PROCEEDINGS BOOK

Abstracts & Full Papers

The 10th International Mediterranean Symposium on Medicinal and Aromatic Plants

25-27 April 2024 / İstanbul - TÜRKİYE

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The 10th International Mediterranean Symposium on Medicinal and Aromatic Plants

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ABSTRACTS & FULL PAPERS

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Dear Colleagues,

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

MESMAP-2 Symposium was held on April 22–25, 2015, in Antalya – Türkiye, which was organized by academicians from Gazi University (Türkiye), Gaziantep University (Türkiye), Kilis 7 Aralik University (Türkiye), Yüzüncü Yıl University (Türkiye), Association of Pharmaceutical Teachers of India (APTI – INDIA) joint with AMAPMED (Association of Medicinal and Aromatic Plants of the Mediterranean). INDUSTRIAL CROPS AND PRODUCTS JOURNAL with a high impact factor from the Elsevier Group, published a special issue covering some of the full papers selected after scientific evaluation. MESMAP-3 Symposium, which was held on April 13–16, 2017 in Girne (Kryneia) in the Turkish Republic of Northern Cyprus (TRNC), was the third event of the MESMAP symposium series on Medicinal and Aromatic Plants. After scientific evaluation, selected full papers were published in the Indian Journal of Pharmaceutical Education and Research (IJPER), indexed by THOMSON REUTERS. MESMAP-4 Symposium, which was held on April 18–22, 2018 in Sherwood Breezes Resort Hotel Antalya, Türkiye, was the fourth event of the MESMAP Symposium Series on Medicinal and Aromatic Plants. Then, the fifth one was the MESMAP-5 symposium, which was organized as a joint meeting with ISPBS-5 at Cappadocia on April 24-28, 2019. After scientific evaluation, selected full papers from the MESMAP-5 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. Afterwards, the MESMAP-6 Symposium was organized on October 15–17, 2021, and this symposium was supported by the TÜBTAK 2223-B National Scientific Meetings Grant Program. After scientific evaluation, selected full papers from the MESMAP-6 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. Then, MESMAP-7 was organized during November 18–20, 2022, and hosted by Dokuz Eylul University and Torbalı (Izmir) Chamber of Commerce, Türkiye. Last year, MESMAP-8 was organized during October 20–22, 2022, in Izmir–Türkiye. After scientific evaluation, selected full papers from the MESMAP-8 Symposium were published in Molecules and the Brazilian Journal of Pharmacognosy, indexed with THOMSON REUTERS. Furthermore, MESMAP-8 was supported by the TÜBTAK 2223-B National Scientific Meetings Grant Program. MESMAP-9 was organized during May 03–05, 2023, in Ankara–Türkiye. After scientific evaluation, selected full papers from the MESMAP-9 Symposium were published in "Phytochemistry Letters" journal by Elsevier, indexed with THOMSON REUTERS.After the nine successful series of MESMAP symposiums with the participation of prominent keynote and invited speakers, worldwide scientists, and young researchers, MESMAP-10, hosted by İstanbul University in İstanbul-Türkiye, was the tenth series of the meeting, and you can find the abstracts in this ABSTRACTS &PROCEEDINGS BOOK. We would like to encourage the participants of MESMAP-10 to submit their full papers to "A Topical Collection by the "PLANT BIOSYSTEMS" - An International Journal Dealing with all Aspects of Plant Biology (Q2)- by TAYLOR & FRANCIS and other contracted journals, including 'Annals of Phytomedicine', 'International Journal of Agriculture, Environment, and Food Sciences," and 'Current Perspectives on Medicinal and Aromatic Plants (CUPMAP). We would like to acknowledge the support of the Turkish General Directorate of Forestry, TURKISH AIRLINES, Gazi University, Gaziantep University, Iğdır University, Walailak University, Kumamoto University, AMAPMED, Association of Pharmaceutical Teachers of India (APTI), Cosmetic Producers and Researchers Associations (KUAD), Aromatic Plants and Spice Producers Association (ABUDER) and distinguished private sector companies, and all the other supporters. The organizing committee hopes that the participants of the MESMAP-10 Symposium had an amazing experience and unforgettable memories to take back to their homes. We would like to thank all our participants from almost all parts of the world for their valuable participation and scientific contribution to MESMAP-10. We are planning to organize the 11th series of MESMAP meetings in INDIA in 2025, and it will be a great honor to see you again at the MESMAP-11 symposium.

Sincerely,
Symposium Chair
Prof. Dr. Nazım ŞEKEROĞLU
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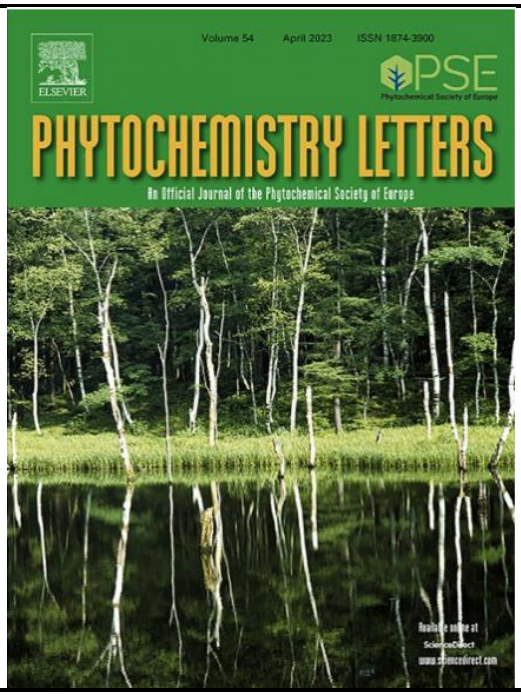

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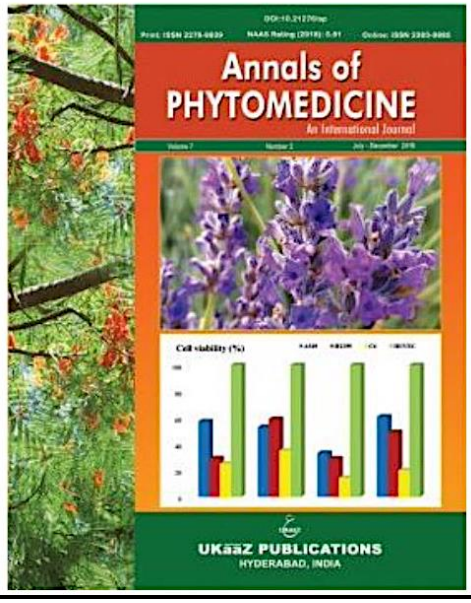

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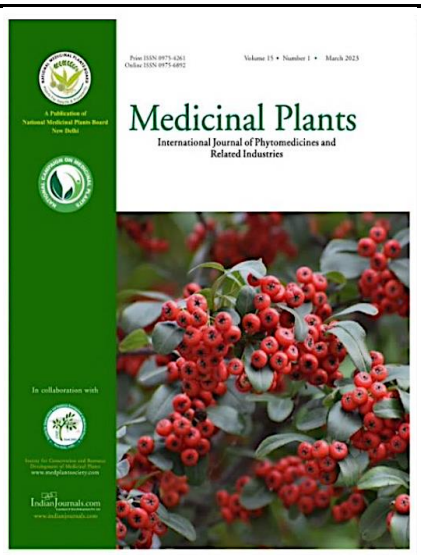

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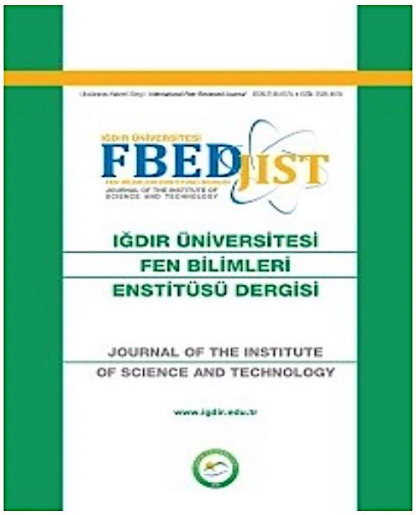

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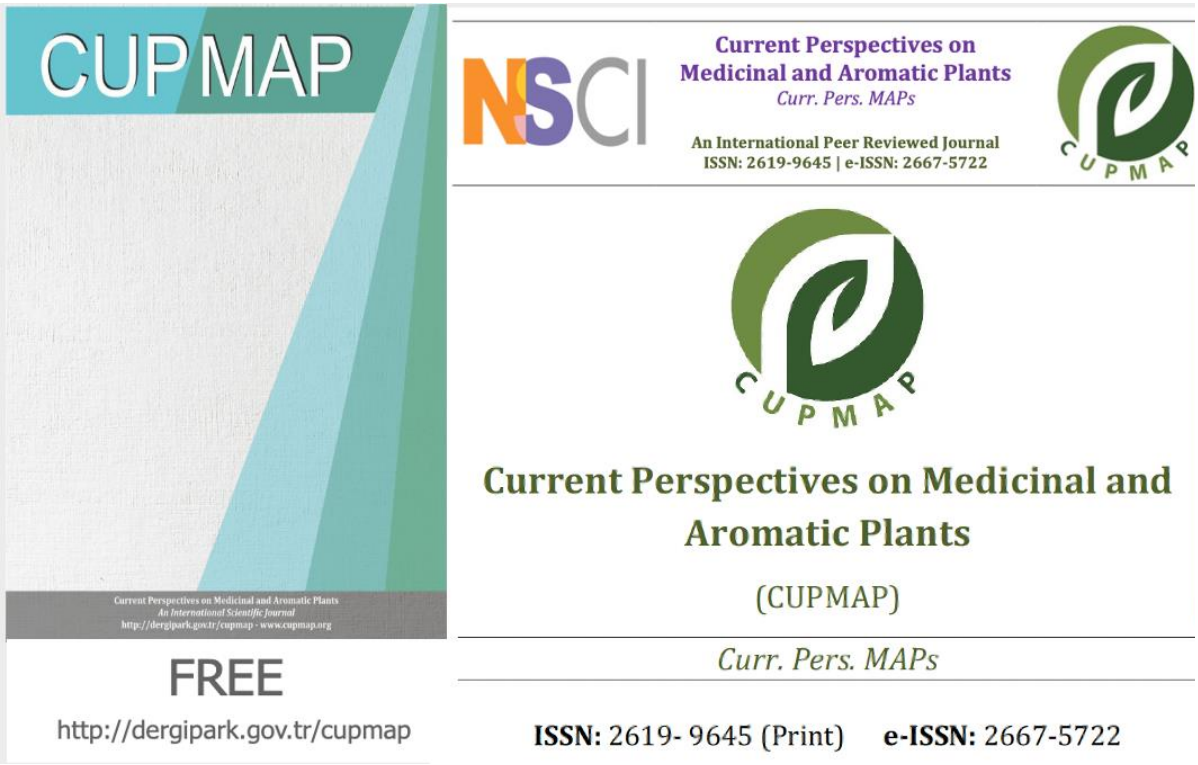

CUPMAP is an open access, peer-reviewed and refereed international journal published by MESMAP scientific group. The main objective of the CUPMAP is to provide an intellectual outlook on the scientific researches on Medicinal and Aromatic Plants. CUPMAP have distinguished goals to promote interdisciplinary scientific studies in which results could easily be used in industrial production on MAPs. This international scientific journal publishes research papers related to Medicinal and Aromatic Plants in the fields of science and technology such as Biology, Molecular Biology and Genetics, Chemistry, Agriculture, Biochemistry, Botany, Ethnobotany, Environmental Science, Forestry, Horticulture, Health Care & Public Health, Nutrition and Food Science, Pharmaceutical Sciences, and so on. CUPMAP publishes original research papers, applied studies, and review articles in MAPs science and technology. Special Issues devoted to important topics in the MAPs science and technology could also be published. CUPMAP Journal publishes Biannually (on June and December) in both print and on-line versions. The publication language of the journal is English. Journal of CUPMAP welcomes article submissions and does not charge any article submission or processing charges. CUPMAP is inviting papers for Volume 7 Issue 1, which is scheduled to be published on June, 2024. **Last date of submission: June 02, 2024.**

MESMAP-10 Sempozyumunda toplam 211 bildiri sunulmuştur, bunlardan 135'sı sözlü, 76 tanesi ise poster sunum şeklinde olup; sunulan **sözlü bildirilerin %66,7'lik kısmı yabancı katılımcılar** tarafından sunulmuştur. Sempozyuma yaklaşık 21 farklı ülkeden bilim insanı katılım sağlamıştır. **Katılım Sağlayan Ülkeler:** Greece, Thailand, Malaysia, Portugal, U.S.A., Sweden, Serbia, Italy, Croatia, India, Poland, Romania, Poland, Algeria, Albania, Morocco, Tunisia, Iran, Azerbaijan, Turkish Republic of Northern Cyprus, and Türkiye.
Sempozyuma katılım sağlayan katılımcılar ve sunum başlıkları 'MESMAP-10 Abstracts & Proceedings Book' kitabının içindekiler kısmında sunulmuştur. MESMAP-10 Sempozyumu aşağıda yer alan YÖK Akademik Teşvik ve Yükselme kriterlerini sağlamaktadır.

İlgili YÖK akademik teşvik yönetmeliği; 17/1/2020 tarihli ve 31011 sayılı Resmî Gazete'de yayımlanan 16/1/2020 tarihli ve 2043 sayılı Cumhurbaşkanı Kararı uyarınca:
(9) (Değişik: RG-17/1/2020-31011-CK-2043/3 md.) Tebliğlerin sunulduğu yurt içinde veya yurt dışındaki etkinliğin uluslararası olarak nitelendirilebilmesi için Türkiye dışında en az beş farklı ülkeden sözlü tebliğ sunan konuşmacının katılım sağlaması ve tebliğlerin yarıdan fazlasının Türkiye dışından katılımcılar tarafından sunulması esastır. Ayrıca etkinliğin uluslararası niteliği haiz olup olmadığı hususunda, ödemeye esas teşkil etmek üzere üniversite yönetim kurulu kararının olması gerekir. Tebliğlerin değerlendirilmesinde tebliğin ilgili etkinlikte sunulmuş ve bunun belgelendirilmiş olması (etkinlik programı ve etkinliğe tebliğde ismi yer alan en az bir araştırmacının katılım sağladığını gösterir belge) esastır. Ayrıca değerlendirme için tebliğin elektronik veya basılı olarak etkinlik tebliğ kitapçığında yer alması ve yayımlanmış tam metninin sunulması gerekir.

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INVITED SPEECHES

GARCINIA MANGOSTANA (A QUEEN OF FRUIT): BIOLOGICAL PROPERTIES IN RELATION TO HEALTH PROMOTION

Jitbanjong Tangpong

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Nowadays, patients use various types of chemical and drug agents for the prevention and cure of disease. Along with their curative effect, almost all drugs have some destructive effects and side effects. Recently, due to the minimal and/or no unwanted side effects, the use of herbal remedies as the drug of choice has become the preferred choice. *Garcinia mangostana*, a queen of fruit, contains various types of polyphenols. It has been used as an herbal traditional medicine from ancient times until now. The biological properties of mangosteen in relation to health promotion effects are well-known. Exploring and understanding the biological properties of mangosteen and its xanthone exerted diverse biological activities such as antioxidant, anti-inflammatory, anti-allergy, anti-bacterial, anti-fungal, anti-malaria, anticancer, and anti-diabetes. Based on these research studies, mangosteen is a beneficial dietary supplement for overall human health.

INVITED SPEECHES

MEDICINAL PLANTS AND THEIR SECONDARY METABOLITES: FROM ANCIENT TIMES TO CONTEMPORARY TIME

Hesham R. El-Seedi

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INVITED SPEECHES

UTILITY POTENTIAL OF RESEARCH ON PLANT *IN VITRO* CULTURES OF MEDICINAL AND COSMETIC PLANTS SPECIES

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Objectives: *In vitro* cultures of medicinal and cosmetic plant species are used as a source of bioactive compounds, an alternative to plant material grown or cultivated in a traditional way (*in vivo*) [1,2]. *In vitro* cultures, apart from being free from contamination and rich in intensively dividing cells, are also a valuable source of plant extracts regardless of the seasons, climatic and edaphic conditions. The obtaining high contents of metabolites in in vitro cultures can be stimulated using various biotechnological methods [3].
Methods: In established in the biotechnology laboratory of Department of Pharmaceutical Botany, Jagiellonian University Medical College *in vitro* cultures of different plant species, like: *Schisandra* sp., *Aronia* sp., *Salvia hispanica*, *Vitis vinifera*, *Centella asiatica* and *Verbena officinalis*, the following culture conditions were tested: selection of the most appropriate medium (Murashige-Skoog, Linsmaier-Skoog and Schenk-Hildebrandt), optimization of type and concentration of plant growth regulators (cytokinins, auxins, gibberellins), choosing the most abundant type of in vitro cultures (organ, callus cultures) and mode of cultivation (solid, agitated, bioreactor cultures e.g. airlift, stirred tank and temporary immersion bioreactors), culture media supplementation with biosynthetic precursors (phenylalanine, tyrosine), application of elicitors (methyl jasmonate, chitosan), and light conditions. Quantification of metabolites was made in methanolic extracts of biomasses using HPLC-DAD and UPLC-MS/MS methods.
Results: The high production of secondary metabolites in studied in vitro cultures was obtained (mg/100g DW), e.g. lignans in *Schisandra chinensis*, *S. chinensis* cv. Sadova, *S. henryi* and S. *rubriflora* (max. 547, 574, 874 and 251), phenolic acids in *Aronia melanocarpa*, *A. arbutifolia* and *A.* ×*prunifolia* (max. 990, 1098, 1615), *Vitis vinifera* (max. 205), *Salvia hispanica* (max. 4651) and *Centella asiatica* (max. 1659). The obtained maximal amounts of compounds were often higher than in analyzed for comparison extracts of soil-grown plants.
Conclusion: The results of the conducted research prove that in vitro cultures of different plant species can be a very good source of bioactive compounds useful in pharmacy and phytocosmetology.

Key Words: Plant biotechnology, plant *in vitro* cultures, innovative natural raw materials

Acknowledgements
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INVITED SPEECHES

PISTACIA LENTISCUS: A CONTEMPORARY "ANCIENT" PHYTOTHERAPEUTIC AND COSMETIC AGENT

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Chios mastic is the resinous secretion obtained from the wounds of the trunk and branches of *Pistachio lentiscus* L. var. Chia, which is endemic to the Greek island of Chios [1]. Since antiquity (500 BC), Chios Mastic has been well recorded for its medicinal and pharmaceutical properties. From 1997, Chios mastic has been identified as a product of Protected Designation of Origin (PDO) while cultivating mastic has been inscribed by UNESCO in 2014 in its Representative List of the Intangible Cultural Heritage of Humanity. In July 2015, mastic was recognized as a traditional medicinal product by the European Medicines Agency (EMA) with two therapeutic indications (mild dyspeptic disorders & skin inflammation/ healing of minor wounds) [2]. In the frame of a continuation study on Pistacia sp. an integrated, complementary bottom-up approach has been designed. This approach includes isolation of active, marker compounds from starting material with fast and state-of-the-art techniques (CPC-UV, SFC-UV-MS); profiling and characterization of composition via multiple analytical methods (HPTLC, HPLC-DAD, UPLC-HRMS & HRMS/MS & NMR); and validation of methods for quality control purposes. Additionally, pharmacokinetic characteristics of major mastic constituents have been determined after a human cohort and metabolomics approaches (LC-MS and NMR) have been implemented for revealing of biomarkers. The current work could be considered as an example of an integrated workflow from the natural entity to human organism.

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INVITED SPEECHES

FOOD SAFETY IN MEDICINAL AROMATIC PLANTS AND SPICES

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Spices and herbs, which are consumed in small quantities, but used in a wide range of foods and food products, represent a unique segment within the food sector. Moreover, being distributed as mostly in their dried, low water activity formats and associated with very complex distribution product chains, specific concerns as regards food safety. Herbs and spices have long been associated with the human culture, and these condiments have been used to flavor our foods since ancient times. Currently, spices and herbs are highly commercialised, similarly to the vast majority of marketed goods, within globalised systems, where the source of cultivation and harvesting may be quite distant from the points of consumption, and spice products may reach the consumers through a series of long and complex food commodity chains. As a result, food safety of herbs and spices became an issue of high priority to protect the health of the consumers. Although herbs and spices are used and consumed only in small quantities, they are added to a great variety of foods, especially to ready to eat (RTE) foods, and therefore, assessment of their intake has become an important topic. Main user of culinary herbs and species is the food processing industry (70-80%), followed by the retail (15-25%) and the food service sector (5-10%).

The vast majority of organic micro-contaminants in spices originate from three main sources: mycotoxins from phytopathogenic fungi, pesticide residues and substances used in food adulteration. Mycotoxins are mostly originated from harvesting and subsequent storage, and are of major concern, because these secondary metabolic substances may exert advert effects, e.g. carcinogenicity or endocrine disruptive effects on humans or other organisms exposed. Potential hazards in spices;aflatoxins and ochratoxin A are the most commonly found mycotoxins in spices. The EU has specified maximum levels in spices like “paprika, chili powder, pepper, nutmeg and turmeric”. European Commission decided to lower the maximum level of ochratoxin A in capsicum spices from 30 µg/kg to 20 µg/kg. This new limit applies to all products already on the market since January. 1, ochratoxin A in spices are very important.

Spices are probably not the first products you associate with Salmonella infections. Due to the long shelf life of spice mixes and the fact Salmonella can survive in dried spices, outbreaks can be widespread.
Allergens; several laboratories identified peanut traces in cumin and later in chili powder. Adulteration with ground peanut shells is most likely the explanation. Early this year traces of (presumably) almonds were found in spices. Confirmatory tests identified the closely related “Prunus mahaleb”, a common spice from the eastern Mediterranean instead of almond. Spices and medicinal plants must not contain foreign substances such as animal hair or insect parts contained in the food as a result of the production, manufacturing, processing, preparation, processing, packaging, packaging, transportation or preservation of spices and medicinal plants, including the primary production stage, or environmental contamination.

Key Words: Food safety, contaminant, mycotoxin, allergens, ochratoxin, aflatoxin

INVITED SPEECHES

ETHNOBOTANY: THE RELATIONSHIP BETWEEN MAN AND PLANT

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Man's relationship with plants is a long-lived one, it has very ancient origins but still lasts unchanged today. Since the dawn of his journey on this Earth, man has always exploited the plant world: from plants he obtains food, protection, materials for the construction and production of artisanal objects and above all substances with a health-promoting and therapeutic action [1]. Plants are a fundamental element of the human world, becoming part of popular traditions and folklore of the various cultures spread across the planet. The most immediate example is demonstrated by the strong ritual character of many plants which were, and still are, used in magical rites to promote health and luck [2]. On these considerations, the multidisciplinary science known as Ethnobotany was born, a science that originally studied "the use of plants by aboriginal populations" [3], but which today has expanded to embrace cultural anthropology, botany, biology, plant physiology, phytotherapy, medicine, ancient history, archaeobotany and pharmacognosy.

It is precisely the synergy between all these disciplines that defines the main character of Ethnobotany, that is the study of the relationship between man-plant-environment [4]. This science places emphasis on the perception that human beings have of plants and on the symbolic and metaphorical meaning that they have in a popular context, involving historical events, customs and habits that are important to reconstruct their origin and provenance. It requires a deep understanding of beliefs, idioms and popular sayings, as they are intrinsically linked to the relationship between man and the plant world. Researchers dealing with Ethnobotany must therefore undertake a journey between cultures, very often making use of cultural mediators capable of understanding their habits, customs, languages and dialects [5]. Ethnobotany aims to recover and safeguard traditional knowledge, shedding light on little-known or endangered cultures, and to create a model for enhancing cultural heritage and sustainable development of the territory to protect its biodiversity. Finally, it makes a great contribution to scientific progress as it can shed light on new uses of plant species in the medical, health and food fields.

Key Words: Ethnobotany, folklore, magical plants, ritual plants, traditional medicine

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INVITED SPEECHES

ISOLATION OF BIOLOGICALLY ACTIVE SUBSTANCES FROM TEA WASTE, PREPARATION OF SEMI-SYNTHETIC DERIVATIVES AND *IN SILICO* STUDY OF TOXICITY, BIOLOGICAL ACTIVITY AND MOLECULAR DOCKING

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Nowadays, tea is widely used to improve the tone of the body in all countries of the world. As we know, tea leaves are rich in various biologically active substances. It has been proven that tea waste contains biologically active substances and can be used as an additional raw source of these substances. In this regard, caffeine and L-threonine from tea waste have been individually obtained and the obtained method has been approved by patent (Patent No. 202100261(EAPO)) by us. For this purpose, tea powder formed during tea production is heated with purified water, filtered, and L-theanine is precipitated from the filtrate using 95% ethyl alcohol. After 1-2 days, the precipitated L-theanine is separated by filtration. Then a saturated 10.6% NaHCO3 solution is added into the cooled precipitate for alkalization, after 24 hours the formed precipitate – tannin is filtered, that is the filtrate is freed from tannin, which is discarded. Ethanol is added into the filtrate freed from tannin and left for a day (caffeine precipitates). Caffeine precipitate is separated by filtration and is mixed with the previously obtained L-theanine. Thus, the target product consisting of L-theanine and caffeine is obtained.

