-	-
106	1730	37.209	2-hydroxy Cyclopentadecanone,	-	-	0,24	-	-	-	-
107	1966	43.009	Sandaracopimaradiene	0,29	-	-	-	-	-	-
108	1995	44.255	Kaur-16-ene (podocarpene)	0,09	-	-	3,76	-	-	-
109	2059	45.789	dehydroabietane	-	-	2,17	-	-	-	-
110	2086	46.436	abietadiene	4,00	-	-	-	-	-	-
TOTAL				93,73	91,81	86,52	82,25	92,11	96,35	96,43

**J.E.:** *Juniperus excelsa*, **J.O.:** *Juniperus oxycedrus*, **C.L.:** *Cedrus libani*, **C.S.P.:** *Cupressus semp. Pyramidalis*, **P.P.:** *Pinus pinea*, **P.N.:** *Pinus nigra*, **P.B.:** *Pinus brutia*

According to Table 2, it's shown that 49 components in *Juniperus excelsa*, 52 components in *Juniperus oxycedrus*, 44 components in *Cedrus libani*, 33 compounds in *Cupressus semp. Pyramidalis*, 49 components in *Pinus pinea*, 42 components in *Pinus nigra*, 39 components in *Pinus brutia* were determined. On the other hand, main components of needle samples have been identified as  $\alpha$ -Pinene (13,41%) and  $\alpha$ -Cedrol (38,72%) in *Juniperus excelsa* and *Juniperus oxycedrus*;  $\beta$ -pinene (10,15%),  $\beta$ -myrcene (12,75%) and limonene (24,83%) in *Cedrus libani*;  $\alpha$ -Pinene (11,52%),  $\delta$ -3-Carene (18,53%) and  $\alpha$ -Terpinolene (9,64%) in *Cupressus semp. Pyramidalis*; limonene (53,78%) in *Pinus pinea*;  $\alpha$ -pinene (12,97%),  $\beta$ -pinene (11,58%), trans- $\beta$ -caryophyllene (14,60%) and germacrene-D (21,15%) in *Pinus nigra* and *Pinus brutia*. The essential oil compounds of cones of samples are shown in Table 3.





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**Table 3.** The composition of the essential oils of samples' cones (%)