In addition, purine alkaloids (caffeine, theophylline, theobromine) were separated from tea waste and identified by TLC, IR-spectroscopy. The isolated alkaloids were exposed to alkaline hydrolysis and the obtained hydrolysis products (caffeidine, theobromidine, theophyllidine) were individually isolated and identified (MS, NMR-spectroscopy). Biological activity and toxicity of these semi-synthetic derivatives were investigated *in silico* based on Pass Online, Protox-II, Swiss ADME programs. It has been discovered that caffeidine and theobromidine inhibit VEGF - vascular endothelial growth factor, and theophyllidine has a hepatotropic effect. Molecular docking of theophyllidine was performed with Auto Dock Vina 4 program. It was determined that the drug-likeness properties of the studied substances were in accordance with Lipinski rules (rule of 5).

Key words: Tea waste, L-theanine, caffeidine, theophyllidine, theobromidine, *in silico* study

INVITED SPEECHES

MEDICINAL PLANTS FOR GYNECOLOGICAL DISORDERS IN TRADITIONAL PERSIAN MEDICINE

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Traditional Persian medicine (TPM) is a holistic medicine which has originated from the land of Persia and has old antiquity. More than 1100 medicinal plants belonging to 66 different families have been mentioned in TPM manuscripts for the management of various medical conditions, especially gynecological disorders. Several gynecological disorders have been implied directly in TPM textbooks including abnormal uterine bleeding (AUB), oligomenorrhea, infertility, and vaginal infections. However, regarding some of them, no direct mention exists but several symptoms have been described that guide us to a specific gynecological condition. Among this category, endometriosis and polycystic ovarian syndrome (PCOS) are noteworthy.

Twenty-three medicinal plants have been mentioned in Canon of Avicenna for controlling AUB like *Myrtus communis* L. and *Punica granatum* L. Anti-inflammatory and estrogenic activities, inhibition of prostaglandins synthesis, and antiproliferative activity on cervical cancer cells have been reported in current studies. *Mentha longifolia* (L.) Huds., *Foeniculum vulgare* Mill., and *Apium graveolens* L. have been recommended for oligomenorrhea in TPM. Their effects on FSH as well as phytoestrogenic activities may play a crucial role in the management of this condition. Some medicinal plants in TPM have been highlighted to be effective for several conditions that are very prevalent in endometriosis including uterine pain and inflammation, oligomenorrhea, and infertility. *Achillea cretica* L. is one of them which reduced the area of foci, cytokine levels, and thickness of the epithelial layer in an experimental model of endometriosis. Clove has been emphasized in TPM as a tonic of uterine and ovaries and is effective for the treatment of oligomenorrhea and infertility. These activities make it a suitable candidate for the management of PCOS. The results of a current study revealed that clove improves disrupted estrous cyclicity, follicular cyst, and level of gonadotropins in a model of PCOS. Conclusively, medicinal plants mentioned in TPM have tremendous potential for the management of gynecological conditions. However, clinical trials are required to confirm their efficacy and safety.

Key words: Herbal medicine, menorrhagia, women's health, sex hormones, Persian medicine

INVITED SPEECHES

POTENTIALS OF METABOLOMICS AND ETHNOPHARMACOLOGY IN DEVELOPMENT OF PHYTOMEDICINAL PRODUCTS

UMESH K. PATIL

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Phytomedicinal products have been recognized around the world for their potential functions in management of a variety of health problems. As interdisciplinary scientific exploration, ethnopharmacology has multidimensional potentials towards development of phytomedicinal products derived from traditionally employed biologically active agents and evidence-based medicines. This includes field observations, descriptions of the utilization and bioactivities of folk remedies, botanical identification of the plant material as well as scientific exploration including phytochemical and pharmacological research. As the consumption of phytoproducts has been increased again in past few years and new botanical products are being introduced into the market, new and efficient methods to assess safety and efficacy remains as a major concern for researchers in phytomedicinal products development. In order to develop a safe and effective phytomedicinal product, it is very important to understand the active phyto-molecules, synergistic actives, other ingredients, safety, effectiveness, possible interactions, and also the possible side effects of phytomedicinal products. The safety and efficacy data for herbal and traditional medicines are presently insufficient to meet the requirements for their worldwide use. Metabolomics and the metabolomic profiling of phytomedicinal products and medicinal plant species have provided new avenues of research in drug development. Metabolomic approaches allow the simultaneous identification of thousands of metabolites present in medicinal plants or phytomedicinal products. This technique is used to identify targeted and untargeted metabolites for scientific validation and the development of phytomedicinal products with involving a variety of high-throughput screening methods. My talk will include, ethnopharmacological aspects of herbal medicine, potentials of metabolomics and a case study with reference to the traditional medicines.

INVITED SPEECHES

DO PLANT PHENOLICS WITH NEUROPROTECTIVE POTENTIAL REALLY WORK? – TOTAL SUM OF *IN VITRO*, *IN SILICO*, *IN VIVO*, AND MOLECULAR DATA

Ilkay Erdogan Orhan[1,2]

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Medicinal plants contain a large variety of phenolic compounds as the major chemical group with desired pharmacological effects for human health. Plant phenolics, due to their significant pharmacological effects, have always been attractive sources for Pharmacognosic research. They have also been shown to present neuroprotection by multiple molecular and pathological mechanisms. With this justification, we have so far reported many coumarins, flavonoids, terpenoids, etc., having promising neurobiological effects, especially towards Alzheimer's disease (AD), in our ongoing research since the 2000s. The possible neuroprotective effects of thirty-seven selected phenolic compounds were tested through combined methods at *in vitro*, *in vivo*, *in silico*, and molecular levels. Their inhibitory effect on cholinesterase (ChE) and □-secretase 1, which is β-site APP cleaving enzyme 1 (BACE1), as well as their antioxidant activity, was assessed using microtiter assays.

Our findings directed us to the next step that rosmarinic acid, gallic acid, and 3-hydroxytyrosol were further examined for their antiamnesic activity *via* passive avoidance test in scopolamine-induced mice, novel object recognition test (NOR), novel tank diving test (NTT), and Y-maze test models on zebrafish (*Danio rerio*). The active inhibitors of ChE were subjected to molecular docking simulations, while *in silico* toxicity of the selected active compounds was assessed. Some of the inhibitory compounds were subjected to the genes associated with AD using a human neuroblastoma (SH-SY5Y) cell line. Anti-aging effects of the selected phenolics were also subjected to *Drosophila melanogaster* (fruit fly) strains. In this talk, the latest data from our team on the neurobiological capacity of selected plant phenolics will be deliberated.

Key words: Plant phenolics, neuroprotection, enzyme inhibition, passive avoidance test, zebrafish.

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INVITED SPEECHES

CHARACTERIZING THE HEALTH PROMOTING PROPERTIES OF ROSEMARY (*SALVIA ROSMARINUS*)

JEREMY J. JOHNSON

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INVITED SPEECHES

BRASSICA NAPUS MONOFLORAL BEE-COLLECTED POLLEN FERMENTED WITH KOMBUCHA-HOW MINERAL COMPOSITION AND PHYTOCHEMICALS/BIOACTIVITY ARE AFFECTED

Aleksandar Ž. Kostić[1], Aleksandra Sknepnek[2], Danijel D. Milinčić[1]
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Bee-collected pollen (BCP), originating from different plants, presents excellent food source. It contains all requested nutrients, as well as phytochemicals, making it suitable as functional food component. However, due to presence of two strong membranes (intine and exine) as well as sporopollenin layer bioavailability of nutrients/phytochemicals, can be limited. Because of this bee performed enzymatic fermentation process transforming BCP to bee bread in the hive. In similar way, it is possible to apply fermentation technology in order to obtain fermented BCP. Recently, kombucha has been proved as good agent for BCP transformation to more bioactive form [1,2]. The aim of research was to prepare kombucha-fermented beverage enriched with monofloral BCP originating from *Brassica napus* (rapeseed) and to determine how mineral profile and phytochemicals/bioactivity of BCP were affected. Three different doses of BCP were applied (5, 10 and 20 g/L) while control sample contained green tea. For mineral composition determination ICP-OES technique was used. In total, 19 elements were quantified in starting BCP whereas kombucha fermented samples were enriched with several benefitial elements (in particular selenium). Arsenic and lead, potentially toxic elements, were absent although they were quantified in traces in BCP. For determination of different bioactive compounds (phenolics and phenyl amides) HPLC- QToF technique was applied. In total, 53 phenolics were identified/quantified in control and kombucha (non)fermented BCP while 13 phenyl amides were also identified. Phenolics belonged to the flavonol aglycones and glycosides (22), phenolic acids and derivatives (15), flavan-3-ols and derivatives (12), flavone aglycone (2) and flavanone aglycone (2). Compared to control and nonfermented samples kombucha fermentation provoked increased content for several compounds belonging to different subgroups except in case of flavan-3-ols which was excepted since tea is the main source of these phenolics. Among phenyl amides different spermine derivatives (8) were predominant.

Key Words: Bee-collected pollen, kombucha fermentation, rapeseed, bioactive compounds, minerals

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INVITED SPEECHES

TRANSLATIONAL RESEARCH - INSIGHT INTO HERBAL MEDICINES FROM NATURE TO BEDSIDE

Ali Yağız Üresin

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INVITED SPEECHES

THE TRENDS AND NEED OF MICROPROPAGATION

Raman Dang

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Micropropagation is one of the classes of plant morphogenesis and embryogenesis. It is a method of plant regeneration through tissue culture. A process of differentiation by which plant organs are formed. This process is much common for mass clonal propagation of plants. It is important to generate substantial mass culture and standardize the extraction procedure. It can be taken to the field as well to multiply. *In vitro* technology is easy to transfer. It will help create new varieties. We can regenerate plant from single cell. Active principles of high value and content can be obtained from Micropropagation. In the present scenario of medicinal plants becoming extinct micropropagation is the only answer to regenerate plants obtain the desired results in terms of their growth and content of active principles.

INVITED SPEECHES

REDUCING THE INVASIVE IMPACT OF *FALLOPIA JAPONICA* BY USING IT AS A SOURCE OF RESVERATROL EXTRACTION

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Fallopia japonica (FJ) also known as *Polygonum cuspidatum (P.cuspidatum)* or Japanese knotweed, is part of the Polygonaceae family and is considered one of the most widely spread invasive plants in Europe, Australia, New Zealand and Northern America, having negative impact on the ecosystem and communities in which it expands and thus representing a threat to biodiversity [1]. This plant proliferates in extremely diverse environments, from pastures to watercourses, forests, railways, disturbed lands or human settlements. Nevertheless, it has been shown that this alien species possesses a distinctive chemical composition, with pharmaceutical features [2] and an under evaluated beekeeping potential as it represents a rich source of nectar for honeybees [3]. Different chromatographic techniques were used for the determination of bioactive compounds from *Fallopia japonica*.

The main compounds belong to the class of polyphenolics, but the most important, this plant contain high amounts of resveratrol, a strong antioxidant, found also in grapes. The amount of resveratrol from the root of *Fallopia japonica* is semnificatively higher [4]. The aim of this presentation is to gather an overview on the high therapeutic potential of *Japanese knotweed* by emphasizing its main compounds and their biological activities, identifying resveratrol, emodin and polydatin as the main compounds that can exert therapeutic effects (antibacterial, antioxidant, anti-inflammatory and anticancer effects, among the most important ones). Also, some future directions are discussed in order to reduce the negative impact of this plant by promoting its functional characteristics, and using it as a source of bioactive compounds extraction.

Key words: Bioactive compounds, chromatography, *Fallopia japonica*, invasive plant, resveratrol.

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INVITED SPEECHES

THE DEVELOPMENT OF THE PRODUCTION OF BOTANICAL NMATERIAL FROM MEDICINAL AND AROMATIC PLANTS IN ALBANIA

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The Development of the Production of Botanical Material from Medicinal and Aromatic Plants in Albania Albania is particularly rich in indigenous species of medicinal and aromatic plants (MAPs), with more than 400 species. They play an important role in everyday life in Albania. Albanians have traditional ethnobotanical knowledge related to the use of local medicinal plants. Mainly, decoctions and infusions are cited as folk medicinal preparations, with the most common applications addressing gastrointestinal and respiratory disorders, as well as illnesses of the urogenital system. The flowers of these plants are known to attract honeybees, and the honey produced is a well-known folk remedy for bronchitis. The demand for MAPs has been increasing globally due to changes in technology, lifestyle, and consumer attitude. Thus, there are opportunities to expand export markets beyond the EU and US. However, the sector also faces several challenges, as demand for higher quality and safety standards in the global markets is increasing. Furthermore, given that currently, most MAPs are exported only partially processed, there is potential to be explored regarding processing, to increase added value. This study aims to analyze recent developments and the strengths and weaknesses of the Albanian MAPs sector, from an agricultural and economic point of view. It should provide recommendations to the stakeholders in the MAPs sector and to political decision-makers.

ORAL PRESENTATION ABSTRACT

ORAL PRESENTATIONS ABSTRACTS

ORAL PRESENTATION ABSTRACT

ARE MEDICINAL PLANTS INFLUENTIAL ON POTENTIALLY ZOONOTIC BACTERIOME IN SWINE?

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Worlwide society confronts in the last years with an increasing number of zoonotic diseases outbreaks due to an intensifying farming sector which facilitates spread, severely impacting on human and animal health, social activities and economies [1]. Development of the organic swine farming, strenghtening the conections between animals and caretakers, could increase the spread of potentially pathogenic, mainly Gram negative bacteria, the animals carry [2]. Medicinal plants are known for their diverse therapeutic effects, including the antimicrobial efficacy [3]. This study aimed at investigating the effects of combined *Calendula officinalis* and *Satureja hortensis* administered orally to pigs on the carried bacteriome. Swine (batch 1: sows=10, fatteners=10 and piglets=10 and batch 2: three identical control groups) from a free-range low-input farm received orally both powdered *C. officinalis* (140 mg/kg bw/day) and *S. hortensis* (100 mg/kg bw/day), for 10 consecutive days (0 to 10). Oral swabs were collected from both batches on days 0, 14 and 28 of the experiment were processed by classical bacteriological methods: broth and agar cultivation, API (Biomerieux, France). Percentages of Gram positive and Gram-negative bacteria were calculated for each sampling. There was a marked ($p<0.05$) effect of the plant combination in sows, where the Gram-positive bacteria decreased by day 14, with a subsequent restauration by day 28. A slight but constant decrease in Gram positive bacteria with a similar but ascending trend of the Gram-negative rods were observed in piglets by day 28; no effects were noticeable in fattening pigs versus the control group. Given the importance of the diet in shaping the bacterial gut population, the results indicated the need for further investigations in tailoring the dose of administered powdered plants plant to obtain the best possible effects in enhancing the gut microbial diversity and structure in pigs of all age categories.

Key Words: Bacteriome, swine, *in vivo*, *C. officinalis*, *S. hortensis*

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ORAL PRESENTATION ABSTRACT

CAROTENOIDS: THE TARGET PHYTONUTRIENTS IN RAMAN SPECTROSCOPY STUDIES

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Carotenoids are lipophilic pigments comprising carotenes (e.g. α-carotene, β-carotene, lycopene) and xanthophylls (e.g. lutein, capsanthin, zeaxanthin), characterized by non-oxygenated and oxygenated polyene molecules, respectively. Carotenoids play ubiquitous role in the photosynthesis by light absorption in the blue spectrum and protection of photosynthetic structures from ROS damage. These compounds are also involved in signaling pathways linked with growth and development regulation, environmental stress responses, symbiotic relations, as well as pollination and seed dispersal processes. Carotenoids are known for their bioactivity and beneficial impact on human health, including anticancer, anti-diabetic, anti-ageing, skin protecting and other effects. Due to their sensitivity to external factors (temperature, light, oxygen, pH) causing isomerization, structural degradation and loss of biological activity, encapsulation technologies are recommended to preserve the desirable traits of carotenoids. The determination of carotenoids in dietary supplements, food and cosmetic products is performed using standard and advanced analytical techniques, mainly HPLC, HPTLC, MS-NMR, and UHPLC. Moreover, carotenoids are studied by vibrational spectroscopy techniques able to detect the light absorption (NIR and FTIR spectroscopy) and the light scattering (Raman spectroscopy) resulting from stretching vibrations of the chemical bonds present in the target molecules. Raman spectroscopy (RS) is superior, rapid, and non-destructive method for studying the physico-chemical traits and molecular interactions in different materials, including complex biological matrixes. To interpret the Raman spectra it is necessary to perform the preprocessing procedures (e.g. normalization, base line correction, smoothing) and subsequent chemometric analyses, mainly regression and classification algorithms. Thanks to the backbone carbon skeleton of conjugated double bonds, the carotenoids are highly sensitive to Raman spectroscopy. In this paper we are reporting our recent results of Raman spectroscopy performances in studies of carotenoid-rich products, and general prospects of RS in food quality assessment.

Key Words: carotenes, xanthophylls, vibrational spectroscopy, chemometrics, encapsualtion

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ORAL PRESENTATION ABSTRACT

ARTEMISIA ARBORESCENS (VAILL.) L.: MACRO- AND MICROMORPHOLOGY, COMPOSITION AND BIOLOGICAL ACTIVITIES OF ITS ESSENTIAL OIL

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The genus *Artemisia* (Asteraceae) includes about 500 species mainly distributed in the temperate zones of the Northern hemisphere [1]. *Artemisia arborescens* (Vaill.) L. is a Mediterranean aromatic shrub, with silver grey-green leaves, covered by a dense tomentum, and a strong scent. It is widely used in the culinary and alcoholic beverages industries and has various ethnopharmacological uses [2]. The secondary metabolites of this species have demonstrated antimicrobial, antiviral, pharmaceutical, phytotoxic and insecticidal activities [3-6]. In the present study we carried out a micro-morphological and anatomical investigation on leaves and young branches of this species, to detect and characterize the secretory structures in which essential oil (EO) is produced. The EO was obtained by steam-distillation and then analysed, showing the presence of *trans*-thujone (24.2%), camphor (18.9%), aromadendrene (6.5%), camphene (6.0%) and 8-cedren-13-ol (5.2%) as major components. Its phytotoxic activity was tested on weed plants (*Lolium multiflorum* Lam. and *Sinapis arvensis* L.) and crops (*Raphanus sativus* L. and *Cucumis sativus* L.). The EO was effective in inhibiting both germination and radical growth of the weeds, *L. multiflorum* and *S. arvensis*. The biological activity of the EO was also assayed against the bacterial plant pathogens *Xanthomonas campestris* pv. *campestris* (Gram-) and *Pseudomonas syringae* pv. *tomato* (Gram+). The EO showed the minimum inhibitory concentration (MIC) when used undiluted [100% v/v], and growth inhibition of 90%, 85% and 76% when diluted 1:10, 1:100 and 1:1000. The antimicrobial activity was also confirmed by the cellular material release assay. In addition, the biofilm formation with or without the EO was also evaluated, resulting in a reduction of 80% of *Xanthomonas campestris* pv. *campestris* biofilm, following the treatment with EO for all the dilution tested. The complex of data shows that *A. arborescens* EO can find application as a potential alternative biocontrol product against weeds and plant pathogens.

Key Words: *Artemisia arborescens*, microscopy, phytochemistry, phytotoxicity, antimicrobial activity

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ORAL PRESENTATION ABSTRACT

BEACH TO BEAUTY: BLENDING SEA LAVENDER AND SEA KNOTGRASS FOR ENHANCED DERMO-COSMETIC SOLUTIONS

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This study investigates the synergistic effects of *Limonium algarvense* Erben (sea lavender) and *Polygonum maritimum* L. (sea knotgrass) extracts as natural cosmetic ingredients. Using ultrasound-assisted extraction, we obtained aqueous extracts at different mixture ratios of 3:1, 1:1, and 1:3, and evaluated their effectiveness against key enzymes relevant to skin health (elastase, lipase, hyaluronidase, collagenase), and antimicrobial properties towards six bacterial strains (*Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi, Staphylococcus aureus*) and four yeast strains (*Candida tropicalis, Candida albicans, Candida parapsilosis, and Candida albicans*). Cytotoxicity was also appraised towards five mammalian cell lines. Chemical characterization was performed using Ultra Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UPLC-MS/MS), complemented by feature finding through Global Natural Products Social (GNPS) Molecular Networking and In Silico annotation via SIRIUS 5. The sea lavender extract was particularly effective against *S. aureus* (MIC=158.74 µg/mL), whereas sea knotgrass extract was more effective in inhibiting collagenase (EC_{50}=16.11 µg/mL) and hyaluronidase (EC_{50}=42.92 µg/mL). The 1:3 ratio mixture exhibited the highest antibacterial activity against *E. coli* (MIC=39.68 µg/mL) and *B. cereus* (MIC=125.99 µg/mL). The 3:1 mixture excelled as an elastase inhibitor (EC_{50}=33.03 µg/mL). None of the extracts or mixtures showed cytotoxicity, maintaining cell viability above 90%. Our analysis identified six distinct networks of compounds, including flavonols, flavans, carboxylic acids, hydroxycinnamic acids, linoleic acids, and amino acids, underscoring the extracts' diverse chemical composition and bioactivity. These results confirm that sea lavender and sea knotgrass extracts, along with their 1:3 and 3:1 mixtures, offer a rich array of bioactive compounds with antimicrobial and enzyme inhibitory properties, making them potent and innovative natural ingredients for the cosmetic industry as anti-ageing and skin-protective products.

Key Words: Sea lavender, sea knotgrass, herbal extracts, antioxidants, antimicrobial, UPLC-MS/MS

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ORAL PRESENTATION ABSTRACT

NUTRITIONAL PROFILE AND PHENOLIC COMPOSITION OF *AMMOPHILA ARENARIA* AND *MEDICAGO MARINA* SEEDS

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Halophyte seeds have gained attention for their potential as functional foods due to their substantial nutritional profile and health-promoting properties [1]. With the goal of enhancing the use of edible halophytic plants in the food industry through biotechnological methods, this study assessed the nutritional (basic composition, fatty acids, and minerals) and antinutritional factors of plant biomass. Additionally, it investigated the phenolic content in both petroleum ether and 70% ethanol extracts derived from this biomass. Higher content in acid detergent fiber (16.4 g/100g DW) and protein content (38.4 g/100g DW) were found in *M. marina*. Linoleic acid was the predominant fatty acid in *A. arenaria*, comprising 64.04% of the total fatty acid detected. Oleic (29.37%) and α-linolenic (25.05%) acids were the main fatty acids in *M. marina*. Both seeds showed low sodium content and high levels of potassium, iron, and zinc. The petroleum ether extract of *M. marina* had a high capacity to inhibit α-amylase, while higher inhibition was observed in ethanol 70% extract of *A. arenaria*. Trypsin inhibitor and phytic acid were not detected in any of the samples. The highest content of total phenolics was detected in the ethanol 70% extract of both species, which also had with the highest number of compounds identified. In summary, *M. marina* and *A. arenaria* seeds exhibit remarkable nutritional properties with a high content of phenolic compounds, particularly in the 70% ethanol extracts. Incorporating these seeds into diets could offer significant health benefits, given their nutritional properties.

Key Words: Sea medick, European beachgrass, mineral composition, functional food

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ORAL PRESENTATION ABSTRACT

THIAMINE CONTENT IN EXTRACTS OF THREE *VERBASCUM* SPECIES

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Vitamins and minerals are micronutrients necessary for human organism in very small amounts but are essential for normal growth, development, and health [1]. Thiamine (vitamin B1) is important for the proper functioning of the nervous system [2] and because of that its content in different plant extracts could contribute to the health effects of these products. *Verbascum* species are rich in several bioactive compounds [3], but we did not find any literature data about their vitamin content.
The flowers of *Verbascum niveum* (VN), *Verbascum speciosum* (VS), and *Verbascum phlomoides* (VP) were collected in the vicinity of Bosilegrad and the voucher specimens were stored in the herbarium of the Faculty of Science and Mathematics, University of Niš (Herbarium codes: *V. phlomoides* – 14506, *V. niveum* - 14615, *V. speciosum* – 14616). The extracts were prepared by percolation with two different solvents (ethanol 50% and distilled water). HPLC method with fluorescence detection was employed for detection and quantification. Identification was performed using standard, and chromatograms were recorded under the same conditions.
According to our results, thiamine was detected in all tested extracts. The detected amounts were between 7.04 µg/g of dry extract and 3.20 µg/g of dry extract, with the highest amount detected for VP water extract and the lowest for VP ethanol extract. Water extracts of VS and VP have higher thiamine content (4.89 µg/g of dry extract and 7.04 µg/g of dry extract respectively) than ethanol extracts (4.65 µg/g of dry extract and 3.20 µg/g of dry extract). In VN ethanol extract the detected amount was 4.17 µg/g of dry extract, and in water VN extract was 3.60 µg/g of dry extract.
Based on the obtained results, the procedures for obtaining these dry extracts ensure the preservation of thiamine, which gives additional value to these extracts for further use in pharmaceutical products.

Key Words: thiamine, HPLC, *Verbascum niveum* Ten., *Verbascum speciosum* Schrad., *Verbascum phlomoides* L.

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ORAL PRESENTATION ABSTRACT

DI-, AND TRITERPENOIDS ISOLATION OF ENDEMIC TWO VARIETIES OF *SALVIA SERICEOTOMENTOSA* WITH ANTICHOLINESTERASES INHIBITORY ACTIVITY

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The genus *Salvia* comprises approximately 1000 species widely distributed worldwide, and has been utilized in traditional folk medicine for centuries. *Salvia* genus is the gene centre of Anatolia and Asia and is a member of the Lamiaceae family with a high endemism ratio is over 50% in Turkiye. The root parts of *Salvia sericeotomentosa* var. *sericeotomentosa* and *Salvia sericeotomentosa* var. *hatayica* plants which are endemic to Turkiye, were collected from Hatay-Arsuz in 2015. The root parts of each *Salvia sericeotomentosa* variety were extracted with dichloromethane.

The extract was subjected to column chromatography separation and isolation. A total of 20 molecules, including 7 diterpenoids, 2 steroids and 11 triterpenoids were isolated and their structures were elucidated by UV, IR, 1D and 2D-NMR and Mass spectroscopic methods. Both dichloromethane extracts and their isolated molecules were examined for anticholinesterase (AChE and BuChE) activity by the Ellman method, and the results were compared with galantamine. Among the terpenoids, a new molecule named hatayic acid with a triterpene structure was discovered from both varieties of *S. sericeotomentosa*. The compound 4-acetyl,15-hydroxy-norkaurene-5-ene having a diterpene structure was isolated as a new molecule only from *Salvia sericeotomentosa* var. *sericeotomentosa*. In addition, kaurenoic acid was obtained from *Salvia* species for the first time in this study. Furthermore, actinidic acid was found for the first time in *Salvia* species growing in Turkiye.

The prepared extracts showed moderate activity against AChE and BChE. Among the isolated compounds, ursolic acid, hatayic acid, erythrodiol-3-acetate and euscaphic acid showed good activity against the AChE enzyme. Among the isolated compounds, 8,11,13-abieta-triene, ferruginol and hatayic acid were found to show good activity against the BChE enzyme. Overall, the results indicated that *Salvia* species and isolated terpenic compounds can be considered promising agents for treating Alzheimer's disease.

Key Words: *S. sericeotomentosa* var. *sericeotomentosa*, *S. sericeotomentosa* var. *hatayica*, isolation, anti-cholinesterase activity, terpenic compounds

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This study is a part of PhD dissertation of Gülbahar Özge Alim Toraman.

ORAL PRESENTATION ABSTRACT

PHYTOCHEMICAL PROFILE OF TUNISIAN WILD ROSEMARY (*ROSMARINUS OFFICINALIS* L.) POST-DISTILLED RESIDUES: CORRELATION BETWEEN POLYPHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY

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Rosemary (*Rosmarinus officinalis* L.) constitutes a reliable and inexhaustible source of biologically active molecules with antioxidant and antibacterial activities which confer beneficial effects for human health. This study was undertaken with the following aims: to identify and quantify the phytochemical profile, to calculate the total phenolic content (TPC), and to assess the antioxidant potential of Tunisian rosemary postdistilled residues. Furthermore, the correlation between polyphenolic compounds and the antioxidant activity was studied. The polyphenolic profile determined by HPLC analysis, showed the presence of eighteen polyphenolic compounds of which carnosic acid and carnosol were the major abundant components (76.36 and 43.53 mg/g DE, respectively), followed by rosmarinic acid and hesperidin (26.02 and 10.60 mg/gDE, respectively) . The TPC, estimated by the Folin-Ciocalteu assay, reached the value of 137.33 mg gallic acid equivalent/g dry extract (mg GAE/g DE). The antioxidant potential, assessed by DPPH and FRAP assays, was 30.78 µg/mL and 46.89 MFe2+/g DE, respectively. The correlation between many identified polyphenolic compounds and the antioxidant activity was found highly significant ($p < 0.05$). These results proved that the post-distilled rosemary residue extracts constitute an effective natural antioxidant due to their high content of bioactive molecules, and seemed to be incorporate in the soft pharmaceutical products, safety foods, and biocosmetics industries, with beneficial effects for human well-being.

Key Words: *Rosmarinus officinalis* L., post-distilled residues, polyphenolic compounds, total phenolic content, antioxidant activity, correlation.

ORAL PRESENTATION ABSTRACT

GC-MS BASED METABOLITES PROFILING, *IN-SILICO* ANALYSIS AND *IN-VITRO* ANTIPROLIFERATIVE ACTIVITY OF ISOLATED BIOACTIVE PHYTOCOMPOUNDS FROM THE CHLOROFORM FRACTION OF *EVOLVULUS ALSINOIDES* L. ON MCF-7 HUMAN BREAST CANCER CELL LINES

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Objective: The study was designed to identify and isolate the main bioactive phytocompounds and to evaluate their *in vitro* antiproliferative effect from the chloroform fraction of *Evolvulus alsinoides* L. aerial parts against MCF-7 breast cancer cell lines using the MTT assay. Methodology: Gas Chromatography– Mass Spectrometry (GC–MS)/ Flash chromatography techniques were used to identify and isolate the main bioactive components. Commercial mass spectral library was used for the depiction of individual phytocomponents in GC-MS analysis. Fatty acids, phytosterols, alkaloids and flavonoids were identified from *E. alsinoides*. Based on GC-MS metabolite profiling, molecular docking studies revealed the potential medicinal activities of phytocompounds like narcissidine, 9- methoxycamptothecin, riboprine, beta-sitosterol, stigmasterol, diethylphthalate from the plant. Bioassay analysis for isolated beta-sitosterol, stigmasterol and eluted fractions were further conducted against MCF-7 breast cancer cell lines using the MTT assay. Result: The binding energies of narcissidine, 9-methoxycamptothecin, riboprine, beta-sitosterol, stigmasterol were found to be -4.42, -4.71, -4.01, -5.24, -4.67 respectively, indicating their affinities towards Estrogen (PDB ID: 6CBZ) receptor alpha. The bioassay results showed a significant ($p < 0.01$) antiproliferative activity of isolated phytocomponents and eluted fractions against MCF-7 cells breast cancer cell lines using the Micro Culture Tetrazolium (MTT) assay. Conclusion: From the present study it was concluded that the herbal drugs can be potentially used to control the proliferation rate of cancer cells. The present investigation may be quite useful as the medicinal plant is highly valued as traditional system of medicine.

Key Words: *Evolvulus alsinoides*, GC-MS, Phytocomponents, *in- silico*, MCF-7, Antiproliferative

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ORAL PRESENTATION ABSTRACT

NARINGENIN AND NARINGIN POLYPHENOLS AS A POWERFUL ANTICANCER AGENT

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Vegetables and fruits can reduce chronic diseases because they contain dietary fiber consisting of different non-starch polysaccharides including cellulose, hemicellulose, gums, lignin, pectin, and β-glucans, which give good nutritional properties to the food. Dietary fiber can significantly prevent, reduce, and treat gastrointestinal, bowel, obesity, diabetes, cardiovascular diseases, and cancer. Fruits and vegetables contain phenolic compounds that can control cholesterol ester accumulation, thus reducing cardiovascular disorders. Besides this, fruit and vegetable intake offer anti-inflammatory and anti-carcinogenic attributes.

Though the incidence of several cancers in Western societies is regulated wisely, some cancers such as breast, lung, and colorectal cancer are currently rising in many low- and middle-income countries due to increased risk factors triggered by societal and development problems. Surgery, chemotherapy, hormone, radiation, and targeted therapies are examples of traditional cancer treatment approaches. However, multiple short- and long-term adverse effects may also significantly affect patient prognosis depending on treatment-associated clinical factors. More and more research has been carried out to find new therapeutic agents in natural products, among which the bioactive compounds derived from plants have been increasingly studied. Naringin and naringenin both are polyphenols present in citrus fruits (grapefruit and oranges). These compounds are widely elaborated for antioxidant, anticancer, anti-inflammatory, and anti-androgenic effects. They have the inhibitory potential against numerous cancer types, including breast, lung, liver, prostate, pancreatic, brain, oral, neck, head, throat, skin, colorectal, bladder, and mammary carcino-sarcoma cancer both in vivo and in vitro. When applied to humans in different clinical studies, these polyphenols, alone or in combination, were proven to have efficacy and safety for cancer patients.

Naringenin exerts its anticancer activities by several mechanisms, inducing or inhibiting different cytokines and enzymes/growth factors like EGF, MAPK, COX-2, STAT3, PKD1, and NF-κB, and hindering different cellular pathways by blocking the phase of the cell cycle. In most cases, the activity of both naringenin and naringin increases dose and time-dependently. Upon further investigation, we conclude that naringin and naringenin can function as anticancer agents by reducing carcinogenesis through pleiotropic processes and could be suitable options to develop innovative and safe anticancer medicines. In order to design naringin and naringenin-nanostructures with surface-modified nanostructures for cancer medication delivery, more experimental and technical techniques are required. The results of this study might provide substantial support for the continued development of naringin and naringenin as a multi-targeted agent for the prevention and treatment of human cancers.

Key Words: Citrus, Naringenin, Naringin, Cancer

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ORAL PRESENTATION ABSTRACT

EXPLORING THE POTENTIAL THERAPEUTIC BENEFITS OF NATURAL SUBSTANCES IN PSORIASIS

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Psoriasis is a chronic inflammatory disease that affects around 2-3% of the world's population, causing skin lesions, pruritus, and pain. Despite its prevalence, it is a condition that still has no cure. The current treatment options include conventional therapies using synthetic substances (such as methotrexate, acitretin, and ciclosporin), as well as biological therapy, all of which present several limitations, including notable side effects (e.g.: nausea, hair loss, infections) [1]. Therefore, nowadays researchers are exploring the potential of natural compounds that could offer viable alternatives for the treatment of psoriasis. This study highlights the main biological activities, including antioxidant, antimicrobial, anti-inflammatory, as well as anticancer properties demonstrated by various classes of active substances from plants. Such compounds have been shown to be highly effective against certain disorders and pathogens given their mechanism of action, which could also make them viable treatment options for psoriasis. The most cited classes of natural products with beneficial roles in the treatment of this condition are polyphenolic acids (e.g.: chlorogenic, gallic and rosmarinic acids), flavonoids (e.g.: astilbin, fisetin, naringin, quercetin), terpenoids (α-bisabolol, chamazulene), coumarins and alkaloids. The current research also discusses plant species used in traditional medicine that could present therapeutic potential in psoriasis [2,3,4].

Furthermore, novel nanocarrier formulations are the subject of ongoing research in an effort to enhance patients' quality of life. Ensuring the stability of the active substance, targeting the site of action, extended-release profile, improved local permeability, efficacy at lower doses, decreased side effects, biocompatibility, biodegradability, and easy drug elimination are just a few of the significant benefits of nanotechnology in the local treatment of psoriasis. Lipid-based nanocarriers, polymeric-based nanocarriers and metal-based nanoparticles are the main types of nanosystems that can be employed in local psoriasis treatment [5]. Among the most notable phytoconstituents included in such systems are phytocannabinoids, bakuchiol, diosmin, curcumin and aloe-emodin.

Key Words: Psoriasis, secondary metabolites, plant species, nanotechnology

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ORAL PRESENTATION ABSTRACT

GREEN AND FRIENDLY SYNTHESIS OF BIOGENIC SILVER NANOPARTICLES WITH POTENTIAL ANTIOXIDANT PROPERTIES

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There is a growing tendency for nanotechnology to be applied in a wide range of fields (biomedical, industrial manufacture, catalysis, optics, electronics) and metal nanoparticles have attracted attention due to their unique properties from both physicochemical and biological standpoints. The existing literature offers numerous methods of obtaining metal nanoparticles, which can be grouped into two main classes: bottom-up (biosynthesis, vapor deposition, sol-gel process, laser pyrolysis) and top-down (mechanical milling, sputtering, chemical etching, laser ablation) approaches. Bionanotechnology emerges as an ecological option, capable of solving important disadvantages of physico-chemical methods of nanoparticle synthesis such as: use of chemicals, high temperature and pressure, expensive equipment, generation of toxic waste. Moreover, the use of plants in the synthesis of metal nanoparticles brings several advantages, including safety, biocompatibility, low cost, and rapid synthesis [1,2].

The objectives of this research were to explore the synthesis, physico-chemical characterization, and *in vitro* antioxidant activity of AgNPs synthesized using two Aronia extracts (aqueous extract and alcoholic extract). After establishing the synthesis conditions, the formation of AgNPs was confirmed by UV-Vis spectroscopy, FTIR, DLS, TEM, EDX and their antioxidant activity was further investigated. In order to obtain a large quantity of AgNPs, the synthesis was carried out using different values of reaction mixture pH, $AgNO_3$ concentration, extract: $AgNO_3$ volum ratio, temperature and reaction time. The obtaining of AgNPs was indicated by the color change from yellow to brown and by the presence of the characteristic SPR band in the 400-500 nm range. Results from EDX, FTIR and the negative zeta potential demonstrate both the presence of metal silver, as well as of phytocompounds at the surface of AgNPs, which act as reducing and stabilizing agents. The AgNPs were spherical, well dispersed and with dimensions < 100 nm. The antioxidant activity determined by lipoxygenase inhibition and DPPH methods provided promising results.

In conclusion, the study demonstrated that Aronia represents a source of phytocompounds that act as ideal candidates for the eco-friendly and rapid synthesis of AgNPs with potential applications in the medical field.

Key Words: Nanoparticles, green synthesis, physico-chemical properties, antioxidant properties

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ORAL PRESENTATION ABSTRACT

PHENOLIC COMPOUNDS AND CYTOTOXIC POTENTIAL OF *HERACLEUM PAPHLAGONICUM* CZECZOTT

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Species of the genus *Heracleum* L., belongs to Apiaceae, are known as Tavşancıl otu, Devesil otu, Baldıran, Çoğşur, Sov etc. in Turkey and use to treat a variety of conditions and diseases such as gastrointestinal diseases, menstrual diseases, hypertension, diabetes, asthma, bronchitis, epilepsy and dysentery etc. both in the world and in our country [1,3,4]. The genus *Heracleum*, which comprises 125 species worldwide, is represented by 23 taxa including 18 species and 7 subspecies in Turkey. Of which 9 are endemic [2].

In the current study, petroleum ether, dichloromethane and methanol extracts of aerial parts of *Heracleum paphlagonicum* Czeczott were investigated for their cytotoxic activity against MDA-MB-231 (human breast cancer cell line), C6 (rat glioma cancer cell line) and NIH-3T3 (mouse embryonic fibroblast; non-cancerous cell line) cell lines by MTT method. According to the results; the methanol fraction is most active extract with 22.29 µg/mL IC50 value on MDA-MB-231 cell line and 24.62 µg/mL IC_{50} value on C6 cell line. Also, in this study, phenolic compounds of methanolic extract prepared from aerial parts of the plant was qualified and quantified by a LC–HRMS analysis. LC–HRMS analysis showed that fumaric acid was the most abundant phenolic acid, whereas luteolin-7-rutinoside was the most abundant flavonoid.

Key Words: *Heracleum paphlagonicum*, Apiaceae, cytotoxic activity, LC-HRMS

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ORAL PRESENTATION ABSTRACT

ANTIBIOFILM EFFECT OF TUNISIAN CULTIVATED MULBERRY (*MORUS ALBA* L.) POLYPHENOLIC COMPOUNDS AGAINST PATHOGENIC BACTERIA

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The emergence of bacteria resistant to many conventional antibiotics poses a serious public health problem. Therefore, the search for new molecules with efficacy to prevent the formation of biofilms is a priority. Historically, mulberry has been effectively used as a traditional medicine in Asia for the treatment of various infectious and internal diseases. It is a rich source of bioactive compounds that can promote human healthy life. This study was undertaken with the aim to estimate the antibacterial activity and antibiofilm effect of Tunisian cultivated mulberry (*Morus alba* L.) leaves extracts. The antibacterial activity was evaluated by micro-dilution method and tested against two Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and two Gram-negative strains (*Escherichia coli* and *Salmonella typhimurium*). The anti-biofilm activity was assessed using a crystal violet test. The mulberry leaf extracts revealed significant antibacterial activity against all bacterial strains. The minimum inhibitory concentration (MIC) varied between 2.03 and 16.25 mg/mL and the minimum bactericidal concentration (MBC) ranged from 8.12 to 16.25 mg/mL. For only the *S. aureus*, *S. epidermidis* and *S. typhimurium* strains tested, the biofilm inhibitory concentration was about (76%, 51.57% and 47.73% respectively) and the biofilm eradication concentration was about (80,02%, 63,40% and 20,41% respectively) for the same pathogens. The plant extract exhibited good anti-biofilm activity (>50%). The results showed that extracts from mulberry leaves had an effective potential as natural antibacterials due to their significant antibacterial and antibiofilm activities and seemed to be useful in pharmaceutical, cosmetics, and food industries with beneficial properties to human health.

Key Words: *Morus alba* L., polyphenolic compounds, pathogenic bacteria, Antibacterial activity, Antibiofilm effect.

ORAL PRESENTATION ABSTRACT

SOME ASPECTS OF *IN VIVO* SAFETY OF EXTRACTS OF THREE *VERBASCUM* SPECIES

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The European Medicines Agency (EMA) recommends the use of *Verbasci flos* in respiratory disorders [1]. In folk medicine practice the whole aerial parts of *Verbascum* species are used to treat skin inflammations and other dermatological problems [2]. EMA considered these indications for the mullein flower and concluded that there is no complete information about the posology to be included in the monograph [1]. Our study aimed to test the safety of flowers and leaves extracts of three *Verbascum* species *in vivo* on the skin of healthy volunteers by measuring biophysical skin parameters. Extracts were made by percolation with different solvents (50% ethanol, distilled water, and 80% propylene glycol) [3]. Ethanol and aqueous extracts were evaporated to dryness and propylene glycol extracts were collected as liquids. Dry extracts were dissolved in water to 2% and propylene glycol extracts were diluted to 10%. *In vivo* non-invasive measurements of the biophysical skin parameters were conducted with Multi Probe Adapter MPA® 9. Tested extracts were applied under patch occlusion (with filter paper, covered with Parafilm®, and then fixed with cotton adhesive Sensifix® tapes) for 24 h on the volar part of both forearms of healthy volunteers. The changes in EI (index erythema), TEWL (transepidermal water loss), and skin pH values before and after the application were observed [4]. The study was approved by the local Ethical Committee (approval number 12-2691/2-3 from 09.03.2023). The values of the measured parameters after the extract application were compared to corresponding basal values using a dependent samples *t*-test and the results showed that there was no significant difference between the measured parameters before and after the application. When it comes to the investigated aspects (influence of extracts on moisture and barrier properties of the skin, erythematous effect), the examined extracts have been tested as safe for topical use.

Key Words: *Verbascum niveum* Ten., *Verbascum speciosum* Schrad., *Verbascum phlomoides* L., biophysical skin parameters, *in vivo* safety

Acknowledgements
Botanical identification of the specimens was performed by prof. Bojan Zlatković and the voucher specimens were stored in the herbarium of the Faculty of Science and Mathematics, University of Niš (Herbarium codes: *V. phlomoides* – 14506, *V. niveum* - 14615, *V. speciosum* – 14616).
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ORAL PRESENTATION ABSTRACT

PHYTOPLASMA DISEASES ASSOCIATED WITH MEDICINAL AND SPICES CROPS IN ASIAN COUNTRIES

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Medicinal plants and spice crops are used in the traditional medicine and our daily culinary as they are rich in many phytochemicals, that provide health benefits and delicious taste to food dishes in Asian countries. Because of their increasing appliance in pharmaceutical, culinary and cosmetic industry, medicinal and spice crops are currently sharing an important role in Asian economy. These crops are prone to several biotic and abiotic stresses of which the diseases caused by phytoplasma infections, impaired the yield and productivity of the phytochemical contents of the affected plants. Phytoplasmas cause diseases in various medicinal plants and spice crops in Asia resulted in serious economic losses and lowers their active constituent's production and quantum along with quality. Epidemics of these diseases have com *Allium sativum* pelled withdrawal or replacing of many medicinal/spice plant varieties from cultivation. Thus, phytoplasma diseases are one of the major constraints in profitable cultivation and production of medicinal and spice crops. So far more than 60 medicinal plant species and 10 different spice crops were reported to be affected with phytoplasma diseases in Asian countries. The most important are *Artemisia sieberi, Allium sepa, A. ativum Cannabis sativa, Capsicum annum, Catharanthus roseus, Foeniculum vulgare, Medicago sativa, Morinda citrifolia, Piper nigrum, Rawolfia serpentina* and *Withania somnifera* etc. The 16SrI, 16Sr II and 16Sr VI groups are the major phytoplasma groups associated with medicinal plants and spice crops in Asia. Phytoplasma diseases of medicinal plants occur all over Asia, however, the majority of reports are available from India, China, Iran, Turkiye, Korea and Thailand. Newly discovered phytoplasma diseases of medicinal plants/spice crops are increasingly being reported attributed to phytoplasma infections. Epidemiological studies are little attempted in case of medicinal plant/spice crop-phytoplasma host combination which is required to be carried out in order to prevent further epidemic spreading. Therefore, the current developed practices in identification and characterization of phytoplasmas infecting medicinal plants/spice crops in Asia should be updated and more concise which would be quite helpful in management.

Key Words: Phytoplasmas, medicinal plants, spice crops, Asia

ORAL PRESENTATION ABSTRACT

IMMUNOSTIMULATING SELENIUM COMPOUNDS VERSUS VEGETAL EXTRACTS: EFFECTS ON THE *IN VITRO* LYSOZYME ACTIVITY

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Lysozyme, an intrinsic component of the immune system, is a naturally occurring enzyme with antimicrobial activity especially Gram-positive but also Gram-negative agents [3], by hydrolyzing the muramyl dipeptide in the bacteria cell wall. It was considered an endogenous antibiotic, differing by type and species dependent characteristics [2]. Some of the medicinal plants were cited to inhibit the anti-lysozyme activity of bacteria and biofilm appearance [1]. The aim of this research was to establish the influence of some selenium or selenium and copper compounds, known as immune modulating preparations and a protein-carotenoid extract from sea buckthorn (*Hippophae rhamnoides*) respectively, on the *in vitro* lytic activity of lysozyme.

The investigations were carried out on serum samples from: a) Rock x Cornish commercial crossbreeds chicken broilers aged 34 days (n = 19) and b) 5-month-old Supercunirom breed male rabbits (n = 19). The agar gel immune diffusion (AGID) method and the *Micrococcus lysodeicticus* test strain were used to define the *in vitro* lysozyme activity. Serial dilutions (1:2, 1:4, etc.) of the tested compounds were used. The groups were compared by Student's t test for statistical significance of the results. Percentages of activity increase versus control were calculated. The concentration of serum lysozyme was higher in rabbits than in chickens and its lytic activity was enhanced by selenium and copper combinations in chickens (183.69 ± 37.91%) and less in rabbits (128.45 ± 84.10%) in a dose dependent manner. At lower dilutions (3:4), the lysozyme activity remained below that of the control treated with saline. Sea buckthorn extract significantly ($t= 7.22$, $p < 0.001$) decreased the *in vitro* activity of lysozyme at both dilutions used (1:2, 1:4). The protein-carotenoid extract of sea buckthorn acted inhibiting on lysozyme activity, proving the need for tailored extraction and treatment protocols depending on the bacteria and host species.

Key Words: Lysozyme, innate immune response, Aves, Mammalia, sea buckthorn extract

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ORAL PRESENTATION ABSTRACT

IMMUNITY IN HORSES: COULD VEGETAL EXTRACTS ALEVIATE PRODUCTIVE EFFORT?