Nr	RRI	r.t.	Compound	J.E.	J.O.	C.L.	C.S.P.	P.P.	P.N.	P.B.
1	917	8.843	Tricyclene	1,58	1,33	0,22	0,38	-	0,24	0,12
2	924	9.111	$\alpha$ -Thujene	0,62	0,61	0,53	0,96	-	-	0,21
3	933	9.331	a-Pinene	30,17	32,89	15,78	39,76	13,97	15,53	15,32
4	942	9.842	a-Fenchene	-	-	-	1,76	-	-	-
5	944	9.864	Camphene	2,71	2,52	2,23	-	0,28	3,73	1,48
6	950	10.107	Verbenene	0,27	0,22	0,18	0,04	0,12	0,76	0,18
7	970	10.923	Sabinene	-	-	-	4,10	-	-	-
8	972	10.989	$\beta$ -pinene	7,50	7,99	17,71	2,76	0,09	6,26	14,61
9	990	11.694	$\beta$ -Myrcene	13,36	11,23	14,85	4,55	2,23	1,02	2,92
10	1001	12.138	a-Phellandrene	0,20	0,19	0,67	0,07	1,59	-	0,15
12	1002	12.140	1,3,8-para-menthatriene	-	-	-	-	-	0,40	-
13	1007	12.391	d-3-Carene	3,70	7,73	1,34	18,22	-	-	23,26
14	1014	12.633	a-Terpinene	0,58	0,54	0,74	0,37	-	0,09	0,23
15	1021	12.945	<i>p</i> -Cymene	0,39	0,42	0,53	0,05	-	0,54	0,80
16	1022	12.996	Trans-Ocimene	-	-	-	0,52	-	-	-
17	1026	13.134	Limonene	5,80	5,39	-	2,78	56,03	11,22	16,30
18	1027	13.139	$\beta$ -Phellandrene	-	-	18,04	-	-	-	-
19	1028	13.217	Eucalyptol (1,8-cineole)	0,05	0,04	0,07	-	-	-	-
20	1048	13.984	$\beta$ -Ocimene <(E)>	-	-	-	0,11	-	-	0,06
21	1056	14.340	$\gamma$ -Terpinene	4,51	3,70	1,13	0,64	-	-	0,40
22	1072	14.958	Capryl alcohol	-	-	-	-	-	-	-
23	1085	15.513	a-Terpinolene	8,80	6,18	3,44	5,87	-	1,12	2,82
24	1086	15.544	<i>a,p</i> -Dimethylstyrene	-	-	-	-	0,32	-	-
25	1098	16.029	Linalool	-	-	0,15	-	0,14	-	-
36	1110	16.480	D-Fenchyl alcohol	0,31	0,24	0,30	-	-	0,20	0,09
27	1114	16.646	a-Thujone	-	-	0,05	-	-	-	-
28	1118	16.830	trans-Sabinene hydrate	-	0,09	0,19	-	-	-	-
29	1123	17.002	a-Campholene aldehyde	0,17	0,21	-	-	0,16	0,93	0,10
30	1135	17.449	Trans Pinocarveol	0,25	0,59	0,45	0,04	0,50	2,60	1,77
31	1138	17.583	Verbenol	0,10	0,09	-	-	0,37	-	-
32	1140	17.668	Camphor	0,64	1,13	0,07	-	-	0,39	-
33	1142	17.733	Z-thujenol	0,32	0,26	0,07	-	-	0,42	-
34	1143	17.741	cis- $\beta$ - Terpineol	-	-	-	-	-	-	-
35	1145	17.825	Camphene hydrate	0,11	-	0,22	-	-	-	-
36	1157	18.304	trans pinocamphone	-	0,05	-	-	-	-	0,75
37	1159	18.389	Pinocarvone	-	0,07	0,12	-	0,10	0,38	0,16
38	1162	18.517	Borneol	0,56	0,37	0,48	0,13	0,10	1,23	0,16
39	1164	18.578	<i>p</i> -Mentha-1,5-dien-8-ol	0,21	0,22	0,14	0,04	0,25	1,93	0,44
40	1168	18.742	Benzoic acid, ethyl	-	-	0,42	-	-	-	-