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Equine represent important actors of human lives, as help for work, participant to sporting activities or simply as companions. The use of horses for various types of exercise could result in stress induced conditions that directly or indirectly prejudice their working performance [1, 2]. The aim of the research was to render several alcoholic vegetal extracts of various sources in alleviating stress effects subsequent to work out on cell mediated immunity in horses. Experimental horses were selected from different working environments: a) draft, agricultural works – n=16, average age 8years, b) inconstant effort, leisure, n=15, average age 7.5 years and c) constant training, endurance, average age 3.5 years. Blood was sampled on heparine (50 UI/ml) before and after the workout. Blood was smeared, stained with DiaQuick Panoptic and leukocyte subpopulations were counted. N/L ratios were evaluated as an indicator of the stress level caused by the workout. Similarly, the *in vitro* leukocyte blast transformation test was implemented using alcoholic extracts (2µl/well, duplicate) of *Taraxacum officinale*, *Symphytum officinale*, *Equisetum palustre*, *Viola tricolor*, *Avena sativa*, *Capsella bursa pastoris*, *Hypericum perforatum*, *Chelidonium majus* L. and the results were statistically interpreted (Student's t test).

The N:L ratio calculated for leisure horses was the highest in this experiment, the animals being subject to inconstant workout and therefore more prone to workout stress. *In vitro* responses were the highest to all tested extracts in endurance horses, and the lowest in draft horses ($p<0.01$-0.001). *C. majus* L. was the most efficient in all categories (draft 30.973± 22.578 and -78.13± 42.6, in leisure 51.63±25.19 and 41.86±22.48 and endurance 51.59±4.83 and 48.06±6.82, before and after the workout, respectively). The effects of plant extracts depended on the type of effort and the level of constant training rather than on the taxonomy of the plant.

Key Words: Horses, effort stress, N:L ratio, blast transformation, alcoholic plant extracts

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ORAL PRESENTATION ABSTRACT

ACCUMULATION OF TERPENE COMPOUNDS IN VALERIAN (*VALERIANA OFFICINALIS* L.)

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Valerian (*Valeriana officinalis* L.) is one of the most important MAPs cultivated in Poland. The raw material collected from this plant are underground organs (rhizome, roots and stolones), applied mainly in the form of dry extracts, for relief of mild nervous tension and sleep disorder (EMA/HMPC/150846/5015 and 340719/2005). According to European Pharmacopoeia (*Valerianae radix* monograph 07/2015:0453) the raw material (whole or fragmented drug) is standardized on the content of essential oil (min. 4 mL/kg DW) and sesquiterpenic acids (min. 0.17% m/m, expressed as valerenic acid). The drug may be highly diversified concerning the content and composition of these compounds, especially essential oils. According to our experiments, carried out on one of the most frequently cultivated in Poland variety (i.e.'Lubelski'), high diversity is observed both between individual plants and in terms of developmental stage of the plants. Moreover, it is distinguished when consider plant organs, between rhizomes, roots or flowers (applied sometimes as repellents in organic crops production). The accumulation of these compounds also depends on the cultivation system, i.e. whether valerian is grown in an annual or 1.5-year system. There are also large differences depending on the term of harvesting the raw material and the method of drying.

Key Words: *Valeriana officinalis*, terpene compounds, accumulation, plant part

ORAL PRESENTATION ABSTRACT

PHYTOCHEMICAL, ANTIMICROBIAL AND *IN SILICO* STUDIES OF COMPOUNDS ISOLATED FROM *PEPEROMIA BLANDA* (JACQ.) KUNTH

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Medicinal plants from Yemen have vast potentials to be explored for their beneficial properties and compound identification, as there are limited scientific evidences available. *Peperomia blanda* (Jacq.) Kunth is an herbaceous perennial plant which grows in Yemen and has been used by the locals as an injury disinfectant. This study was performed to assess the antimicrobial potentials of extracts and compounds present in *P. blanda*. Bioassay-guided isolation protocols were performed on the whole plant using solvent systems with varying polarities. Anitimicrobial assays were performed using selected Gram-positive and Gram-negative bacterial strains and two fungal strains to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts, fractions and isolated compounds. Structures of isolated compounds were elucidated using spectroscopic methods such as nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography mass spectroscopy (LCMS), ultraviolet (UV) spectroscopy and infrared (IR) spectroscopy. MIC were recorded within the range of 62−250 µg/mL. In addition, assessment of the pharmacotherapeutic potential was also performed on the isolated compounds using the Prediction of Activity spectra for Substances (PASS) software, and different activities of compounds were predicted. Molecular docking, molecular dynamics simulation and molecular mechanics /Poisson−Boltzmann Surface Area (MM-PBSA) calculations have proposed the binding affinity of these compounds toward methylthioadenosine phosphorylase enzyme, which may explain their inhibitory actions.

Key Words: *Peperoma blanda*, bioassay-guided isolation, antimicrobial, Prediction of Activity spectra for Substances (PASS), medicinal chemistry

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ORAL PRESENTATION ABSTRACT

ANTIBACTERIAL AND ANTIBIOFILM ACTIVITIES OF TUNISIAN WILD THYME (*THYMBRA CAPITATA* L.) ESSENTIAL OILS AGAINST PATHOGENIC BACTERIA

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The excessive use of antibiotics in several fields, such as agriculture, food and pharmaceutical industries and medicine leads to the emergence of multi-resistant bacteria and the evolution of antimicrobial resistance genes with serious consequences on human health. The bacteria adopted many drug-resistance strategies such as biofilm production. Therefore, controlling bacteria biofilm formation's still a challenging issue that requires discovery and analysis of effective and safe alternative antimicrobials that may be used for the prevention of antibiotic resistance and infection recurrence. Thym essential oils have been used for hundreds of years as natural medicines to combat a multitude of pathogens, including bacteria, fungi, and viruses. The present study was designed to investigate the phytochemical composition, to evaluate the antibacterial activity and anti-biofilm effect of wild Tunisian thyme essential oils (TEOs). The chemical composition of TEOs was analyzed by GC-MS. The antibacterial activity was evaluated against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus cereus*) and two Gram-negative strains (*Escherichia coli* and *Salmonella typhimurium*). The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by the micro-dilution method. The anti-biofilm activity was evaluated using the crystal violet test. Twenty-one compounds were identified in TEOs which represent 99.8% of the oil. The main component of the TEOs that define the chemotype was carvacrol (85.41%). The TEOs have shown interesting antibacterial activity against all tested bacterial strains Furthermore, the studied TEOs showed potential anti-biofilm activity. The percentages of inhibition varied from 57.92% to 83.72%. Similarly, in the eradication activity, the tested TEOs were able to eradicate the bacterial preinstalled biofilms with rates attending the 88.41%. Overall, the results demonstrate that the thyme EOs presents strong antibacterial and antibiofilm activities and could be, due to its high content of carvacrol, explored for food and pharmaceutical industries.

Key Words: Thyme essential oils, phytochemical profile, pathogenic bacteria, antibacterial activity, anti-biofilm activity.

ORAL PRESENTATION ABSTRACT

PROTECTIVE POTENTIAL OF *ANNONA SQUAMOSA* SEED ISOLATES IN OVARIAN CANCER

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Overview and Objective: A vast group of disorders known as cancer can start in almost any organ or tissue in the body when abnormal cells multiply uncontrollably, transcend normal boundaries to infect other bodily parts, or spread to other organs. In its biannual survey revealed a shocking statistic that more women than men are being diagnosed with cancer in India. 1.57 million people are predicted to be impacted by the disease in 2025, up from 1.46 million this year. The three most prevalent cancers in women were ovarian, cervix, and corpus uteri, with breast cancer having the highest incidence. The primary objective of this research is to ascertain the anticancer potential of an isolate obtained from the hydroalcoholic extract of Annona Squamosa seeds, and to pinpoint the exact component accountable for its ability to combat Ovcar cancer cells.

Materials and Procedures: The LCMS method was used to undertake phytochemical analysis on ovarian cancer cell lines after the hyroalcoholic extract of *Annona squamosa* seeds was purified using column chromatography to produce two isolates, I-1 and I-2. Using the LCMS approach, the phytochemical analysis revealed 15 distinct molecular weight molecules. At a dosage of 100 µg/ml, the extract showed average in vitro anticancer efficacy against cell lines that represent ovarian cancer.

Conclusion: LCMS method of phytochemical analysis revealed a broad variety of phenols and flavonoids that have comparatively prominent anticancer properties in AS seed isolate I-2.

ORAL PRESENTATION ABSTRACT

PHENOLIC AND GLUCOSINOLATE PROFILES OF *DIPLOTAXIS TENUIFOLIA* (L.) DC HYBRID MARTE GROWN WITH/WITHOUT BIOFORTIFICATION TREATMENTS

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The distinctive sharp and peppery flavor of wild rocket (*Diplotaxis tenuifolia* (L.) DC) leaves has gained attention in recent years, leading to their extensive use in various culinary preparations. In addition, the leaves serve as a rich source of nutrients and bioactive compounds such as vitamins, pigments, phenolics, and glucosinolates. Due to the antioxidative, anticancer, and anti-inflammatory effects of the mentioned constituents, the wild rocket has potential application in diverse fields, such as medicine. The aim of this study was to determine the influence of biostimulant Kelpak (*Kelp Products Ltd.*), alongside iron and potassium-enriched foliar fertilizers on the quantities of phenolics and glucosinolates (GLSs) in the leaves of the hybrid Marte F1. To compare the effects, a control group with no treatments was established. The quantification of twenty-three plant phenolics and assessment of the relative content of four glucosinolates was conducted in 70% methanol extracts using an ultra-high-performance liquid chromatography (UHPLC) system, coupled up with a quadrupole time-of-flight mass spectrometry (Q-ToF-MS). Regarding total phenolic compound content, the treatments involving potassium application recorded the highest quantity, approximately 3244 mg/kg of fresh weight (FW), whereas the control group exhibited the lowest (2197.1 mg/kg FW). The most abundant compounds in wild rocket leaves were quercetin-3,4'-di-*O*-hexoside-3'-*O*-(6"-sinapoyl)-hexoside (ranging from 646.8 to 693 mg/kg FW among treatments), and quercetin 3,7,4'-tri-*O*-hexoside (294.7-554.6 mg/kg FW). Moreover, quercetin-3-*O*-(2"-feruloyl-hexoside)-3'-*O*-(6"-sinapoyl-hexoside)-4'-*O*-hexoside was present at a level above the limit of quantification (LOQ) as well as quercetin but only in the control sample. Furthermore, the relative GLSs content of glucosativin, glucoerucin, neoglucobrassicin, and DMB-GLS was determined. Among the identified, glucosativin was represented as the predominant ranging from 73.4% (control group) to 89.5% (Kelpak tretments). In conclusion, the application of foliar fertilizers provoked the increase of phenolics and glucosinolates content in the leaves of the hybrid Marte F1, resulting in an improved bioactive profile.

Key Words: Wild rocket, phenolics, glucosinolates, UHPLC- Q-ToF-MS

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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 as well as the Science Fund of the Republic of Serbia, #GRANT No. 7744714.

ORAL PRESENTATION ABSTRACT

EFFECTS OF AN ORGANIC BIOFERTILIZER AND A SEAWEED LIQUID EXTRACT ON THE GROWTH, THE BIOMASS PRODUCTION AND THE PHYSIOLOGICAL STATUS OF AQUAPONICALLY GROWN BASIL (*Ocimum basilicum* var. *Genovese*)

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Meeting the ever-rising demand for Medicinal and Aromatic Plants is a growing challenge especially in countries marked by water scarcity. Hence, adopting ecologically friendly agricultural practices such as aquaponics could be an alternative as it offers numerous benefits that include higher yields and reduced water consumption when compared to soil-based cultivation. Moreover, applying seaweed-based products as biostimulant has become a common practice in agricultural production systems for their plant growth-promoting and enhancing effects. Consequently, the present study aimed to determine the effects of an organic biofertilizer (OF) and a seaweed liquid extract (SLE) on the growth, biomass production and physiological status of basil (*Ocimum basilicum* var. *Genovese*). For this purpose, an experiment was conducted under greenhouse at the National Agronomic Institute of Tunisia where plants were grown aquaponically according to the Nutrient Film Technique. The OF and the SLE which was obtained from the green macroalgae *Ulva rigida* were applied by foliar spraying. Additionally, a non-applied seaweed-based biostimulant served as a control.

Based on our experimental data, it was demonstrated that the application of the seaweed-based biostimulants did not affect most of the measured growth parameters. Nevertheless, the biomass production was positively affected by the SLE: Indeed, this treatment increased the shoot fresh and dry weights as well as the root fresh and dry weights by 48.05%, 41.26%, 14.3% and 25.26% respectively. Similarly, the relative water content (78%) as well as the photosynthetic pigments (increment by 22.6%, 24.88%, 25.03% and 32.92% of chlorophyll a, b total chlorophyll and carotenoids respectively) were enhanced by the application of these biostimulants. Overall, results of this study showed that the application of seaweed-based products could be successfully used as biostimulant in basil to enhance the biomass production as well as the photosynthetic status of aquaponically grown plants.

Key Words: Basil, Aquaponics, Seaweed, Biostimulants

ORAL PRESENTATION ABSTRACT

HEAVY METAL AND TRACE ELEMENT CONCENTRATIONS IN ABOVE-GROUND PARTS OF WIDELY USED MEDICINAL PLANTS IN CENTRAL ASIA IN TERMS OF HUMAN HEALTH RISK

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Kyrgyzstan, a mountainous country in the northeast of Central Asia, is a unique setting for this study. It is bordered by China to the southeast, Kazakhstan to the north and west, and Tajikistan and Uzbekistan to the west and south. The country's extreme environmental and climatic conditions have led to the growth of a diverse range of plant species. Some of the medicinal plants grown here also thrive in the mountainous regions of surrounding countries and Russia. These plants are sold in the markets of Kyrgyzstan and are widely used throughout Central Asia.

The present study aimed to determine heavy metal and trace element levels of medicinal plants commonly used by local Kyrgyz people. Aluminium (Al), boron (B), calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), and zinc (Zn) levels in above-ground parts of 46 medicinal plants sold in herbal markets of Bishkek/Kyrgyzstan were measured by ICP-OES. The concentrations of Al, B, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb, and Zn were determined as 10.824–780.495, 1.530–156.184, 1000.075–20207.346, 0.020–0.881, 0.011–9.947, 0.041–95.479, 61.020–824.012, 897.523–117246.861, 198.379–3250.612, 6.024–402.820, 24.846–2816.736, 0.200–6.363, 0.048–9.452, and 11.557–359.472 mg kg^{-1}, respectively. Since they were collected from polluted and mining areas, heavy metal accumulation of some studied plant samples was determined as slightly higher. The human risk assessment values of all of them were less than 1 based on the health risk assessment performed on the studied plants. When collecting medicinal plants to alleviate discomfort, it's advisable to choose areas that are far from potentially contaminated industries and sites. For instance, rural areas located far from mining zones and areas near clean rivers and mountainous sites are preferred.

Key Words: Herbal medicine, mineral content, heavy metal pollution, health risk assessment, RDA

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This study was funded by the Scientific Research Commission of Kyrgyzstan-Türkiye Manas University (Project No: (KTMU-BAP-2020.FB.06).

ORAL PRESENTATION ABSTRACT

EFFICACY ASSESSMENT OF THYME ESSENTIAL OIL AS AN ALTERNATIVE CHEMICAL TREATMENT TO PRESERVE FRESH PINEAPPLE JUICE

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Fresh pineapple juice exhibits a shelflife of a few days limited by the development of yeasts and molds. An approach was implemented to assess the efficacy of treatments of juice. The treatment chosen was the addition of *Thymus leptobotrys* (Tl) and *Thymus maroccanus* (Tm) essential oils (EO) to fresh pineapple juice. These extracts were mainly composed of the monoterpene carvacrol, and inhibited fungal growth in culture medium. The efficacy of the treatments was assessed in fresh pineapple juice harboring its natural microbial population. The extracts TlEO at 0.25‰ or TmEO at 0.05‰ limited fungal growth below 5 log CFU/mL during 14 days.

Key Words: Shelflife, *Thymus* Esssential Oil, Carvacrol, Thymol, Antifungal properties.

ORAL PRESENTATION ABSTRACT

INVESTIGATION OF THE ANTIOXIDANT POTENTIAL OF *PISTACIA LENTISCUS* AERIAL PARTS EXTRACTS FROM MOROCCO

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In recent years, herbal antioxidant products have gained recognition for their efficacy in treating various diseases. The high levels of antioxidants, particularly phenolic content, found in these products have attracted significant interest in fields such as medicine and food science due to their natural antioxidant activity. *Pistacia lentiscus*, or "lentisk," a species of the Anacardiaceae family, grows wild in the Mediterranean region. Its aerial parts have a long history of use in traditional medicine for treating various ailments, including degenerative diseases, owing to their antioxidant potential.

This study aimed to evaluate the antioxidant potential of phenolic extract from Moroccan Pistacia lentiscus aerial parts, using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition method, Ferric Reducing Antioxidant Power Assay (FRAP), and Total Antioxidant Capacity (TAC) assays. The results showed that the phenolic extracts exhibited significant antioxidant activity in leaves, with IC50 values of 0.373 ± 0.037 mg/mL for DPPH, 2.80 ± 0.6398 mg/mL for TAC, and an EC50 value of 0.51 ± 0.007 mg/mL for the FRAP assay. The flowers showed IC50 values of 0.23 ± 0.022 mg/mL for DPPH, 0.93 ± 0.23 mg/mL for TAC, and an EC50 value of 3.47 ± 1.41 mg/mL for the FRAP assay. Generally, the phenolic extracts from Moroccan Pistacia lentiscus aerial parts show an important antioxidant activity, making them promising as natural antioxidants against free radical damage and a potential source of bioactive compounds.

Key Words: *Pistacia lentiscus*, phenolic extracts, antioxidant activity.

ORAL PRESENTATION ABSTRACT

GERANIUM (*PELARGONIUM* SP.) WITH HIGH ESSENTIAL OIL AND MEDICINAL AROMATIC CHARACTERISTIC

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Geranium originates from Anatolia, South Africa and Australia. It is in the Geraniaceae family. There are approximately 300 species in perennial, shrub and succulent forms. It is produced both as an indoor and outdoor ornamental plant, as a bedding plant and as a plant suitable for use in hanging baskets. It is very popular in our country as well as around the world. Geranium, which also has high essential oil and medicinal aromatic content, is used and evaluated in the pharmaceutical, food and cosmetic industries. Geranium is one of the most cultivated and traded species among all flowering potted plants. There is a high production of geranium (130 million units) (AIPH-International Horticultural Products Association, 2022). Important Commercial Species are Pelargonium zonale, Pelargonium peltatum, Pelargonium graveolens and Pelargonium grandiflorum. Pelargonium sidoides and Pelargonium graveolens are produced to benefit from their medicinal and essential oil properties and are used in the pharmaceutical, food and cosmetic industries. Therefore, geranium produced for the ornamental plant and medicinal aromatic sector is important in terms of providing employment for the local people and raw materials for the industrial sector.

Key Words: Geranium, production, trade, medicinal aromatic, essential oil

ORAL PRESENTATION ABSTRACT

BIOCHEMICAL PROFILE AND IN VITRO BIOLOGICAL ACTIVITIES OF DIFFERENT PARTS OF THREE SPONTANEOUS EDIBLE PLANTS IN SOUTHERN TUNISIA

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Plants have demonstrated potential as valuable biological sources for exploring promising therapeutic agents in cancer treatment. Many effective anti-cancer drugs, or their analogues, are currently derived from plants, with numerous others undergoing clinical trials. This study aims to investigate the phytochemical screening, antioxidant, and antiproliferative activities of different parts of three widely used spontaneous edible plants in Tunisian ethnomedicine. Aqueous and ethanolic extracts from *Allium roseum* L. (leaves), *Asphodelus tenuifolius* Cav. (vegetative part, leaves, roots), and *Scorzonera undulata vahl* (flower, leaves, tubers) were analyzed for various secondary metabolites (polyphenols, flavonoids, condensed tannins). High-performance liquid chromatography (HPLC) was performed on the ethanolic extracts. Three tests were used to evaluate their antioxidant activity. The antiproliferative activity was assessed using the MTT test on breast cancer cells (MCF-7). The metabolic profiles of the polyphenolic extracts revealed quinic acid, 1,3-di-O-caffeoyquinic acid, luteolin, naringin, apigenin, rutin, chlorogenic acid, and epicatechin as primary and prevalent components among the different plant parts. All plant parts exhibited remarkable antioxidant activities, with the highest antioxidant capacity detected in the ethanolic extracts rather than in the aqueous ones for all tested plants. The in vitro anti-proliferative activity showed a dose-dependent inhibitory effect on MCF7 cell growth, particularly with both *A. roseum* and *S. undulata* (leaves) ethanolic extracts (0.45 and 3.9 mg/ml). Overall, this study supports the potential development of functional food and chemotherapeutic agents from the studied Tunisian plants.

Key Words: Spontaneous plants, edible, Antioxidant, Anticancer, MCF-7

ORAL PRESENTATION ABSTRACT

ELASTASE AND LIPOXYGENASE INHIBITORY EFFECTS AND PHYTOCHEMICAL ANALYSES ON CULTIVATED SAMPLE OF *MOMORDICA CHARANTIA* L. IN SAMSUN PROVINCE (TÜRKİYE)

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Momordica charantia L. is an important medicinal plant species from the Cucurbitaceae family, known in English as "bitter melon, balsam pear, or bitter gourd". It is an annual, herbaceous, climbing plant with a stem up to 1-2 m in length. The fruits, which resemble a lumpy shuttle, turn orange when ripe. The fruits are about 10 cm wide and 20 cm long, flat, and bear 20-30 seeds that turn brown as they ripen. The seeds of the plant are rich in fixed oil and protein in addition to high amounts of vitamins C and A, beta-carotene, alpha-carotene, potassium, magnesium, and zinc. On the other hand, the plant is also rich in components such as cucurbitane-type triterpenoids, triterpene glycosides, phenolic acids, flavonoids, volatile oil, saponins, fatty acids, and proteins. Although there are many studies on the positive effects of the fruits and oily macerate of *M. charantia* on skin wounds, the number of studies on the inhibitory effects against the lipoxygenase (LOX) enzyme is very few. According to our literature search, there is only one study on *M. charantia* growing naturally in Türkiye.

On the other hand, no study on elastase inhibition of naturally grown or cultivated samples in our country has been found so far. Therefore, there is no study examining the LOX and elastase inhibitory effects on cultivated pomegranate in our country. For this reason, elastase and LOX inhibitory effects of the extracts in different polarities prepared from the seeds, seed pods, and leaves of the bitter melon cultivated in Samsun province were investigated. The inhibitory effect of all extracts on elastase was found to be below 20%, while only the leaf extract showed a 57.82±2.04% inhibitory effect against LOX. The phytochemical content of the leaf extract was analyzed by liquid chromatography-mass spectrometry (LC-MS). In this paper, the elastase and LOX inhibitory effects of the plant and the results of the LC-MS analysis will be presented.

Key Words: *Momordica charantia*, elastase, lipoxygenase, enzyme inhibition, LC-MS analysis

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ORAL PRESENTATION ABSTRACT

MEDICINAL AND AROMATIC PLANTS IN POLAND

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Poland is one of the biggest producers of herbal raw materials in Europe. They originate from both wild-growing and cultivated plants. In the first group, about 200 species are commercially harvested, mainly horse chestnut (*Aesculus hippocastanum* L., seeds), elderberry (*Sambucus nigra* L., fruits), birch (*Betula pendula* Roth, leaves) and nettle (*Urtica dioica* L., leaves/herbs). The most commonly cultivated species are valerian (*Valeriana officinalis* L.), thyme (*Thymus vulgaris* L.), peppermint (*Mentha* x *piperita*), purple coneflower (*Echinacea purpurea* (L.) Moench.), caraway (*Carum carvi* L.), milk thistle (*Sylibum marianum* (L.) Gaertner) and chamomile (*Matricaria chamomilla* L.). Recently, intensive research has been carried out on introducing wild-growing species into cultivation, concerning both our native plants, i.e. stinging nettle (*Urtica dioica* L.), bison grass (*Hierochloë australis* (Schrad) Roem et Schult) and bastard balm (*Melittis melissophyllum* L.) and foreign species (*Rhodiola rosea* L., *Saposhnikovia divaricata* Schischkin, *Rhaponticum carthamoides* (Willd.) Iljin). In Department of Vegetable and Medicinal Plants WULS-SGGW works related to the protection of the genetic resources of wild and cultivated species of MAPs, has been carried out. In the frame of these activities, we cooperate with the National Centre for Plant Genetic Resources: Polish Genebank, and with the European Cooperative Programme for Plant Genetic Resources (ECPGR). In our MAP's collection several hundred objects are maintain, originating from Poland, Mongolia, Romania, Georgia, Ukraine and others. The objects are made available through Polish Genebank to research institutions, breeding companies and the so-called amateurs.

ORAL PRESENTATION ABSTRACT

PROTECTIVE EFFECTS OF *PISTACIA ATLANTICA* Desf. OLEO-GUM-RESIN ON OSTEOPOROSIS IN OVARIECTOMIZED RATS BY REGULATING OPG/RANKL/RANK SYSTEM

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Background: Osteoporosis is a chronic bone disease characterized by a decrease in bone mass and a disturbance in bone microstructure. *Pistacia atlantica* Desf from the *Anacardiaceae* family, has long been used by native people in the Middle East and Mediterranean regions for several therapeutic indications such as osteoporosis and fractures.

Objective: The aim of this study was to evaluate the effects of *P. atlantica* oleo-gum-resin on the gene expression and histological parameters on osteoporotic rats induced by ovariectomy (OVX).

Methods: Sham or OVX operations were performed on 63 female Wistar rats. Rats were orally treated with *P. atlantica* gum and its nanoencapsulated form for eight weeks. The rats were divided into 9 groups with 7 rat in each group as follows: sham group, OVX control group, and OVX rats with treatments (*P. atlantica* gum and its nanoencapsulated with doses of 50, 100, 200 mg/kg). Stereological analysis was performed by evaluating total bone density, cortical bone thickness, trabeculated bone formation, as well as calculating, the number of osteocytes, osteoblasts, and osteoclasts. The expression of osteocalcin, osteoprotegerin (OPG), receptor activator of nuclear factor kappa-B (RANK), and receptor activator of nuclear factor kappa-B ligand *(*RANKL*)* were evaluated by real-time polymerase chain reaction.

Results: Treatment with either *P. atlantica* gum or its nanoencapsulated form at 100 and 200 mg/kg doses significantly increased the total bone volume, cortical bone thickness, trabeculated bone formation, as well as the numbers of osteoblasts of callus compared to the control rats. All three doses of gum and its nanoencapsulated form significantly upregulated mRNA expression of osteocalcin and OPG, and decreased RANK and RANKL in the tibia bone callus compared to the control group.

Conclusions: This study showed that *P. atlantica* has a protective effect on bone loss in OVX-induced osteoporotic rats by upregulation of OPG and downregulation of RANKL and could be considered as an anti-osteoporotic adjuvant for human osteoporotic disorders. However, clinical trials are needed to confirm its efficacy in the management of postmenopausal osteoporosis.

Key Words: *Pistacia atlantica,* osteoporosis, Persian medicine, stereology, mRNA gene expression, herbal medicine

ORAL PRESENTATION ABSTRACT

ASSESSMENT OF PLANT EXTRACTS FOR TACKLING CYTOCHROME P450-BASED INSECTICIDE RESISTANCE IN AGRICULTURAL PESTS AND DISEASE VECTORS

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Arthropods, vectors of diseases such as malaria and leishmaniasis, are responsible for over 17% of infectious diseases[1] and the damage of 18-26% of global crop production[2]. Plants synthesize potent chemical compounds as a defense mechanism against phytophagous arthropods. Historically, indigenous plants were employed for repelling phytophagous, blood-feeding arthropods, and household pests[3], however synthetic pesticides gained market dominance due to their efficacy and shelf stability. Yet, their extensive use led to environmental impacts and the development of insecticide resistance in target species[4]. The upregulation of cytochrome P450 enzymes (CYPs) is one of the main mechanisms used by insects to metabolize and thus detoxify insecticides[5]. These enzymes have been the target of non toxic enzyme inhibitors, such as piperonyl butoxide. Here, to discover new drug scaffolds, extracts were rationally chosen based on target-associated characteristics and prior knowledge of major compounds, streamlining the process, and enabling early discovery of active extracts that were sourced from an established in-house extracts and natural compounds library. More than 70 extracts and compounds were screened in a high-throughput *in vitro* enzymatic assay. Their inhibition potential was tested against major CYP metabolizers from agricultural pests (e.g. the *Bemisia tabaci* CYP6CM1) and mosquitoes (e.g. the *Anopheles gambiae* CYP9K1), by using the O-deethylation of the fluorogenic substrate 7-ethoxycoumarin[6]. The results revealed 14 moderates to potent CYP inhibitors. All hits were analyzed with UPLC-ESI-HRMS/MS (Orbitrap) in positive and negative ionization mode to elucidate their chemical profile in terms of major constituents. Furthermore, lead extracts were fractionated using FCPC and were evaluated further together with pure compounds. Certain substances from the chemical group of terpenes, flavonoids and terpenophenolics were found to be significantly potent. We aspire total extracts or enriched fractions, even pure compounds, to be used as biopesticide add-ons for low toxicity anti-resistance insecticide formulations to control "difficult-to-manage'' pests.

Key Words: CYP450, insecticide resistance, LC-HRMS/MS, Oleaceae, Fabaceae, Cannabaceae

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ORAL PRESENTATION ABSTRACT

NEW APPROACHES FOR COMPREHENSIVE QUALITY ASSESSMENT OF *CANNABIS SATIVA* L. WITH FOCUS ON CANNABINOIDS

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Cannabis sativa L. and its bioactives are continuously attracting scientific, commercial, and public interest. Cannabinoids are considered the most valuable constituents due to their therapeutic potential, though the plant contains various other metabolites in a highly complex composition [1]. In view of the widening exploitation routes for *C. sativa* due to its phytochemicals, especially non-psychoactive cannabinoids, reliable quality assessment of the plant material is crucial to ensure consistency in products, practices, and eventually therapeutic outcomes [2]. Effective extraction and determination of cannabinoids, including those of minor levels but increasing biomedical relevance, is highly desirable in this regard, promoting informed utilization of cannabis resources [3]. In this work, extraction procedures were comprehensively investigated, including critical comparison of the majority of reported solvents. Combining the observations about cannabinoid selectivity and phytochemical fingerprints by high-performance thin layer chromatography (HPTLC), specific solvents were proposed according to the application scope. In parallel, streamlined analytical methods based on liquid chromatography (LC) were developed and validated for quantitation of target cannabinoids. For in-depth sample characterization, high-resolution mass spectrometric (HRMS) platforms were employed, leading to the annotation of a multitude of minor cannabinoids in addition to other compound classes. Metabolomics workflows were further followed to unbiasedly probe the underlying causes of variation among *C. sativa* samples. In a primary study of its kind, a large panel of hemp inflorescences from different harvesting years, varieties, and cultivation regions of Greece were studied using advanced metabolite profiling and pattern recognition techniques. Overall, targeted and untargeted analysis in conjunction with optimized extraction provided comprehensive quality assessment and characterizatinon of *C. sativa* with a view to supporting human health. The results of this work are envisioned to enhance our understanding of the complex phytochemistry of cannabis, and contribute to standardization efforts in this rapidly expanding field.

Key Words: *Cannabis sativa* L., cannabinoids, quality assessment, extraction optimization, metabolite profiling, mass spectrometry

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ORAL PRESENTATION ABSTRACT

EVALUATION OF THE PERFORMANCE AND SAFETY OF VERSION II ACIDOSALUS SUPPOSITORIES COMPARED TO BIOAPIFIT ANTI-HEMORRHOIDAL OINTMENT IN THE TREATMENT OF HEMORRHOIDAL DISEASE

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Objective / Purpose: randomized, comparative study is meant to evaluate the efficacy and safety of probiotic based Acidosalus hemorrhoidal suppositories compared to Bioapifit anti hemorrhoidal ointment to relieve the symptoms of hemorrhoidal disease of grade I to III such as bleeding, itching, defecation discomfort and pain.

Materials and Methods: The experimental group consisted of 40 participants was treated with Acodosalus® suppositories that are applied rectally once a day (before bedtime) for 10 consecutive days. The control group also consisted of 40 participants was treated with Bioapifit® ointment applied externally three times a day onto clean perianal area and rectally once a day for 10 consecutive days. The evaluation of the patients before and following the therapy was done in terms of pain (0-10), defecation discomfort (0-10), bleeding severity (0-4), anal itching severity (0-4) and overall subjective discomfort (0-10). For statistical evaluation Statistica 11.0 software package was employed.

Results: Acidosalus suppositories applied rectally for ten days promoted significant decrease of all the symptoms while in the end of the treatment overall subjective discomfort decreased for app 89.6%. Clinical cure was confirmed in 82.5% of the patients. Ten days of rectal application of Bioapifit ointment resulted in significant decrease of all the symptoms of hemorrhoidal disease at third day of the treatment while in the end of the treatment overall subjective discomfort decreased for more than 97.5%. Clinical cure was observed in 87.5% of the patients. None of the patients in either Bioapifit or Acidosalus group experienced any adverse effect during the treatment and follow up period for both medical devices.

Conclusion / Discussion: Physical parameters like low pH, high osmolarity/low water activity, high viscosity, greasiness and coating effect as well as lubricating and astringent effect of both products resulted in the alleviation of the symptoms of hemorrhoidal disease such as bleeding, itching, irritation and pain as well as wound infection due to: the creation of the protective coating on the damaged perianal and rectal mucosa enabling its recovery and preventing further irritation; the creation of unfavorable conditions for the growth, adhesion and multiplications of the pathogens (low pH, high osmolarity, low water activity, coating effect); alleviation of pain and discomfort during defecation due to lubricating effect.

Key Words: Hemorrhoidal disease, herbal macerate, honeybee's products, probiotic yeasts

ORAL PRESENTATION ABSTRACT

INVESTIGATION OF ANTIMICROBIAL PROPERTIES AND CHEMICAL COMPOSITIONS OF POLAR EXTRACTS FROM SELECTED *SALVIA* SPECIES

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Medicinal and aromatic plants have been used in folk and traditional medicine since the early ages of humanity and have been the source of many scientific researches. *Salvia* species, known as sage and of which our country is the gene center, have been scientifically demonstrated to possess various effects such as antimicrobial, spasmolytic, antioxidative, anti-inflammatory, anticholinesterase, and it is one of the species that is still being investigated. In this study, the aerial parts of S. *recognita*, *S. syriaca* and *S. chrysophylla*, two of which are endemic *Salvia* species growing in Anatolia, were separately extracted with ethanol and water to obtain infusion and decoction. The phytochemical contents of all extracts were analyzed using the LC-HRMS method and compared for the first time. High levels of rosmarinic acid and hispidulin-7-*O*-glucoside were observed in all extracts of all three species except *S. syriaca* infusion. The main compounds salvianolic acid B, hispidulin-7-*O*-glucoside, caffeic acid, and fumaric acid were detected in EtOH extracts, in different ratios. Homogentisic acid and salicylic acid were mainly seen in *S. recognita* EtOH extract, and hyperoside was seen at the highest rate in *S. syriaca* EtOH extract. Apigenin and luteolin were detected at higher levels in EtOH extracts, being highest in *S. chrysophylla*, compared to water extracts. Among the decoction extracts, salvianolic acid B and salicylic acid compounds were identified as major components in *S. recognita*. It was observed that all extracts of *S. syriaca* mainly contain quercetin and its routine compound. Additionally, the antimicrobial potentials of all extracts were investigated against various Gram-positive (*S. aureus*, MRSA, *E. faaecalis*, *B. subtilis*) and Gram-negative (*K. pneumonia*, *E. coli*, *P. aeruginosa*, *P. mirabilis*) bacterial strains by using the standard microbroth dilution method. *S. syriaca* and *S. chrysophylla* EtOH extracts were more effective against *Staphylococcus aureus*. It was determined that *S. recognita* ETOH extract had the highest activity against MRSA, and all three ETOH extracts exhibited good activity against *Bacillus subtilis*. In conclusion, the rich contents, and antimicrobial properties of the three species, especially the EtOH extracts, were revealed and an important step was created for natural source drug research with *Salvia* species.

Key Words: *S. recognita*, *S. syriaca*, *S. chrysophylla*, Chemical compositions, antimicrobial activity

ORAL PRESENTATION ABSTRACT

EVALUATION OF COMMERCIAL *PINUS PINASTER* AIT., *PINUS MASSONIANA* D. DON. (PINE TURPENTINE) ESSENTIAL OIL SAMPLES ACCORDING TO EUROPEAN PHARMACOPOEIA 10.0 CRITERIA

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Pine turpentine *(Pinus pinaster* Ait., *Pinus massoniana* D. Don.) oil is known to be used in many fields including medicine, cosmetics, agriculture and art. Its antiseptic properties have been proven which dates back to 19th century.[1] Furthermore, it is known that pine turpentine oil has anthelmintic and cathartic properties in healthcare.[2] This study focuses on both qualitative and quantitative analysis to compare the quality factor of the pine turpentine essential oil in different brands on the Turkish market. Pine turpentine essential oil monograph is found in European Pharmacopoeia 10.0, which has been accepted as the guidebook in pharmacy field for the analysis methods of active substances as well as excipients used in drug preparation. European Pharmacopoeia is legally recognized as a standard and authoritative reference in both European and several non-European countries including Turkiye. In this evaluation study, 14 different oil samples were collected from pharmacies and non-pharmacy-oriented markets. The evaluation is performed according to "Turpentine Oil" monograph in European Pharmacopoeia 10.0.[3] The series of analysis that the mentioned monograph states are: Appearance, Thin-Layer Chromatography, Relative Density, Refractive Index, Optical Rotation, Acid Value, Peroxide Value, Fatty Oils and Resinified Oils and GC-MS for phytochemical profile. These tests were conducted for each sample, separately.

The obtained results showed that none of the samples on the Turkish Market meet the standards of European Pharmacopoeia 10.0 criteria. Expressed in terms of percentage, the rate of meeting the criteria from pharmacy-oriented samples is 56% and from non-pharmacy-oriented samples is 49.4%. Therefore, the necessity of audity for quality control of the pine turpentine oil samples on the market has been stated. The current study is believed to contribute to the literature of pine turpentine oil sample standards with obtained analysis results.

Key Words: *Pinus pinaster* Ait., *Pinus massoniana* D. Don. Pine turpentine oil, Essential oil, European Pharmacopoeia 10.0, GC-MS

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ORAL PRESENTATION ABSTRACT

EXPLORING THE ANTIMICROBIAL POTENTIAL OF *LAVANDULA DENTATA*'S ESSENTIAL OIL AND PHENOLIC EXTRACTS

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Excessive antibiotic use has been identified as a major contributor to the spread of resistance in a variety of infections. As a result, alternative remedies such as essential oils and polyphenol extracts derived from plants may provide a viable solution to reduce the negative effects of antimicrobials while increasing their efficacy. The primary objective of this study was to evaluate the antimicrobial properties of *Lavandula dentata* essential oil and phenolic extracts using the microdilution method, against a variety of pathogenic microorganisms (*Escherichia coli, Staphylococcus aureus, Enterococcus hirae*) Determination of minimal inhibitory concentrations (MIC) revealed that lavender's essential oil demonstrated an inhibitory effect on all bacterial strains in different concentrations. In fact, the highest anti-bacterial activity was observed against *E. coli* (0,6 mg/mL) followed by *S. aureus* and *E. hirae,* both exhibiting a minimal inhibitory concentration of 1,5mg/mL. For the minimal inhibitory concentration of phenolic extracts, results revealed that the highest antibacterial potential was exhibited against *E. Hirae* (0,5 mg/mL) followed by *E. coli* and *S.Aureus* (2mg/mL). These results suggest that the essential oil and phenolic extracts obtained from *L. dentata* certainly exhibited inhibitory effects on the proliferation of the tested microorganisms. Therefore, *L. dentata's* potential use as an antibacterial agent in the pharmaceutical industry could constitute an alternative to antibiotics.

Key Words: *Lavandula dentata*, Essential oil, Phenolic extracts, Anti-bacterial activity, Minimal Inhibitory Concentration (MIC)

ORAL PRESENTATION ABSTRACT

ANTIFUNGAL, ANTIOXIDANT ACTIVITIES AND CHEMICAL CHARACTERIZATION OF BROWN ALGAE FROM MOROCCO

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The current study aimed to investigate, for the first time, the antifungal and antioxidant properties of methanolic extracts and volatile compounds derived from four brown algae: *Bifurcaria bifurcata*, *Cystoseira humilis*, *Ericaria selaginoides*, and *Ericaria mediterranea*. The antifungal activity of methanolic extracts was assessed, showing the most potent inhibition at a concentration of 3 mg/ml for *E. selaginoides* and *B. bifurcate* against *B. cinerea*, with inhibition radii of 14.8 ± 0.01 mm and 13.90 ± 0.08 mm, respectively. Against *P. digitatum*, inhibition radii were 8.60 ± 0.12 mm and 8.70 ± 0.08 mm, respectively. The antioxidant activity was observed across all species, particularly in *E. selaginoides*, which exhibited a high phenolic compound content of 36.92 ± 1.68 mg GAE g-1 PS. The analysis of methanolic extracts using HPLC-MS revealed 9 phenolic compounds. In *C. humilis* and *E. selaginoides*, 5 compounds were identified, dominated by Deoxyschisandrin (40.67%) and Fucophlorethol (26.44%), respectively. Additionally, GC-MS analysis of methanolic extracts detected 20 volatile compounds, with *E. selaginoides* exhibiting 13 compounds primarily composed of eucalyptol (40.49%), Bornéol (11.93%), α-pinene (10.84%), and camphor (10.34%). The volatile compounds of the seven algae showed a strong antioxidant and antifungal activity against both fungal species at the concentration of 3 mg/ml, where an inhibition radius of 23.6 ± 0.11 mm was registered by *E. selaginoides* against *B. cinerea*. Whereas against *P. digitatum*, an inhibition radius of 16.4 ± 0.09 mm was registered by *C. humilis*. A total of 65 volatile compounds were identified in the seven species of algae by CPG-MS analysis, dominated by aromatic hydrocarbons (Hydroxyeremophilone, 1-Ethyl-2-methylbenzene, Mesitylene….), carboxylic acids (palmitoleic acid, palmitic acid, lauric acid, oleic acid, etc.), terpenes (Linalool, p-Cymene, Nerolidol, etc.) and phenols (Durohydroquinone, Alpha -cyclocostunolide Phenol, 2,4-bis-(1,1-dimethyl ethyl), …). Since the volatile compounds of algae are an inexpensive resource with strong antifungal and moderate antioxidant capacity, their use during postharvest opens a new way of biological control.

Key Words: Volatile compounds; Antifungal activity; Antioxidant activity; Biological control, Brown algae, Biochemical analysis.

ORAL PRESENTATION ABSTRACT

OPTIMIZATION OF DRYING METHODS AND EXTRACTION CONDITIONS OF OLS FOR THE PRODUCTION OF OLEUROPEIN AND OLEACEIN ENRICHED EXTRACTS

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Olive by-products are at the center of commercial interest due to their high content in bioactive compounds. Particularly, olive leaves have attracted attention for their varied phenolic content. The EFSA's 2012 approval of a health claim regarding olive oil polyphenols underscores their beneficial properties. Oleuropein (OLE), a primary phenolic compound abundant in olive leaves, exhibits various health benefits, including anti-inflammatory, antioxidant, and cardioprotective properties. However, studies on optimizing OLE extraction processes remain inconclusive, lacking a comprehensive approach based on critical parameters that affect OLE content. Oleacein (OLEA), another significant phenolic compound derived from OLE, is abundant in olive oil, while its presence in olive leaves is minimal. This study focuses on optimizing drying methods (ambient air, oven 140 ^{o}C, microwave and freeze drying) and extraction conditions (extraction solvent, temperature, use of salts) of OLs to produce OLE-enriched extracts and explores alternative plant sources for oleacein recovery. Microwave and high-temperature oven drying are effective methods for the production of OLE-rich extracts, while freeze-drying yielded OLEA-rich extracts. Herein, we report for the first time the biotransformation of OLE into OLEA in OLs under freeze-drying conditions. Notably, enzymatic reactions play a crucial role in determining the chemical composition of OLs extracts. The quantification of OLE and OLEA was performed by HPLC DAD analysis. Moreover, high purity (99.2%) OLE and OLEA isolation was achieved, after pilot scale extraction, MPLC and preparative HPLC DAD chromatography. NMR and QTOF-HRMS supporting data are provided for the structural elucidation of the isolated secoiridoids.

Key Words: OLs, OLE, OLEA, drying methods, extraction conditions, enzymes

ORAL PRESENTATION ABSTRACT

EVALUATION OF THE THERAPEUTIC PROPERTIES OF *CHICORIUM SPINOSUM* L. ON CARRAGEENAN-INDUCED INFLAMMATION IN MICE

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Cichorium spinosum L. is a wild plant that possesses a wide range of therapeutic properties. The objective of this research was to investigate the anti-inflammatory potential of *C. spinosum*. The *in vivo* evaluation of the anti-oedematous effect of the hydroethanol extract of *C. spinosum* was carried out using the experimental model of mouse paw oedema induced by 1% carrageenan. This inflammatory agent was administered by injection into the subplantar region of all experimental animals, one hour after pre-treatment. This induced acute inflammation. The mice were distributed as follows: the inflammation control group (Ci) received distilled water, the Cs50, Cs150 and Cs250 groups received hydroethanol extract of C. spinosum at 50, 150 and 250 mg/kg respectively, and the standard group (STD) represented the reference treatment of diclofenac at 50 mg/kg. Measurements of the percentage increase and inhibition of oedema were used to assess the attenuation of inflammation. The mouse paw was removed for further histological study. The percentage increase in oedema showed a highly significant ($P \leq 0.001$) decrease in the Cs150 and Cs250 groups from the 2nd hour after injection of carrageenan compared with the control group (Ci). Histology of the paw clearly supported the appearance of oedema in the mice, showing a virtually regular appearance with moderate damage due to the oedematous agent in the Cs250 group. Finally, the findings of our research suggest that *C. spinosum* extract has an anti-inflammatory effect, probably because of its bioactive molecules such as polyphenols and others. This study could eventually lead to the development of natural anti-inflammatory treatments.

Key Words: *Cichorium spinosum* L, anti-inflammatory activity, oedema, histology, carrageenan, mice

ORAL PRESENTATION ABSTRACT

BIOLOGICAL ACTIVITIES OF *PISTACIA LENTISCUS* EDIBLE OIL FROM OURIKA REGION (MOROCCO)

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Pistacia lentiscus, also known as lentisk, belongs to the Anacardiaceae family and is flourishing in the wild throughout the Mediterranean region. The fruit of *Pistacia lentiscus* has a history of traditional medicinal use for various ailments, attributed to its antioxidant and antimicrobial properties. This study investigates the antioxidant and antibacterial activities of edible oil extracted from *Pistacia lentiscus* fruits collected from Ourika, Morocco. The oil traditionally extracted was tested for its antioxidant activity using the Ferric Reducing Antioxidant Power Assay (FRAP) and Total Antioxidant Capacity (TAC) assays.

The findings reveal that the edible oil exhibits high antioxidant activity, with an EC50 ranging from 3.27 ± 0.086 mg/ml in the FRAP assay and an IC50 ranging from 0.22 ± 0.019 mg/ml in the TAC assay. Additionally, the oil's antibacterial activity was assessed against two bacterial strains using the microdilution method. The minimum inhibitory concentration (MIC) results demonstrate moderate activity against *Escherichia coli* and A*ctinobacteria*, with a 2.5 mg/ml MIC for both strains. Overall, the results indicate that the edible oil from *Pistacia lentiscus* fruits showed significant antioxidant activity and inhibited the growth of *Escherichia coli* and *actinobacteria significantly.*

Key Words: *Pistacia lentiscus*, edible oil, Antioxidant activity, Antibacterial activity

ORAL PRESENTATION ABSTRACT

IMPACT OF A 28-DAY ADMINISTRATION OF MOROCCAN *ROSA DAMASCENA* FLOWERS EXTRACT ON BLOOD BIOCHEMICAL PARAMETERS, TISSUE HISTOLOGY, AND RODENT COGNITION

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Rosa damascena is one of the most important species of Rosaceae family in the world. Morocco has a rich history of cultivating *Rosa damascena*, a plant highly valued for its medicinal properties. Today, the production of rose-based products plays a significant role in Morocco's economy, supporting numerous local communities as a vital source of livelihood. The objective of this study is to investigate the sub-chronic toxicity of the hydroethanolic extract of *Rosa damascena* flowers (RDFE) from Dades Valley (Morocco) in mice in order to evaluate its safety profile. During the sub-chronic toxicity study, the extract was administered orally at doses of 200, 400, and 800 mg/kg and the distilled water was given to the control group daily to Swiss albino male mice for 28 days respectively. The general behaviors and body weight of the mice were observed daily. Cognitive tests (Open Field test and Splash test) were conducted before the sacrifice, while biochemical analysis and histopathological examinations of the liver and kidneys were conducted at the end of the treatment period. In the sub-chronic study, the RDFE extract induced no mortality or treatment-related adverse effects with regard to body weight, general behaviors, relative organ weights, and biochemical parameters. Histopathological examination of vital organs showed normal architecture suggesting no morphological alterations compared to control group. Furthermore, RDFE does not induce cognitive impairment in mice. Therefore, the present study revealed the absence of sub-chronic toxicity after oral exposure to RDFE. However, further studies evaluating long-term effects are needed in order to have sufficient safety evidence for its use in humans.