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			ester							
41	1171	18.824	cis Pinocamphone	0,03	0,08	0,11	-	-	0,36	0,70
42	1174	18.966	Terpinen-4-ol	0,40	0,99	2,07	1,47	-	0,42	0,63
43	1183	19.277	<i>p</i> -Cymen-8-ol	0,08	0,07	0,20	0,04	0,13	0,53	0,29
44	1188	19.490	$\alpha$ -terpineol	0,79	0,57	3,44	0,22	4,24	6,80	1,84
45	1192	19.678	cis-dihydro-Carveol	-	-	-	-	0,43	-	-
46	1193	19.704	Myrtenol	0,20	0,18	0,46	0,03	-	2,12	0,70
47	1194	19.736	cis-dihydrocarvone	-	-	-	-	0,55	-	-
48	1198	19.883	2-Cyclohexen-1-ol	-	-	-	-	0,39	-	-
49	1203	20.158	trans-Piperitol	-	-	0,03	-	-	-	-
50	1206	20.204	Verbenone	0,18	0,18	0,09	-	0,16	1,62	0,13
51	1209	20.317	4,7-Dimethylbenzofuran	-	-	-	-	0,05	-	-
52	1216	20.558	trans-Carveol	0,04	0,08	0,02	-	1,64	0,87	0,04
53	1228	20.992	cis-Carveol	-	-	-	-	0,82	0,15	-
54	1241	21.471	Carvone	-	-	-	-	0,95	0,28	-
55	1242	21.507	Carvacrol methyl ether	0,09	0,05	0,05	-	0,11	-	-
56	1273	22.601	Phellandral	-	-	0,07	-	0,18	-	-
57	1284	23.041	Bornyl acetate	1,34	1,16	1,28	0,65	0,28	1,85	0,12
58	1295	23.483	Perilla alcohol	-	-	-	-	0,12	-	-
59	1298	23.545	trans Pinocarveylacetate,	-	-	0,06	-	-	-	-
60	1299	23.582	Carvacrol	-	-	-	-	-	0,25	-
61	1324	24.470	Myrtenyl acetate	-	-	0,02	-	-	-	-
62	1337	24.904	$\delta$ -Elemene	-	-	-	-	-	-	-
63	1349	25.286	$\alpha$ -Terpinyl acetate	-	-	-	3,50	-	-	-
64	1350	25.307	$\alpha$ -Cubebene	0,03	0,03	-	-	-	0,17	0,51
65	1349	25.330	$\alpha$ -Longipinene	-	-	0,21	-	0,75	-	-
66	1369	26.005	Longicyclene	-	-	-	-	-	-	0,16
67	1371	26.059	$\alpha$ -Ylangene	-	-	-	-	0,07	-	-
68	1375	26.208	$\alpha$ -Copaene	-	-	-	0,09	-	0,19	-
69	1383	26.487	Geranyl acetate	-	-	-	-	0,08	-	-
70	1386	26.688	Sativen	-	-	-	0,05	-	-	0,06
71	1389	26.727	7-epi-Sesquithujene	-	-	-	0,03	-	-	-
72	1404	27.205	Junipene	-	-	0,44	1,28	1,73	0,39	3,37
73	1411	27.454	$\alpha$ -Cedrol	-	0,79	-	-	-	-	-
74	1412	27.475	$\beta$ -Funebrene	0,74	-	-	0,52	-	-	-
75	1417	27.688	<i>trans</i> - $\beta$ -Caryophyllene	0,22	0,23	0,25	0,61	2,82	12,51	4,25
76	1430	28.062	Cis Thujopsene	0,15	0,13	-	0,04	-	-	-
77	1436	28.254	Iso amyl benzoate	-	-	0,12	-	-	-	-
78	1452	28.804	Dimethyl Phthalate	0,25	0,15	0,13	-	-	-	-
79	1453	28.805	$\alpha$ -Humulene	-	-	-	0,51	0,52	2,14	0,60
80	1456	28.903	$\beta$ -Farnesene (E)	-	-	0,05	-	-	0,10	0,03
81	1473	29.486	10 $\beta$ -Cadina-1(6),4-diene	-	-	-	0,06	-	-	-