Key Words: *Rosa damascene* Flowers, Sub-chronic toxicity, Biochemical, Histological, Morocco, Cognitive tests.

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ORAL PRESENTATION ABSTRACT

ETHNOBIOLOGICAL INVESTIGATIONS IN KIRŞEHIR (TÜRKİYE)

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The ethnobiological investigations were made in order to determine the plants and animals used by the people in the locality of Kırşehir and which have a traditional function in their life. For this purpose, the center of Kırşehir and 44 villages have been visited between 2021-2022. During the field works, plant specimens were collected and the various ethnobotanical information belonging to these plants was provided. The plant specimens are kept in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE). The use of animals that have a role in people's lives was also recorded. The information was obtained through open and semi-structured interviews with the local people. According to the results of this study 153 taxa recorded with ethnobiological usage. Totally 145 taxa which have ethnobotanical usage, were identified. The plants are mostly used as folk medicine (64 taxa), food (69 taxa), fuel (4 taxa), broom (7 taxa), dye (3 taxa) and other usages (15 taxa). In addition, information about 8 animal taxa was also recorded. In this study, it was determined that traditionally plants and animal resources are still used in the region.

Key Words: Ethnobiology, Medicinal plants, Edible plants, Ethnobotany, Kırşehir, Türkiye.

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The authors would like to thank personnel of the Republic of Türkiye, Ministry of Agriculture and Forestry Department of Kırşehir and the Republic of Türkiye, Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks.

ORAL PRESENTATION ABSTRACT

EVALUATION OF THE NEUROPHARMACOLOGICAL PROPERTIES ON ALZHEIMER'S DISEASE AND THE INHIBITORY ACTION ON THE ACETYLCHOLINESTERASE ACTIVITY OF PASSIFLORA EDULIS

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Alzheimer's disease is a neurodegenerative disease that causes progressive and permanent deterioration of nerve cells that slowly destroys thinking and memory abilities. Medicinal plants are currently considered a promising source for identifying new therapies against the spread of this disease. Passiflora edulis seeds are by-products of industry potential resources for pharmaceutical applications, possess valuable biological activities related to Alzheimer's disease. The objective of our research was to create a stilbene-rich extract of the seeds of Passiflora edulis, to study its HPLC-DAD-MS profile, and a qualitative study by HPTLC and its ability to inhibit acetylcholinesterase activity in vivo and in vitro, to evaluate in vivo anti-Alzheimer's activity with two protective and parallel protocols, as well as to perform in silico evaluation of potential stilbene compounds on AChE and BChE activities. In addition. the results of phytochemical and HPTLC analyses of the PEAS fraction revealed the presence of secondary metabolites, phenolic compounds that are intimately associated with the biological activity of the extract, HPLC-MS analysis revealed the presence of phenolic compounds with trans-piceatannol (3,4,3′,5′- tetrahydroxy-trans-stilbene) (PE-1), bulrush B (PE-2) and bulrush A (PE-3) as major constituents, An improvement in memory and behavior was noted in mice, confirmed by histological study of the brain, and fluorescent immunohistochemical for amyloid plaques. edulis seeds and their stilbenes may all prove to be promising candidates for the development of novel AChE and BChE treatment drugs for neurodegenerative diseases in general and Alzheimer's disease in particular

Key Words: *Passiflora edulis*, Alzheimer's disease, Stilbene, Acetylcholinesterase, *in vivo*

ORAL PRESENTATION ABSTRACT

EVALUATION of ANTIOXIDANT POTENTIAL of *SILENE BEHEN* L. EXTRACTS OBTAINED BY TWO DIFFERENT EXTRACTION METHODS

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The genus *Silene* (family: Caryophyllaceae) is represented by 150 taxa [1]. The different parts of the members of the genus are used to treat diabetes, eczema, colds and inflammation [2,3]. The aim of this work was to evaluate the effects of maceration and ultrasound-assisted extraction on the antioxidant potential of *S. behen* leaves. The total phenolic and flavonoid content of the extracts were analzyed by spectrophotometric methods. The antioxidant capacity was assessed by various assays (radical scavenging activity, reducing activitiy, and metal chelating). The results showed that ultrasound-assisted extraction had higher antioxidant activity than maceration extraction. These restults could provide data support for future research of *S. behen*.

Key Words: *S. behen*, extraction methods, antioxidant

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ORAL PRESENTATION ABSTRACT

COMPARISON OF CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF *HYPERICUM PERFORATUM* MACERATES PREPARED WITH AVOCADO, HEMP, COCONUT, AND OLIVE OILS

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Hypericum perforatum L., Hypericaceae, is renowned globally for its therapeutic properties such as wound healing, antidepressant, and antimicrobial effects. It has been acknowledged by major health regulators like the European Medicines Agency and the World Health Organization [1]. The study focuses on the impact of different oil bases on the properties of *H. perforatum* oil macerates, using oils which are avocado (h4), hemp (h5), coconut (h1), and olive from Sapanca (h2) and Antalya (h3). The oils were sourced commercially and the *H. perforatum* was collected from Sapanca, Türkiye. *H. perforatum* was macerated each oil separately using methods from the German pharmacopeia [2]. The study involved analyzing fatty acid profiles via GC-FID/MS, and various *in-vitro* assays to evaluate the bioactivities of the macerates. These included measuring total phenolic and flavonoid contents, CUPRAC, DPPH and ABTS activities, and enzyme inhibitory activities against acetylcholinesterase, butyrylcholinesterase, and tyrosinase. The results indicated specific fatty acid prevalence in the oils: oleic acid was dominant in (h2), (h3), and (h4); linoleic and oleic in (h5); and lauric and myristic in (h1). The phenolic content was higher in (h3) (36.84±2.33 µg PEs/mg) and /h1) (35.75±0.95 µg PEs/mg), whereas the flavonoid content was higher in (h4) (43.93±0.51 µg QEs/mg) and (h2) (40.79±0.38 µg QEs/mg). None of the oils showed activity in the DPPH and ABTS assays, but h2 exhibited higher activity in the CUPRAC assay. Hemp oil (h5) displayed notable inhibitory activities against AChE, BChe, and tyrosinase with inhibition range values of 46.22±0.13%, 28.87±0.23%, and 47.25±0.69%, respectively. The study hypothesizes that hemp oil (h5) may contain high levels of phloroglucinols, which could explain its robust biological activities. Ongoing analysis aims to further uncover the distinct chemical and therapeutic properties of these macerates, particularly those using (h5), to enhance our understanding of their potential benefits in various medicinal formulations.

Key Words: Hypericum perforatum, oil macerate, hemp seed oil, fatty acid composition, GC-FID/MS, in-vitro biological effects.

ORAL PRESENTATION ABSTRACT

BOTANICAL MARVELS OF THE MEDITERRANEAN: UNRAVELING SALVINORIN A AND ITS NEUROBIOLOGICAL MECHANISMS

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For thousands of years, psychedelic plants have been integral to spiritual practices and medical treatments. The current surge interest in psychedelic substances for mental health treatment necessitates research into their neurobiological mechanisms. Studies suggest their potential in anxiety reduction, cognitive enhancement, and addiction treatment when administered appropriately. Certain Salvia species indigenous to the Mediterranean and surrounding regions contain Salvinorin A, a unique psychedelic compound. Research demonstrates the presence of Salvinorin A in Salvia species such as *S. divinorum, S. recognita, S. cryptantha, S. glutinosa, and S. multicaulis,* indigenous to the Mediterranean region. Salvinorin A is a selective kappa opioid agonist with no activity at the 5-HT2A serotonin receptor, the primary site of activity for classic hallucinogens. Animal studies have delineated its pharmacological, behavioral, and discriminative effects, sparking interest in investigating kappa opioid mechanisms in neurological disorders, psychiatric disorders, pain, and substance addiction. Salvinorin A's psychedelic effects include heightened perceptions, vibrant colors, body distortions, hallucinations, dysphoria, and feeling of disconnection from reality. High doses may lead to temporary memory loss and altered consciousness. Prolonged use may result in side effects akin to those observed with LSD use, including depression and symptoms resembling schizophrenia. Salvinorin A stands as the first non-nitrogenous selective κ opioid receptor agonist and the initial non-alkaloidal hallucinogen. Its potent κ opioid receptor agonist properties suggest that its hallucinogenic effects stem from κ opioid receptor activation. Modifications to the Salvinorin A scaffold have also facilitated the development of selective μ opioid receptor agonists, hinting at the potential for manipulating its chemical structure for designing novel receptor-specific ligands. Further investigations involving κ agonists and antagonists are likely to shed light on the therapeutic potential of the κ opioid receptor–dynorphinergic system in treating psychiatric disorders associated with hallucinations, such as schizophrenia and Alzheimer's disease, as well as various mood disorders.

Key Words: Psychedelic plants, Salvinorin A, neurobiological mechanisms, κ-opioid receptor, mediterranean botany, psychiatric disorders

ORAL PRESENTATION ABSTRACT

MEDICINAL PLANTS FOR THE MANAGEMENT OF INFLAMMATORY BOWEL DISEASE IN TRADITIONAL PERSIAN MEDICINE

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Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, is a chronic inflammatory condition in intestines with a complicated pathophysiology. Current pharmacotherapy can partially manage the disease; however, not all patients are satisfied with the treatments. Traditional medicines can play a promising role in the management of chronic diseases, such as IBD. Traditional Persian Medicine (TPM) is one of the oldest medical doctrines with a long history, turning back to thousands of years ago. Current research provides an overview of medicinal plants introduced in TPM, for the management of IBD. In the TPM textbooks, e.g. "*the Canon of Medicine*" and "*Treasury of Drugs*", terms such as "*Zaheer*", "*Sahj*/*Qorhe Amaa*" describe conditions similar to IBD and thus, related treatments can be considered as possible IBD therapies. Two main groups of medicinal plants are recommended in TPM for IBD, including those with a high content of mucilage and those containing high amounts of phenolic compounds, especially tannins. Fenugreek, linseed, frankincense, pomegranate, apple, and mastic are amongst the medicinal plants recommended in TPM for IBD which are also investigated in modern preclinical and clinical studies and have demonstrated significant beneficial effects. Anticolitis effects of these plants are mediated via different mechanisms, including but not limited to, modulation of nitric oxide-related pathways, Toll-like receptors, endogenous antioxidant enzymes like superoxide dismutase and catalase, and inhibition of pro-inflamamtory cytokines. Overall, TPM suggests a large number of medicinal plants for the treatment of IBD, some of which are also confirmed in modern studies. Thus, this medical doctrine is worth being investigated in future studies to provide a higher level of clinical evidence in order to suggest more treatment options as complementary and alternative therapies in IBD.

Key Words: Persian Medicine, colitis, IBD, Iran, intestine, ulcer

ORAL PRESENTATION ABSTRACT

COMPARING THE CHEMICAL COMPOSITION AND CYTOTOXIC ACTIVITY PROFILES OF NEW *FERULA* PLANTS FROM *MERWIA* SECTION GROWING NATIVE IN TÜRKİYE

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Ferula is a genus that belongs to the Apiaceae family with more than 200 members and grows widely in Asia and Mediterranean countries[1]. The roots and resin of *Ferula* species have been used for cancer treatment by local people and herbalists since ancient times[2,3]. The plants are rich with oleo-gum resin, including miscellaneous biologically active secondary metabolites such as sesquiterpenes, sesquiterpene ethers, sesquiterpene esters, and sulfur-containing compounds[4,5]. Recently, *Ferula* species of the *Merwia* section of the subgenus *Narthex* growing in Anatolia have been explored, and two new taxa, *Ferula latialata* Akalın, Miski, and Tuncay, and *Ferula turcica* Akalın, Miski, and Tuncay were added to the Flora of Türkiye[6]. These *Ferula* species were investigated in terms of their secondary metabolites and cytotoxic activities. Dichloromethane and methanol extracts of the plant materials were obtained by sequential Soxhlet extraction. Analytical thin-layer chromatography and LC-MS analyses were used to compare the secondary metabolite profiles of extracts. The cytotoxic activities of the extracts were screened through cell viability in vitro MTS bioassay. Human umbilical vein endothelial (HUVEC) cell lines were used to identify the cytotoxic effect of extracts on the non-cancerous cell lines. The DCM extracts of *Ferula latialata*, *Ferula turcica*, and *Ferula szowitsiana* showed significant cytotoxic activities against leukemia (K-562) and breast (MCF-7) cancer cell lines differently. This study helps to understand that the extracts from three close species from the same section significantly differ in chemical constituents and cytotoxic activities. Thus, preliminary investigation of the cytotoxic compounds of the *Merwia* section using bioassay-guided fractionation and elucidation of their structures using spectroscopic techniques led to the chemotaxonomic evaluation of *Ferula* species from the *Merwia* section growing in Anatolia and identification of two new species. Phytochemical and bioactivity investigations of the *Ferula* species of the *Merwia* section growing in Türkiye are currently in progress.

Key Words: *Ferula*, *Merwia*, *Cancer*, Cytotoxic activity, Sesquiterpene ethers

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ORAL PRESENTATION ABSTRACT

THE IMPORTANCE OF THE QUALITY OF MEDICINAL PLANTS UTILIZED BY PUBLIC: MARKET EVALUATION WITH DIFFERENT LINDEN SAMPLES

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Pharmacopoeias are official sources with monographs that outline the requirements for components, either synthetic or natural, that are utilized medicinally or as an excipient in a composition. The state government has approved these source books, which have been updated over time based on the developments in technology and the additional, supplementary ingredients to natural products. The only way to guarantee the quality, dependability, and efficacy of medicinal plants and the products derived from them is to assess these materials in accordance with recognized criteria [1]. Linden (*Tilia* L.) is a deciduous tree of the family Malvaceae, found in the temperate zone of the Northern Hemisphere. The drug is obtained by collecting the flowers together with the bracts [2]. Linden flowers are still used as diaphoretic, diuretic, antispasmodic and sedative to relieve the symptoms of the common cold [3, 4]. Linden is recorded in European Pharmacopoeia, Turkish Pharmacopoeia, British Pharmacopoeia, Iranian Pharmacopoeia and many other national pharmacopoeias and German Commission E monographs.

Within the scope of the study, macroscopic and microscopic examinations, thin layer chromatography analysis, foreign matter, loss on drying and total ash tests were carried out on 21 different plant materials obtained from local markets, herbalists and some cultural gardens in Samsun, Türkiye. The tests conducted in this study were carried out according to the Turkish Pharmacopoeia (2017). None of the samples fully met the standards given in the Turkish Pharmacopoeia (2017), showing the low quality of herbal drugs sold in the market and used for medicinal purposes by public.

Key Words: Pharmacopoeia, Quality control, Herbal teas, Linden

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ORAL PRESENTATION ABSTRACT

THE BEE PRODUCTS AND THE BROAD-SPECTRUM ACTIVITIES

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The honeybee (*Apis mellifera* L.) products include honey, propolis, royal jelly (RJ), bee venom, bee pollen, and beebread. These products have been used in traditional medicine for thousands of years, and there is an increasing interest in their applications in modern medicine. For instance, bee pollen has served to prevent and treat many chronic diseases, especially metabolic disorders, and in particular diabetes, obesity, hyper-dyslipidemia, and other cardiovascular disorders. The health and nutritive values of bee pollen was attributed to its physicochemical compositions, i.e. (water, protein, and lipid content) and techno-functional properties (protein solubility, carbohydrate solubility, and emulsifying ability) promoting its wide implications [1,2]. Bee venom, on the other hand, has attracted a lot of attention due to its wide range of bioactive components such as melittin, and secapin (**Fig 1**). In the current talk, we will explore the possible antioxidant and apoptotic activities of melittin against paraquat-induced lung injuries in mice. Melittin exerted potentially protective antioxidant effects by increasing superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities and decreasing lipid peroxidation and nitric oxide (NO) levels. Melittin also exhibited anti-apoptotic effects by increasing B-cell lymphoma-2 (Bcl-2) and survivin expressions. Moreover, it declined the expression level of Ki-67 in the lung tissue [3]. Recently, beehive air therapy is recognized as a potential remedy for treating asthma, bronchitis, lung fibrosis, and respiratory tract infections. Countries in which beehive air therapy is currently authorized include Germany, Hungary, Slovenia, and Austria. However, scientific evidences of its efficacy are lacking which warrants further chemical and biological analyses as a proof of concept [4]. Similarly, the synergistic interaction of RJ with commonly used cancer chemotherapy is discussed, either through its ability to ameliorate the adverse effects of the drugs, or through the enhancement of the drug anticancer-potential. These unique properties of RJ, besides possessing minimal toxicity, make it the best choice to be combined with anticancer drugs. The significant protection properties of RJ against different types of cancer was also attributed to its active compounds. Equally interesting, RJ alleviates menopausal symptoms by readjusting the hormonal concentration, promoting the reproductive performance in polycystic ovarian syndrome, counteracting infertility, and reducing the oxidative stress of the rats productive systems [5].

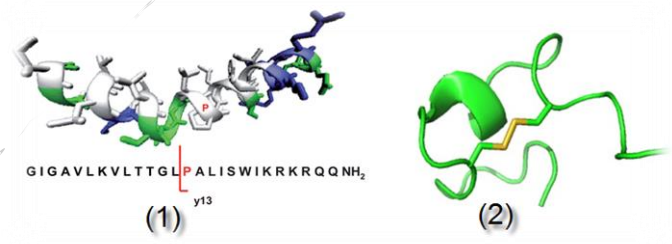

Fig 1. bioactive compounds from bee venom; (1) melittin and (2) secapin

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ORAL PRESENTATION ABSTRACT

INVESTIGATING OF THE PHARMACOLOGICAL POTENTIAL OF SAGE (*Salvia* spp.) ESSENTIAL OILS: ANTIPROLIFERATIVE AND CHOLINESTERASE INHIBITORY ACTIVITIES

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The genus *Salvia* L., a prominent member of the Lamiaceae family, comprises a wide range of species worldwide, with over 100 species identified in Turkiye, many of which are endemic to the region. Salvia species are rich in essential oils (EOs) and have a long history of use in folk medicine for various purposes such as wound healing, pain relief, sedation, expectorant, cholesterol-lowering, sore throat relief, inflammation, and enhancing cognitive function. Ethnobotanical records indicate that sage species has been utilized for its sedative, diuretic, antihyperglycemic, antiviral, antioxidant, anticancer, and antiseptic properties in Anatolian folk medicine. This research aimed to investigate **antiproliferative and cholinesterase inhibitory activities EOs of some sage species, including** *Salvia aramiensis* Rech.f. (SA), *Salvia fruticosa* Miller (SF), and *Salvia officinalis* L. (SO). The aerial parts of these sage species were dried and subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to obtain the EOs. Yields of EOs were 0.39%, 0.35%, and 0.47%, for SA, SF, and SO, respectively. Inhibitory activities of the EOs against cholinesterase enzymes (AChE and BChE) were determined using a slightly modified version of Ellman's method [1,2]. Galanthamine hydrobromide (Sigma, USA) was used as the reference drug in both experiments, which were performed in triplicate. The percentage of inhibition of AChE and BChE was determined and IC_{50} values were calculated. The EOs of SO exhibited the highest AChE and BChE inhibition, with the lowest IC_{50} value of 33.10±1.32 and 42.09±1.76 µg/mL, respectively. EOs of SA showed the lowest cholinesterase activity against AChE (74.50±2.08 µg/mL), while SF demonstrated the lowest cholinesterase activity against BChE (65.01±0.98 µg/mL, $p< 0.01$). Namely, tested EOs displayed remarkable cholinesterase inhibitory effects. Furthermore, the antiproliferative activity of the EOs was screened on colon cancer cell lines, including HCT-116 and HT-29. The cell lines were treated for 48 h and 72 h with 20 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL dilutions of *the* EOs, which were diluted in DMSO. Cell proliferation was assessed using the MTT assay, as previously described [3,4]. The EOs of Salvia spp. exhibited a promising dose- and time-dependent inhibition of cell growth on the cancer cells with low IC_{50} values with a high safety profile against the normal Vero cell line compared to the positive control Doxorubicin. Among the tested EOs, SO demonstrated the most potent antiproliferative activity both on HCT-116 and HT-29 with IC_{50} values of 38.02±0.40 and 22.36±2.07 µg/ml ($p< 0.01$), respectively, whilst EOs of SA was showed the lowest activity on the cells. Consequently, this study provides insights into the potential pharmacological properties of Sage essential oils, particularly in the context of their impact on cell proliferation and cholinesterase enzymes. Accordingly, it can be concluded that EOs of SA, SF, and SO could be promising neuroprotective and antiproliferative agents, which should be verified through further in vivo studies.

Keywords: Salvia spp., Sage, neuroprotective, antiproliferative, bioactivity, colon cancer, essential oil, Anatolian folk medicine

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ORAL PRESENTATION ABSTRACT

EVALUATION OF CYTOTOXIC AND ANTI-INFLAMMATORY ACTIVITIES OF SOME *XANTHOPARMELIA* (VAIN) HALE SPECIES DISTRIBUTED IN TÜRKİYE

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The history of using lichens in the treatment of diseases dates back quite far. Through research, ethnobotanical uses have been identified for approximately 60 different lichen taxa. *Xanthoparmelia* (Vain) Hale (Parmeliaceae) is the largest genus of foliose lichens, represented by more than 800 species worldwide. Traditional uses of the *Xanthoparmelia* genus include treatment of impetigo, oral ulcers (thrush), dental caries and gum problems, syphilitic rashes, healing of snakebites, rheumatism, aphrodisiac effects, treatment of male impotence, blurry vision, uterine bleeding, wound healing, and chronic dermatitis. Usnic acid, gyrophoric acid, psoromic acid, fumarprotocetraric acid, protocetraric acid, stytic acid, norstic acid and salazinic acid are the most common lichen acids in *Xanthoparmelia* species and have many biological activities.

In our study, 5 species of *Xanthoparmelia* (*X. verrucilifera, X. glabrans, X. loxodes, X. stenophylla, and X. conspersa*) were extracted using 70% methanol at room temperature. The cytotoxic effect of the extracts on RAW 264.7 cells was determined, and then the levels of nitric oxide, TNF-α, and PGE2 were measured. Additionally, MCF-7 and MDA-MB-231 cell lines were used to determine their cytotoxic effects. According to the results obtained, the *X. verrucilifera* extract was evaluated as more active compared to other extracts both in terms of its effect on nitric oxide levels and its effect on inflammatory cytokines. All extracts were found to be more effective at higher concentrations in a dose-dependent manner. Furthermore, lichen extracts strongly inhibited viability in both cell lines at concentrations of 500 and 1000 µg/mL.

Key Words: *Xanthoparmelia*, cytotoxic, lichens, anti-inflammatory

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ORAL PRESENTATION ABSTRACT

GC-MS ANALYSIS OF *TROPAEOLUM MAJUS* L. (GARDEN NASTURTIUM) VOLATILE COMPONENTS AND THEIR BIOLOGICAL ACTIVITIES

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Essential oil from *Tropaeolum majus* L. aerial parts, a plant native to North Western Algeria, was obtained by hydrodistillation. The oil volatile components were identified by a combination of gas chromatography/flame ionization detection (GC/FID), GC-mass spectrometry (GC-MS) techniques, and NMR spectroscopy. Nine components representing 92.0 % of the essential oil total (GC/FID chromatogram) were identified. The most abundant compounds were benzyl isothiocyanate (82.5 %), benzene acetonitrile (3.9 %) and 2-phenylethyl isovalerate (2.9 %). Higher content in nitrogen- and sulfur-containing compounds accounting to 86.4 % of the volatile fraction composition of *T. majus* were quantified. Antioxidant capacity was assessed by *in vitro* tests using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric-reducing antioxidant power (FRAP) assay. The volatiles showed strong activity against the radical DPPH, compared with ascorbic acid. In FRAP assay the ability of volatile fraction to reduce ferric ions was determined. Furthermore, volatile components exhibited a strong antimicrobial activity, especially against two strains and five fungi: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Fusarium oxysporu*, *Fusarium solani*, *Fusarium redolens*, *Aspergillus flavus* and *Alternaria dauci*a. The findings showed that the studied volatiles have antimicrobial activity, and thus great potential for their application in food preservation and natural health products.