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82	1476	29.608	$\alpha$ -Amorphene	-	0,22	-	0,12	-	-	-
83	1481	29.708	Germacrene-D	0,65	0,87	-	1,55	-	-	0,05
84	1486	29.898	$\beta$ -Selinene	-	-	-	-	0,12	-	-
85	1495	30.182	$\alpha$ -Chamigrene	-	0,12	-	0,06	-	-	-
86	1499	30.328	$\alpha$ -Muurolene	0,08	0,10	-	0,13	-	-	-
87	1508	30.601	$\beta$ -Bisabolene	0,06	-	-	-	-	-	-
88	1512	30.705	Butylated hydroxytoluene	-	-	0,13	-	-	-	0,07
89	1523	30.764	$\gamma$ -Cadinene	-	0,07	-	-	-	-	-
90	1516	30.850	Bornyl isovalerate	0,08	0,32	-	-	-	-	-
91	1524	31.060	$\delta$ -Cadinene	0,29	-	-	0,25	-	0,38	-
92	1543	31.668	cis- $\alpha$ -bisabolene	-	-	1,98	-	-	-	-
93	1584	32.915	Caryophyllene oxide	-	-	-	-	0,58	3,54	0,14
94	1590	33.153	Viridiflorol	-	-	-	-	-	-	-
95	1593	33.179	Diethyl Phthalate	2,30	1,62	-	1,69	0,51	0,79	1,87
96	1598	33.371	Guaiol	-	-	-	-	0,31	-	-
97	1605	33.480	$\alpha$ -Cedrol	-	5,83	-	1,72	-	-	-
98	1656	35.070	$\alpha$ -Cadinol	6,28	-	-	-	-	-	-
99	1966	43.009	Sandaracopimaradiene	-	-	-	-	-	0,22	-
100	2059	45.789	dehydroabietane	-	-	0,46	-	-	-	-
101	2086	46.436	abietadiene	-	-	4,16	-	-	-	-
102	2108	46.969	Sclareol	-	-	-	-	-	2,61	-
TOTAL				97,19	98,14	95,95	97,77	93,79	87,28	97,89

J.E.: *Juniperus excelsa*, J.O.: *Juniperus oxycedrus*, C.L.: *Cedrus libani*, C.S.P.: *Cupressus semp. Pyramidalis*, P.P.: *Pinus pinea*, P.N.: *Pinus nigra*, P.B.: *Pinus brutia*

Table 3 show that 45 components in *Juniperus excelsa*, 48 components in *Juniperus oxycedrus*, 50 components in *Cedrus libani*, 43 compounds in *Cupressus semp. Pyramidalis*, 39 components in *Pinus pinea*, 41 components in *Pinus nigra*, 41 components in *Pinus brutia* were determined. Moreover, main compounds of cone samples have been determined as  $\alpha$ -Pinene (30,17%) and  $\beta$ -Myrcene (13,36%) in *Juniperus excelsa* and *Juniperus oxycedrus*;  $\alpha$ -pinene (15,78%) ,  $\beta$ -pinene (17,71%),  $\beta$ -myrcene (14,85%) and  $\beta$ -phellandrene (18,04%) in *Cedrus libani*;  $\alpha$ -pinene (39,76%) and  $\delta$ -3-Carene (18,22%) in *Cupressus semp. Pyramidalis*;  $\alpha$ -pinene (13,92%) and limonene (56,03%) in *Pinus pinea*;  $\alpha$ -pinene (15,53%), Limonene (11,22%), and trans- $\beta$ -Caryophyllene (12,51%) in *Pinus nigra*;  $\alpha$ -pinene (15,32%),  $\beta$ -pinene (14,61%),  $\delta$ -3-Carene (23,26%) and limonene (16,30%) in *Pinus brutia*.

When all the results are examined, it is shown that  $\alpha$ -pinene is the main component in all samples. On the other hands main compounds of *Juniperus excelsa* and *Juniperus oxycedrus* was determined the same in both cones and needles. Also in *Pinus pinea* only limonene was identified as main components with more than 50 percent.

### Conclusion

In this study, essential oil components of needles and cones of some coniferous trees in Türkiye were determined. The essential oil components of these coniferous species, which have been studied separately in the literature, were given collectively and compared with each other. When all the samples are examined, we come across  $\alpha$ -Pinene,  $\beta$ -pinene,  $\beta$ -Myrcene,  $\delta$ -3-Carene, Limonene, trans- $\beta$ -Caryophyllene, Germacrene-D and cedrol compounds as the main components.



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It is known that these components are used in the field of cosmetics and medicine and there are many biological studies on them. On the other hand, different parts of coniferous trees have been used in the treatment of many diseases since ancient times in traditional medicine among the people. In this respect, it is thought that more scientific studies should be carried out on these tree species, which are mostly used in the timber, furniture and construction sectors in Türkiye, and natural products should be produced from these species and their scientific and economic value should be increased.

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