Key Words: *Tropaeolaceae* ; *Tropaeolum majus* L., volatiles, GC, GC-MS, NMR, antimicrobial activity, antioxidant capacity

ORAL PRESENTATION ABSTRACT

ZEBRAFISH AS A POWERFUL TOOL IN PHARMACOGNOSY

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Research on natural product drug discovery plays a crucial role in the pharmaceutical industry by facilitating the isolation of novel compounds with diverse structures and a wide range of bioactivities. Bioactivity-guided isolation studies in which the assays are based solely on cells and/or enzymes do not always accurately reflect the bioactivity in living organisms. To overcome this limitation, the field requires more powerful assays that utilize biologically intact models. Zebrafish (*Danio rerio*) emerges as a highly promising model in pharmacognosy research due to its unique advantages, such as small size, transparent embryos and larvae, high reproductive rate, and ethical adherence to the principles of replacement, reduction, and refinement (3R) compared to other *in vivo* models. This approach facilitates the generation of accurate and reliable data on bioactivity screening studies via various disease models, including those related to the cardiovascular system, metabolic syndrome, neurological disorders, immune system dysfunction, and inflammation. This presentation offers insights into bioassay-guided isolation, particularly emphasizing zebrafish-based bioactivity screening studies. The utilization of zebrafish models in pharmacognosy holds promise for natural product discovery. Advancements in preclinical studies using zebrafish models facilitate the development of novel drug candidates.

Key Words: Pharmacognosy, natural product discovery, bioactivity screening, zebrafish model, *in vivo* studies

ORAL PRESENTATION ABSTRACT

OBESITY: THE IMPORTANCE OF PHYTOTHERAPY IN TREATMENT

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Obesity has experienced widespread prevalence recently, becoming one of the health issues faced by populations worldwide. The aim of this study is to raise awareness about it. To achieve this, a questionnaire was distributed to 50 obese individuals, divided into two parts: the first part included general questions to identify the individual, and the second part focused on specific questions regarding obesity and its phytotherapy. The results showed that the majority of the respondents were women, aged 15 to 55, coming from different professional backgrounds, with most being university students, residing in urban areas. They also indicated that the majority of them were unaware of the Body Mass Index (BMI) (58%) and that most of them had started gaining weight during childhood (44%). Furthermore, our study revealed that poor dietary habits were the main cause of the spread of this disease (90%). All respondents were aware of the various diseases associated with it, including diabetes and high blood pressure, which prompted the majority of them to take measures to preserve their health. They opted for several methods to achieve this, including diet, exercise, healthy eating, and the use of medicinal plants (6%), the most commonly used being: incense, green tea, mugwort, ginger, cinnamon, fenugreek, basil, rosemary, and turmeric; as they confirmed the effectiveness of most of these plants.

Key Words: Obesity, BMI, diseases, diet, medicinal plants, effectiveness

ORAL PRESENTATION ABSTRACT

ASSESSMENT OF ALLELOPATHIC POTENTIAL OF A SPONTANEOUS PLANT ON VARIOUS OASIS WEEDS

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Allelopathic effects of *A. campestris* leaves were studied on *C. annuum* (Chili) and associated weeds: *Setaria verticillata*, *Phragmites communis* and *Cynanchum acutum*, through the pulverization of its aqueous extracts (at 5, 10, and 20%), in pot experiments. Results indicated that the water extracts inhibited the germination potential, germination rate, seedling height, root length ; Water level ; fresh weight and dry weight of the three plants, with increasing extract concentration further enhancing the inhibitory effect. The pulverization treatment was futile for chilli growth, with organ length remaining steady at all concentrations. However, it has been very harmful to weeds, which were totally scorched at 20%. The allelopathic effects of the water extracts from *A. campestris* leaves on the three receptor plants varied in strength from strong to weak as follows: *S. verticillata* > *C. acutum* > *P. communis*. Thereby, *P. communis* exhibited strong resistance to the allelopathic effects of *A. campestris* and could be cultivated in areas where *A. campestris* occurs. The significant impact of the water extract from *A. campestris* leaves on the seed germination and seedling growth of *S. verticillata* was evident. Thus, *A. campestris* emerges as a species rich in allelochemical compounds, given its allelopathic potential. The possibility of using its aqueous extract by spraying shows that it could be an ecofriendly approach to exploit its valuable allelochemicals.

Key Words: *A. campestris*, allelopathic effect, pulverization

ORAL PRESENTATION ABSTRACT

DEVELOPMENT OF A NUTRITIOUS BEVERAGE UTILIZING PUMPKIN BY-PRODUCTS

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Incorporating a healthful beverage enriched with particular seeds into one's dietary regimen holds pivotal promise in averting malnutrition and mitigating a range of afflictions. Noteworthy for their versatile therapeutic attributes, pumpkin seeds, classified under the *cucurbitaceae* family, emerge as a valuable botanical resource. This inquiry embarked on the formulation of a nutritious juice, enhanced with either pumpkin seeds or flax seeds, followed by a rigorous examination of its physical, chemical, and microbiological attributes. The foundational blend, comprising spinach, banana, fresh cucumber, ginger, and water, was meticulously blended to attain the desired texture. For the enriched variant, a precise measure of either pumpkin seed or flax seed powder was incorporated and blended to achieve optimal consistency. Discerning the physicochemical properties revealed variations in viscosity, density, soluble sugar content, and total protein content. Notably, the flax seed-enriched juice exhibited the highest viscosity at 905.54 mPa/s, while the control juice demonstrated the lowest at 789.55 mPa/s. All samples exhibited similar densities, approximating 20 g/cm³. The enriched juices demonstrated substantially higher total protein content (15.2 g/100ml for pumpkin seed-enriched and 15.6 g/100ml for flax seed-enriched) compared to the control juice (7.7 g/100ml). In addition to these findings, the pH analysis, extended over a period of 7 days, revealed intriguing dynamics. The control juice exhibited stability over the initial 2 days, maintaining a pH between 5.7 and 5.6, but then exhibited a steady decrease, reaching 3.8 by day 7. In contrast, the flax seed-enriched juice displayed superior stability, with a pH reduction of only 1 unit between day 0 and day 7 (from 5.8 to 4.8, respectively). Notably, the pumpkin seed-enriched juice showcased the highest pH after 7 days of conservation, registering at 5.22, underscoring its stability in comparison to the other variants. These findings collectively underscore the considerable influence of pumpkin seed and flax seed enrichment on the physicochemical attributes of the juice, and highlight the notable stability exhibited by the pumpkin seed-enriched juice in terms of pH over a 7-day conservation period.

Key Words: Enriched juice blends, pumpkin & flax seed powder, microbiological analysis, nutritional enhancement

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ORAL PRESENTATION ABSTRACT

KARYOLOGICAL ANALYSIS OF SOME *CENTAUREA* TAXA

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Centaurea L. (Asteraceae) is a well-known genus in ethnomedicine due to its various pharmacological properties. Although the genus *Centaurea* is taxonomically problematic, the number of taxa of the genus is increasing day by day, especially in Asia Minor, indicating that the genus is still evolving in terms of speciation. Various methods and approaches have been applied by different researchers for a long time to solve taxonomic problems in the genus *Centaurea*. One of these approaches is karyological analysis. KAMERAM program was used for karyotype measurements of the examined taxa and the taxa were compared in terms of asymmetry indices (CV_{CL}, CV_{CI}, AI and M_{CA}). In this study, chromosome morphologies of taxa are presented. The basic chromosome numbers of *Centaurea inexpectata* Wagenitz (*x*=11), *Centaurea athoa* DC. (*x*=10), *Centaurea stapfiana* (Hand.-Mazz.) Wagenitz (*x*=9) and *Centaurea babylonica* (L.) L. (*x*=8) located in different sections within the genus *Centaurea* are consistent with previous reports. The karyotypes had a predominance of metacentric (m) chromosomes. Among the taxa examined, *C. babylonica* was found to have the highest asymmetry index. Comparison of the karyotypes can provide valuable information about phylogenetic relationships and chromosome evolution between related species.

Key Words: Asteraceae, karyomorphology, Türkiye.

ORAL PRESENTATION ABSTRACT

CYTOTOXIC POTENTIAL OF *DORYCNIUM SANGUINEUM* EXTRACTS

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In recent years, many researchers have focused on medicinal plants as they are natural resources that represent significant potential in the development of new and pharmacologically active compounds. A wide range of disorders, from diabetes to cancer, are treated and/or prevented by using plant extracts/phytochemicals obtained from different plant parts (leaves, flowers, bark or others) via various techniques. There are 13 species of the genus *Dorycnium* worldwide, including 7 species in Turkey, and it is a member of the Fabaceae. There are relatively limited investigations on *Dorycnium* species, even though numerous studies on the various biological activities of Fabaceae species have been conducted. This study aims to investigate the cytotoxic potential of endemic *Dorycnium sanguineum* extracts using different extraction methods. For this purpose, the extracts obtained by maceration and infusion techniques were applied to different cancer cell lines (HL-60 and DLD-1). The cytotoxic potential of the extracts was evaluated via the MTT test and their cytotoxic potentials were determined by comparing the absorbance values of the control group. It was found that the extracts had dose- and time-dependent cytotoxic effects and the methanol maceration extract had the highest potential. Although this is a pioneering study evaluating the cytotoxic capacity of *D. sanguineum* extract, further research is required to elucidate the molecular basis of this potential.

Key Words: Endemik, MTT, Kızıl Kaplanotu

ORAL PRESENTATION ABSTRACT

DETERMINATION OF HEDERACOSIDE C AND A-HEDERIN CONTENT OF VARIOUS *HEDERA HELIX* L. EXTRACTS

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Hedera helix L. has anti-inflammatory, anti-spazmodic and antibacterial properties. Its effect on bronchitis has been proved and been in use for the treatment of mild infections of upper respiratory tract especially on patient with excessive secretion of viscous mucus. Pharmacological properties of *H. helix* L. has been attiributed to hederacoside C and α-hederin. Obtainment of standardized extracts with high pharmaceutical value starts with high quality plant material itself along with extraction solvent.

In this study, *H. helix* L. leaves from two different locations -Yalova and Rize/Turkey- were used in order to determine climate effect on hederacoside C and α-hederin content. *H. helix* from Rize/ Turkiye (HHR) subjected to maseration with 30% ethanol and 80% methanol while *H. helix* from Yalova (HHY) macerated with distilled water, methanol, 80% ethanol, 30% ethanol and hexane: dichloromethane:80%methanol sequentially. All of the maceration procedures took place in shaking incubator for 3 days. HHY also went under infusion and decoction in order to mimic traditional use. All of the extracts freeze-dried following evaporation of organic solvent.

RP-HPLC was used for quantification of hederacoside C and α-hederin simultaniously in all aforomentioned extracts. C-18 column (150mm x 2,1mm, 5 micron) was used for the measurments and gradient Acetonitrile:Water (H_3PO_4, pH: 2) was chosen as mobile phase. In conclusion HHY showed higher yield in terms of hederacoside C yield than HHR. Amoung the tested extracts, 80% methanol from sequential maceration, 80% methanol and infusion extract showed higher hederacoside C content than others.

ORAL PRESENTATION ABSTRACT

SUSTAINABLE RAW MATERIAL FOR PHARMACEUTICAL, COSMECEUTICAL AND FOOD INDUSTRIES: BLACK CUMIN OIL RESIDUE

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Black cumin (*Nigella sativa* L.) is a of important medicinal and aromatic plant which is known for its therapeutic properties and nutritional benefits. Cold-pressed oil of the plant seeds has gained attention in various industries, including pharmaceuticals, cosmeceuticals, and food in the last decades. During the cold -pressed oil production black cumin oil generates a significant amount of residue with low oil content. This sustainable by-product is thought as a sustainable raw material for numerous industries. In this study, chemical and biological analysis of the residues were made. According analysis results, we found that black cumin residue after cold-pressed oil extraction had more mineral content and phenolic content than the pure black cumin oil. Overall, this study highlights black cumin cold-pressed oil residue as a promising and sustainable raw material with multiple applications in pharmaceutical, cosmeceutical, and food industries, paving the way for innovative and environmentally conscious product development.

Key Words: Black cumin, by-product, phenolic content, oil, residue, mineral content

ORAL PRESENTATION ABSTRACT

COMPARATIVE MORPHO-ANATOMICAL CHARACTERIZATION OF SOME MEDICINAL AND AROMATIC LAMIACEAE SPECIES FROM ALGERIA

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The family Lamiaceae is a key group within Eudicots including many traditional medicinal herbs. The traditional uses of Lamiaceae speciesrange from perfumery to phytoteraphy due to their efficient essential oils. However, in Algeria, the taxonomy of this group is still controversial due to high polymorphism of taxa expressed at morphological and ecological levels. Plant materials from *Lavandula*, *Origanum*, *Rosmarinus* and *Thymus* species were sampled in various bioclimatic locations in Algeria, then were subjected to morpho-anatomical investigation. The objective of our work is the taxonomic characterization and the evaluation of secretory structures polymorphism. A significant diversity and density of secretory tissues has therefore been demonstrated. The external secretory tissues (glandular trichomes and simple hairs) as well as the internal secretory tissues (secretory ducts) are evaluated in all the studied species. We therefore attempt to understand the evolutionary implications.

Key Words: Labiatae, Algeria, anatomy, morphology, taxonomy, evolution

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This research on rare and endemic plants is part of the PRFU project D00L05UN160420230001, at the Laboratory of Organismic Biology and Physiology.

ORAL PRESENTATION ABSTRACT

CHANGES IN ANTIBODIES TITERS OF CHICKENS INJECTED WITH *CALENDULA OFFICINALIS* AND *ECHINACEA ANGUSTIFOLIA* EXTRACTS

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Cell-mediated reactions represent one of the major components of the immune response against environmental microbiome in all species [1]. When deficient, the adequate restoration of the cell-mediated immunity could involve immune stimulating or modulating compounds, inclusding plant extracts [2]. This research aimed at quantification of antibody dynamics and evaluation of antihemation antibody classes against thymo-dependent antigens in chickens subjected to *in vivo* stimulation with alcoholic extract of *Calendula officinalis* and *Echincea angustifolia*. Four equal experimental groups (n=7) of 47 days old chickens were subjected to the experimental protocol on days 0 and 7: group I - treated with 0.5 ml of an alcoholic extract of *C. officinalis*, II - treated with 0.5 ml of an alcoholic extract of *E. angustifolia*, III- solvent control 70º alcohol, IV- environment control. All groups were injected with 0.5 ml of a 5% sheep red blood cell suspension (days 0 and 7). Blood was sampled at the beginning, on days 7 and 14 of the experiment on otting gel and the whole sera as well as sera treated with 2-mercapto ethanol were tested for the antibody titers by the hemagglutination test. The results were statistically interpreted (Student's t test). Both extracts significantly ($p<0.01$-0.001) increased the antibody titers (I - 0.60, 1.81, 3.57 and II - 0.17, 1.51 and 3.10 respectively) in the treated groups, while the main component was IgG. The IgM values decreased more over time in the *C. officinalis* but not in the *E. angustifolia* treated group. The results and their statistical significance strongly supported the positive impact of the two plant extract in enhancing the cell-mediated response in chickens, further studies to tailor the *in vivo* dosage being needed.

Key Words: *Calendula officinalis*, *Echinacea angustifolia*, chickens, blast transformation, alcoholic plant extracts

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ORAL PRESENTATION ABSTRACT

SAFETY AND EFFICACY OF VERSION II ACIDOSALUS® PESSARIES COMPARED WITH BIOAPIGYN® PESSARIES FOR THE RELIEF OF BACTERIAL VAGINOSIS SYMPTOMS.

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Objective / Purpose: The objective of the study was assessment of the clinical efficacy and safety of a 7-day treatment of vulvo-vaginal disorders with version II Acidosalus® pessaries compared to Bioapigyn® pessaries used as comparative group.

Materials and methods: 80 females were randomly selected into two groups (each of 40 participant) and treated once a day seven days with either version II Acidosalus® pessaries or Bioapigyn® pessaries. All the patients were subjected to gynecological examination, measurement of vaginal pH, Pap test, native (wet) mount preparation and KOH test, self-assessment and clinical assessment of the symptoms at baseline and following the therapy.

Results: At baseline all 80 participants had abnormal pH values up to 7.5. Following the treatment with Acidosalus pessaries and Bioapigyn pessaries the mean value of the percentage of the total score of vulvo-vaginal disorders decreased for 86.3% and 84.3%, respectively. The percentage of total score for vulvo-vaginal disorders assessed by the investigator decreased for 93.3% and 92.9% following the treatment with Acidosalus pessaries and Bioapigyn® pessaries, respectively. Vaginal pH dropped for 12.3% and 13% after the treatment with Acidosalus pessaries and Bioapigyn pessaries, respectively. Native wet mouth test showed normalization of the vaginal flora following the treatment with all two products with no significant difference between the products. Clinical cure rate was 90% for Acidosalus pessaries and 82.5% for Bioapigyn pessaries. Although, Acidosalus pessaries performed slightly better, there was no significant difference in the treatment efficiency among two tested products. There were no adverse effects reported during the study and monitoring period for neither of the product.

Conclusion/Discussion: Both Acidosalus®and Bioapygin® products were highly efficient in alleviation of the symptoms of vulvo-vaginal disorders caused by elevated vaginal pH. The products created unfavorable conditions for pathogenic growth, adhesion and replication through the lowering of vaginal pH value, promoting the growth of lactobacilli, creating the environment with low water activity and creating a protective layer on the damaged mucosa that creates a physical barrier to the entrance of the pathogens into the cells and enables the recovery of the vaginal mucosa.

Key Words: Acidosalus, Bioapygin, probiotic yeasts, vulvo-vaginal disorders, vaginal pH

ORAL PRESENTATION ABSTRACT

PHYTOFORENSICS AS A TOOL FOR FINDING ADULTERANTS AND CONTAMINANT IN FOOD SUPPLEMMENTS, PLANT EXTRACTS AND BIOLOGIC SAMPLES

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Pollution of the environment has been a continuous challenge for modern life. Phytoforensics and phytoscreening have been adopted as a mean to identify environmental and plant contamination. In the recent years due to the increase in demand for food supplements, numerous suppliers and distributors of products that are not verified in terms of quality have appeared on the market. Rapid and modern hyphenated techniques (such as PDA-UPLC and/or LC-MS/MS) may be a solution for the identification of pesticides, pharmaceutical residues, toxic compounds or synthetic enhanchers in food supplements. Our research used a Transcend TLX-1 Vanquish Flex system coupled with the Orbitrap Exploris 480 high-resolution mass spectrometer and Compound Discoverer 3.2 software for the identification of compounds, part of the CENEMED platform. A fast method was developed (4 minutes), and the chromathograms were subjected to Compound Discoverer software for evaluation in targeted and untargeted modules. The peaks with a rating of at least 7.4 were taken into account and verified against Thermo m/z Vault, NIST, and Chem spider databases. Several plant extracts included or not in food supplemets (*Tribulus terrestris* L., *Lespedeza capitata*, *Perilla frutescens* var *frutescens* f. *crispidiscolor*, *Agastache mexicana*) were screened for the presence of hallucinogen substances, drug residues, and pesticides. The results indicated that the extracts were free of contaminants, however traces of pesticides were detected in *Tribulus* and *Agastache* samples. On the other hand, some biological samples from patients with attempted suicide indicated the presence of some hallucinogenes, pesticides and tranquilizers. All in all, the used methods proved to be fast and reliable and could represent a solution for effective screening of contaminants.

Key Words: Contaminants, toxic compounds, food supplements, intoxication

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ORAL PRESENTATION ABSTRACT

THE ASSSESSMENT OF ENZYME INIBITION EFFECTS OF MORIN AND ITS BETA-CYCODEXTRIN COMPLEX

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Morin is a polyphenol isolated from the leaves and fruits of the *Moraceae* Family, from *Maclura pomifera* and other vegetal sources. These plants are often use in traditional medicine for their antidiabetic, anti-inflammatory, antibacterial, antihypertensive and neuroprotective effects. The aim of our study was the assessment of the enzyme modulatory ability of morin and the complex of morin with beta-cyclodextrin. The enzyme modulating actions were tested on lipoxygenase, alpha-amylase, alpha-glucosidase and acetylcholine esterase. The enzyme activity with or without morin was evaluated by spectrophotometric methods. Lipoxygenase is an enzyme involved in fatty acids oxidation and could induce oxidative stress and inflammation. Alpha-amylase and alpha-glucosidase are enzymes involved in carbohydrates digestion. The inhibition of these enzymes is a good option to reduce post-prandial hyperglycemia. Polyphenolic compounds have the potential to delay post-prandial blood glucose and reduce starch digestion. Acetylcholine esterase catalyses the hydrolysis of acetylcholine and decreases the quantity of this neuromediator. By blocking this enzyme, the acetylcholine will increase and neurons activity is improved. Depending on dose the morin and its beta-cyclodextrin complex reduces or block the lipoxygenase activity. Morin reduces the activity of alpha-amylase and alpha-glucosidase, but the effects are lower than that induced by acarbose. The inhibitory effects are more important for alpha-glucosidase than alpha-amylase. Morin and its beta-cyclodextrin complex reduce the acetylcholine esterase activity, and this effect is more important for the morin - beta-cyclodextrin complex. The effects of morin and its beta-cyclodextrin complex on these enzymes are important in therapy for the patients with diabetes and cognitive impairments.

Key Words: morin, enzyme inhibition, beta-cyclodextrin, antioxidant, acetylcholine esterase

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ORAL PRESENTATION ABSTRACT

INFLUENCE OF PGPB STRAINS ON THE GROWTH, BIOMASS PRODUCTION AND ANTIOXIDANTS OF BASIL (*Ocimum basilicum* var. *Genovese*) FERTILIZED WITH FISH EFFLUENT ORIGINATING FROM AN AQUAPONIC SYSTEM

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Plant Growth Promoting Bacteria (PGPB) are widely used as microbial biostimulants to improve the growth of horticultural crops as well as Medicinal and Aromatic Plants. Indeed, PGPB can upregulate the expression of genes related to cell growth and are responsible of the enhancement of nutrient acquisition, thus could be considered as a better alternative to chemical fertilizers which cause harm to human health and to the environment. For this purpose, the effects of the inoculation with 2 PGPB strains namely *Rhizobium sullae* (RSU9), *Agrobacterium* sp. (JB4) and their dual combination (RA) on basil (*O. basilicum* L. var. *Genovese*) plants fertilized with fish effluent originating from an aquaponic system were determined. Thus, a pot experiment was conducted under glasshouse and many parameters relative to the plant growth (height, branches and leaves number), biomass production (shoot and root fresh and dry weights and the dry matter contents), water status (relative water content and electrolyte leakage) as well as the antioxidant compounds (phenolics, flavonoids and tannins) and activity were assessed.

Main results demonstrated that plants inoculated with the strain RSU9 showed an enhancement of their shoot dry weight by about 6% while the plants inoculated with the combined strain RA significantly increased their root dry weight and the branches number by 36.07% and 25.23% respectively. Additionally, RSU9 significantly enhanced the relative water content (63.6%), meanwhile the highest electrolyte leakage (79.49%) was noted in plants inoculated with RA. This last treatment induced an important improvement (by 99.72%) of the phenolic content, and slightly enhanced (by 2.16%) the tannin content. Besides, the inoculation with the strain JB4 improved the flavonoids content by 9.86%. Overall, results of this study suggest that the use of PGPB could be a promising and environmentally friendly approach for the production of basil plants fertilized with fish effluent originating from an aquaponic system.

Key Words: Basil, PGPB, antioxidants, growth, fish effluent

ORAL PRESENTATION ABSTRACT

RECOVERY, REFINEMENT AND STABILIZATION OF PRESERVING OF HIGH ADDED COMPOUNDS VALUE FROM PUMPKIN BY-PRODUCTS

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PulpIng is an European PRIMA project aimed to the valorisation of Pumpkin by-products as a sustainable approach to reduce food waste in the framework of circular economy in food industries. This fruit *Pumpkin* has been ranked among the most consumed foods in the world, which represents 602 kilos per second, with a world production of 26 million tons per year. However, a massive amount of biomass was unused, because only the pulp is consumed. In this context, the present study aims to optimize the extraction of antioxidants from pumpkin peels by-products (Cucurbita maxima var. Batati, Tunisian variety) to intensify their phenolic composition and to increase their antioxidant capacity. Moreover, the encapsulation of the refined peels extract was optimized using an experimental design plan (RSM design). In addition, physicochemical and biological attributes of encapsulated bioactive compounds from peels, were assessed. Main results displayed that, after performing the statistical analysis, the optimal parameters for extracting antioxidants from the peels were: 12.2% ethanol for 11.2 min at 55°C. Concerning the formulation of high added value compounds from peel pumpkin: Maltodextrin, gum arabic, and concentrated phenolic extract exert a significant influence on both the phenolic content and antioxidant activity. For instance, the system incorporating the lowest concentration of extract in conjunction with maltodextrin and arabic gum demonstrated a significant DPPH scavenging ability, reaching an impressive activity of 74.5%. Once formulation was validated, the optimized encapsulated refined peel extract was characterized for its antibacterial capacity against several bacterial pathogens. In fact, *S. typhimurium* exhibited the highest sensitivity, followed by *E. faecalis*, *P. aeruginosa*, and *S. aureus*. These results are promising for diverse applications such as the incorporation of this formulation to obtain functional foods and nutraceutical products which contribute to enhance health benefits.

Key words: Pumpkin by-product, RSM approach, High added value compounds, Circular economy

ORAL PRESENTATION ABSTRACT

PLANTS USED AS WOUND HEALERS IN FOLK MEDICINE IN TÜRKİYE

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Wound healing is a physiological and dynamic process regulated by molecular, cellular, and humoral mechanisms. The process mainly occurs in four stages; proliferation, inflammation, hemostasis, and tissue remodeling. The skin, the body's biggest organ, acts as a protective barrier against harmful stimuli such as bacteria, UV radiation, allergies, and irritants. It serves as the crucial boundary between the body's internal and exterior environments [1,2]. The restoration of the skin regenerates itself upon injury. The recovery process involves the interaction of many cellular elements (monocytes/macrophages, fibroblasts, keratinocytes, and endothelial cells) and extracellular matrix components including collagen and fibronectin, which help the wound edges come together. Various variables including mechanical stress, infection, or poisonous chemicals can greatly impact the skin's capacity to repair itself [3]. Ethnobotanical investigations conducted using ancient treatment approaches are documented to aid in the advancement of pharmaceuticals. Knowledge about the traditional use of these medicinal or wild plants has been passed down through generations. This study examined theses at the National Higher Education Center and ethnobotanical studies from different regions of Turkey focusing on the usage of regional flora for wound therapy. Extensive ethnobotanical research has identified numerous plants utilized for their healing properties in traditional wound care in Türkiye. Due to their numerous favorable physiological advantages, they have been utilized on the skin for cosmetic and medical purposes for a long time [4]. This study demonstrates that the majority of plants have been utilized as healers for treating wounds.

Key Words: Ethnobotanical research, Medicinal plants, Wound healing, Folk medicine, Türkiye

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ORAL PRESENTATION ABSTRACT

CROCETIN LOADED NANOGELS FOR SKIN CANCER TREATMENT VIA THE TRANSDERMAL ROUTE

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Objectives: The objective of this research is to isolate crocetin from the seeds ethanolic extract of *Nyctanthes arbortristis* Linn to develop crocetin-loaded nanogel to enhance its delivery topically to skin tissues for potential treatment benefits.

Methods: Crocetin loaded nanogels were formulated using a ionic gelation method and optimized by Box-Behnken design. The In vitro cytotoxicity studies were performed in the B16F10 and A-357 cell lines. Permeation studies were performed on mice skin to assess transdermal delivery. DMBA- Croton oil Swiss albino mice model was employed for in vivo assessment. Immunohistochemistry (IHC) was performed to assess NFKBp65 and HDAC1expression semi-quantitatively.

Results and Conclusion: The size of the vesicles for the formulations was between 202±14.2 nm-374±127 nm, while the entrapment efficiency was between 61.22±0.23% - 85.16±0.24%, and the drug release % after 8 h was between 48±0.82%- 76±0.52%. In vitro studies demonstrated the potential of the nanogel in significantly inhibiting the proliferation of skin cancer cells, evidenced by its cytotoxic effects on A375 and B16F10 cell lines. Additionally, Ex vivo studies of the nanogel showcased efficient penetration into deeper skin layers, promising enhanced drug delivery to the tumor site. Furthermore, animal studies conducted on DMBA-induced skin cancer animal models corroborated the therapeutic efficacy of the crocetin-loaded nanogel, revealing marked suppression of tumor growth compared to conventional treatment by marketed gel or untreated groups. These findings supported the potential of this crocetin nanogel formulation as a promising strategy for skin cancer therapy, offering targeted delivery, reduced systemic toxicity, and improved treatment outcomes.

Key Words: *Nyctanthes arbortristis* Linn, Crocetin, Chitosan nanoparticles, Skin cancer, DMBA

Acknowledgements
This research is supported by UGC Non-NET fellowship (Enrollment No.- Y19454001)

ORAL PRESENTATION ABSTRACT

DETECTION AND CHARACTERIZATION OF PHYTOPLASMAS AND THEIR POSSIBLE VECTORS IN THYME (*Thymbra spicata* L.) IN HATAY PROVINCE OF TÜRKİYE

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Thyme is an important crop and export product of Turkiye and accounts for 70% of world trade. Although there are some reports on fungal and viral diseases of thyme plants, limited information is available on phytoplasmas and their potential vectors. Recently some symptoms resembling phytoplasmas on thyme in Hatay province of Turkiye were observed like proliferation, paleness, leaf curling, dwarfing, reddening, yellowing drying of plants, and flower deformations (flowers resembling stars). In the present study, 85 thyme, 10 bindweed (*Convolvulus arvensis*) and 34 potential phytoplasma vector insects belong to *Cicadellidae* family collected from thyme fields in Hatay province tested by Nested-PCR/RFLP analysis for phytoplasma presence and characterization. Among 85 tested thyme plants, 20 were found positive for phytoplasmas as well as 2 bindweeds and 15 insects by nested PCR. Based on RFLP analysis of 16S rDNA sequences, all phytoplasma positive samples were found related to '*Candidatus* Phytoplasma solani' (16SrXII-A) and '*Ca*. P. asteris' (16SrI). Although '*Ca*. P. solani' was previously detected in thyme plants, this study is the first report for the presence of *Ca*. P. asteris worldwide. Detection of the same group of phytoplasmas in thymes, weeds and possible vector insects collected from the thyme fields indicates that these phytoplasmas are most likely transmitted to thyme from different host plants in the environment. Due to phytoplasma diseases of medicinal and aromatic plants severely reduce yield, quality of crops and causing changes in the composition of secondary metabolites, the impact of phytoplasma infections on medicinal and aromatic plants should be taken into account in promoting good agricultural pratices for cultivation and propagation of these plants.

Key Words: Thyme, phytoplasma, weeds, vectors, nested-PCR, sequencing

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ORAL PRESENTATION ABSTRACT

EXPLORING THE POTENTIAL USE OF ARTHROCNEMUM MACROSTACHYUM BIOMASS AS A SALT SUBSTITUTE IN GOAT CHEESE

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This study investigated incorporating *Arthrocnemum macrostachyum* biomass as a salt alternative in fresh goat cheese. We introduced dried biomass at 4 g/L into cheese batches with reduced salt levels of 8 g/L (B1) and 4 g/L (B2), comparing them to a 12 g/L salt control (C). The cheese and *A. macrostachyum* biomass were scrutinized for microbial safety (E. coli, coagulase-positive Staphylococci, 30°C microorganisms, molds, and yeasts), mineral content, and phenolic and flavonoid levels. Moreover, the cheeses were examined for physical and chemical characteristics over 8 days at 4°C. The biomass contained notable quantities of total phenolics (23.76 GAE/g DW) and flavonoids (10.35 mg QE/g DW), with Na, K, Mg, Ca, and Fe being the predominant minerals. The addition of biomass didn't affect cheese processing and yielded a product safe in terms of microbiology, enriched in minerals, and with enhanced bioactive compounds. Notably, the cheese with biomass (B2) presented optimal functional and physical-chemical attributes. Storage time significantly influenced the pH and dry weight. The findings indicate that substituting traditional salt with *A. macrostachyum* biomass in goat cheese could reduce sodium levels while augmenting bioactive phenolic and flavonoid content, potentially offering antioxidant health advantages.

Key Words: Halophytes, functional additives, functional food

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ORAL PRESENTATION ABSTRACT

UNDERSTANDING THE ROLE OF NITRIC OXIDE AND OPIOID SYSTEMS IN THERAPEUTIC EFFECTS OF PERSIAN BISTORT ON THE RAT MODEL OF ACETIC ACID-INDUCED COLITIS

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Background & Objectives: Nitric Oxide (NO) is a messenger molecule produced by the iNOS enzyme during inflammation. It can be both pro-inflammatory and anti-inflammatory and is associated with disease activity in ulcerative colitis. Inhibiting iNOS can improve symptoms of experimental colitis. Mu opioid receptors can decrease symptoms of colitis by suppressing inflammation. Persian bistort, *Persicaria bistorta* (L.) Samp., (*Polygonaceae*), is a plant used in Traditional Persian Medicine for colitis. This study examines whether NO and opioid pathways play a role in its therapeutic effects.
Methods: Bistort root was authenticated (voucher No.: PMP-1239), and an aqueous extract was prepared using a freeze-dryer. The extract's gallic acid content was measured by HPLC. Colitis was induced in 24 rats (ethics approval code: IR.TUMS.MEDICINE.REC.1400.954) through rectal administration of 4% acetic acid. The rats were divided into nine groups, sham, disease control, 700 mg/kg of the extract (PFX) (oral), Aminoguanidine (AG), i.p., N(ω)-nitro-L-arginine methyl ester (L-NAME), i.p., Naloxone, i.p., AG +PFX, L-NAME +PFX, and Naloxone +PFX, and given medications for two consecutive days. They were euthanized on day four. Distal colons were examined microscopically, and TNF-α, TLR-4, and MPO were measured using ELISA kits. RT-PCR was used to measure NF-κB, IL-1β, and IL-6. SPSS.22 was used for data analysis with ANOVA and Tukey's post-hoc test. A significance level of $p < 0.05$ was considered.
Results: The concentration of gallic acid was found to be 2.08 mg/g. After inducing colitis in the control group, a significant increase was observed in the levels of TLR-4, TNF-α, MPO, NF-κB, IL-1β, and IL-6 compared with the sham group ($P<0.001$). Although the levels decreased in the PFX group, which showed the therapeutic activity of the extract, the levels remained elevated in the other groups. This suggests the involvement of both NO and Opioid systems in the effects of the PFX.
Conclusions: The results of the current mechanistic study suggest the significant but complex involvement of the NO and Opioid systems in the therapeutic effects of the bistort rhizome extract. They are involved in the regulation of inflammation and the immune response, and they have potential therapeutic implications. However, their exact mechanisms and effects in IBD require further investigation to fully understand and effectively target these pathways for treatment.

Key Words: Inflammatory Bowel Diseases, Persian medicine, Toll-Like Receptors, Phytopharmacology

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"This is part of the Ph.D. thesis of Niusha Esmaealzadeh which was supervised by Dr. Roodabeh Bahramsoltani at the School of Persian Medicine, Tehran University of Medical Sciences, Tehran, Iran."

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ORAL PRESENTATION ABSTRACT

INFLUENCE OF SOLVENT POLARITY ON PHYTOCHEMICAL PROFILE, ENZYME INHIBITORY ACTIVITY, TOTAL PHENOLIC COMPOUNDS, AND ANTIOXIDANT ACTIVITY OF BLUEBERRY (*VACCINIUM CORYMBOSUM* L.): A COMPREHENSIVE CORRELATION ANALYSIS BETWEEN CHEMICAL PROFILES AND BIOACTIVITY

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Plant products are a virtually unlimited reservoir of bioactive compounds that can be used to develop new candidates for plant-based medicines or functional foods. With their known health-beneficial effects and rich phytochemical content, fruits are an excellent source for the discovery of plant phytochemicals, and blueberries, which are rich in nutrients and polyphenols, have a promising place among them. Herein, phytochemical profiles, enzyme inhibitory activities, antioxidant capacity, and total polyphenol contents (TPC) of polar and non-polar extracts of dried *Vaccinium corymbosum* L. fruits were examined, along with the statistical analysis to determine the main components that are accountable for bioactivity. The phytochemical composition of the extracts was analyzed by LC-MS/MS and GC-MS techniques using 53 phenolic and seven triterpenoid standards. A total of 23 phenolics were detected in both extracts, while no triterpenoids were detected. The inhibitory potential of the extracts was investigated against certain enzymes targeted in the treatment of neurodegenerative disorders (tyrosinase, acetylcholinesterase, and butyrylcholinesterase), ulcers (urease), hyperpigmentation (elastase, and collagenase), and hypertension (angiotensin-converting enzyme).
The maximum enzyme inhibitory activity against ACE (99.75%) and urease (91.66%) was obtained from the non-polar extract. On the other hand, the polar extract showed low ACE inhibitory activity (7.04%), and no activity was observed against urease. The non-polar extract also showed the highest β-carotene bleaching capacity (91.15%), DPPH radical scavenging activity (IC_{50} of 92.92 µg/mL), and TPC (42.13 mg GAE/g). The relationship between each specific phenolic and bioactivity was investigated using principal component analysis and Pearson correlation analysis.

Key Words: *Vaccinium corymbosum* L., Enzyme inhibitory activities, antioxidant capacity, phytochemical profile, Pearson correlation, principal component analysis

ORAL PRESENTATION ABSTRACT

EFFECT OF DIFFERENT ORGANIC FERTILIZERS AND HARVEST TIMES ON THE ESSENTIAL OIL COMPOSITION OF SWEET BASIL (*OCIMUM BASILICUM* L.)

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The effects of different organic fertilizers (vermicompost, guano, biomobilizer- P bacteria, aminoacid and fluid fertilizer) and three harvest times at the same growing season on the essential oil composition of *Ocimum basilicum* L cultivated in 2021 under Eskisehir ecological conditions were evaluated. The experiment was arranged in a randomized complete block design with three replications. Essential oil composition was determined using GC-FID/GC-MS.

The main essential oil compound was linalool and varieted between the different organic fertilizer applications and harvest times. Analysis of the essential oil components showed that the linalool were more pronounced at the biomobilizer- P (bacteria) application compared to control plots. All applications were mainly higher at the first harvest time except of the control plots and the application of fluid fertilizer. Organic fertilizers and especially biomobilizer- P (bacteria) application could be recommended for higher plant quality of *Ocimum basilicum* L.

Key Words: sweet basil, *Ocimum basilicum* L., organic fertilizer, harvest time, essential oil composition.

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ORAL PRESENTATION ABSTRACT

UTILIZING RESPONSE SURFACE METHODOLOGY TO IMPROVE CONSISTENCY IN PHYCOCYANIN EXTRACTION

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Introduction and Objective: This research endeavors to improve the extraction process of Phycocyanin from Spirulina platensis PCC 7345, a water-soluble blue pigment known for its health benefits. Utilizing the Quality by Design (QbD) approach—a variation of response surface methodology—this study identifies Target Product Profiles (TPPs) and Critical Quality Attributes (CQAs) such as concentration and purity.

Methods: The extraction process adopts the Box-Behnken design, guided by risk assessment using Ishikawa diagrams to ensure consistent outcomes. Techniques, including FTIR analysis, SDS-PAGE, and gas chromatography, are employed to validate the quality and molecular composition of the extracted Phycocyanin.

Results: The Phycocyanin obtained exhibits robust antioxidant activity, with IC50 values for DPPH, ABTS, and Nitric Oxide Scavenging activity measured at 40.70µg/ml, 23.25µg/ml, and 17.74µg/ml, respectively. Furthermore, its ability to inhibit α-Amylase and α-glucosidase enzymes in a concentration-dependent manner, as evidenced by IC50 values of 72.24µg/ml and 82.45µg/ml, underscores its therapeutic potential. The application of the QbD approach ensures a high-quality extraction process for Phycocyanin, and the observed antioxidant and enzyme inhibitory properties suggest promising applications in health management. Conclusion: This study contributes valuable insights for optimizing the extraction process and utilizing Phycocyanin effectively, emphasizing its potential as a bioactive compound with multifaceted health benefits.

Key Words: Antioxidant potential, Phycocyanin, Quality by Design (QbD) approach, Response Surface Methodology, Target Product Profiles (TPPs).

ORAL PRESENTATION ABSTRACT

BIOLOGICAL ACTIVITIES OF *THYMUS SATUREIOIDES* AND *THYMUS PALLIDUS* ESSENTIAL OILS

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Plant medicinal properties have garnered a lot of attention in recent years due to their pharmacological activities, and economic viability. Indeed, plant extracts and essential oils possess antifungal, antibacterial, and antiviral properties and have been screened on a global scale as potential sources of novel antimicrobial compounds, agents promoting food preservation, and alternatives to treat infectious diseases.

The main objective of this study is to investigate the biological activities (*Antioxidant, Antibacterial*) of essential oils obtained by hydrodistillation extraction using Clevenger apparatus from aerial parts of *Thymus satureioides* and *Thymus pallidus* .For the purpose of achieving this objective, the micro dilution plating method was utilized for antibacterial activity while the antioxidant ability was assessed using DPPH,FRAP,ABTS assays.

The results showed that *T. satureioides* exhibited a high antioxidant activity, as measured by DPPH ABTS with IC50 values of 0,51 mg/mL and 0,06 mg/mL respectively while FRAP results showed no activity. However, *Thymus pallidus* essential oil showed an inhibitory concentration (IC_{50}, EC_{50}) higher than 2mg/mL for all the tests. On another note, determination of minimal inhibitory concentrations (MIC) revealed that *Thymus satureioides* essential oil demonstrated an inhibitory effect on all bacterial strains in different concentrations. In fact, the highest anti-bacterial activity was observed against *E. coli* (0,06 mg/mL) followed by *E.hirae* (0,5mg/ml), *S. Aureus* (1,5), and finally Pseudomonas with the lowest activity (5 mg/ml).

The results indicated that *Thymus satureioides* essential oil showed a higher biological activity in comparison with *Thymus pallidus* due to its rich chemical composition. These findings suggest the thyme EO they can be used as natural and valid agents in agriculture, food, pharmaceutical and cosmetic industries for the treatment of microbial and fungal infections or contaminations.

Key Words: Phenolic extract, *Thymus satureioides*, *Thymus pallidus*, antibacterial activity, antioxidant activity, human pathogenic bacteria.

ORAL PRESENTATION ABSTRACT

PHYTOCHEMICAL COMPOSITION AND *IN VITRO* BIOLOGICAL ACTIVITIES OF FUMARIA *OFFICINALIS* FROM A TUNISIAN ARID REGION

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Throughout history, humans have utilized plant resources for sustenance, medicinal purposes, and religious rituals. Among these resources, plants offer a wealth of bioactive compounds renowned for their aromatic and medicinal properties.

This study aims to assess the biochemical composition and evaluate the *in vitro* antioxidant, anti-inflammatory, antidiabetic, antiproliferative, and antibacterial activities of leaf extracts of a spontaneous plant growing in a Tunisian arid region, *Fumaria officinalis.* Various extraction methods (maceration and decoction) and solvents (water and ethanol) were employed to obtain these extracts. The biochemical composition was determined by measuring phenolic, tannin, flavonoid, and alkaloid contents, revealing a significant richness in these compounds. Antioxidant activity was evaluated using three tests, with the decoction extract exhibiting the highest total antioxidant activity. *In vitro* anti-inflammatory activity showed moderate activity for both aqueous and ethanol extracts obtained by maceration. Evaluation of *in vitro* antidiabetic activity showed that the aqueous maceration extract presents the most important activity. However, the antibacterial activity of *Fumaria officinalis* against both gram-positive and gram-negative bacteria showed limited efficacy. Besides, results of the antiproliferative activity assessment revealed an important inhibitory effects on the proliferation of breast cancer cells (MCF-7).

In summary, this research aims to highlight the various properties of *Fumaria officinalis* extracts and their potential applications, particularly in the field of healthcare and pharmacology.

Key Words: *Fumaria officinalis*, biochemical composition, anti-inflammatory activity, antidiabetic activity, antiproliferative activity, antibacterial activity.

ORAL PRESENTATION ABSTRACT

BIOPESTICIDES FOR MANAGING DISEASES OF MEDICINAL PLANTS: CURRENT SCENARIO AND FUTURE PROSPECTS

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India is a paradise of large number of medicinal plants. Most of the medicinal plants are growing wild in forest and only few are cultivated for their use in pharmaceuticals industries. Like other plants , medicinal plants too are prone to many diseases caused by fungi, bacteria, viruses, phytoplasma and nematodes. Out of several diseases reported on medicinal plants, soil-borne diseases caused by fungi cause considerable loss in productivity and quality of the produce. Biopesticides based on living microbes and their bioactive compounds have been promoted as replacements for synthetic pesticides for control of plant diseases. However, lack of efficacy, inconsistent field performance, low shelf life and strict regulatory requirements by CIBRC has generally relegated them to niche products. Significant increases in market penetration have been made, but microbial pesticides still only make up a small percentage of disease control products. Thiry four microbes have been included in the schedule to the Insecticide Act 1968 for production of microbial based biopesticides.. While working with some important antagonistic microbes (*Trichoderma* spp.), we have documented the biocontrol ability of these organisms at field level as well as up to the extent of commercialization.

We have started promoting the usage of biopesticide formulations as a component of integrated farming practices with involving farmers of eastern Uttar Pradesh in order to produce pesticide residue free crop. The research on biocontrol agents (BCAs) can be fruitful only when we commercialize and register the product based on superior strains. Biopesticide registration require data on technical and formulation related information such as biological characteristics, pathogenic contaminants, other microbial contaminants, bioefficacy, toxicity, container compatibility and self life etc. To achieve this, certain norms specified by Central Insecticides Board are to be followed. Till date, about 970 microbial based biopesticides products are registered with CIBRC (http://cibrc.nic.in/bpr.doc) under section 9(3B) and 9(3) of the Insecticides Act, 1968 Government of India). None of the biopesticides registered in India have level claim of controlling the diseases of medicinal plants . During the presentation emphasis will be given on organic cultivation of medicinal plants using microbial pesticides in order to increase the farmers income and also to provide pesticide residue free quality raw material to pharmaceutical industries.

ORAL PRESENTATION ABSTRACT

INVESTIGATING THE THERAPEUTIC ACTIVITIES OF NOVEL SELAGIBENZOPHENONE B DERIVATIVES AS PARP-1 INHIBITORS IN THE TREATMENT OF CASTRATION-RESISTANT PROSTATE CANCER

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Selaginella, a diverse genus with a rich medicinal history, has been used in traditional remedies, including for cancer treatment [1]. Based on our previous research, we have identified the potential of some of the Selagibenzophenone B (SB) analogs to selectively target prostate cancer cells [2]. Through in silico studies, we have designed novel SB derivatives and found that they reveal PARP-1 as a promising target. In the case of castration-resistant prostate cancer (CRPC), a type of cancer that is notoriously resistant to conventional treatments [3], there is hope in targeted therapies that focus on inhibiting PARP-1 [4]–[6]. Our objective is to discover compounds that can effectively inhibit PARP-1. The cytotoxic impact of SB derivatives on CRPC and human normal prostate cells was assessed through the SRB assay. Wound healing and colony formation assays were conducted to evaluate the cell migration and invasion capacities of the lead compounds (with $IC_{50} \leq 10$ µM and SI >2). Gene expressions at the mRNA level were examined via Q-PCR to comprehend the type of cell death induced by the derivatives. The potential of these compounds to bind PARP-1 for inhibition was determined by molecular docking analyses. Molecular dynamics simulations were employed to determine the binding free energy of protein-ligand complexes. The lead SB derivatives exhibited selective inhibition of cell survival, migration, and colony formation in prostate cancer cell lines compared to healthy cells. In addition, molecular docking and molecular dynamics studies revealed a high binding affinity between these compounds and PARP-1. The lead compounds were also found to be druggable based on Lipinski and Veber filter assessments. In essence, SBs have the capacity to function as inhibitors of PARP-1 and could be progressed as promising candidates for anticancer treatment. Our team is currently conducting further analyses to confirm their effectiveness and understand their mechanism of action.

Key Words: Castration-resistant prostate cancer, PARP-1, Selagibenzophenone

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ORAL PRESENTATION ABSTRACT

HEALTH PROMITING EFFECTS OF TURKISH PINE HONEY BY SERVING PRINCIPAL BIOACTIVE COMPOUNDS

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Pine honey is a valuable honeydew honey and it is mainly produced in *Pinus brutia* forests [1]. The fact that *Pinus brutia* is most common in Turkey brings great wealth in terms of pine honey production. Pine honey is progressively valued thanks to its potent biological properties [2]. In this research, several principles and bioactive constituents of pine honey were identified analytically through several omic approaches, and the health-promoting effects were discussed by attributing to the observed compounds. LC-MS phenolic analysis proved that protocatechuic acid is the major phenolic compound in pine honey. It is a strong antioxidant and it is well known to have anti-inflammatory, anti-hyperglycemia, antibacterial, anti-asthma, and anti-ulcer properties. ICP-MS analysis indicated considerably higher potassium in pine honey. Potassium helps reduce blood pressure, protect against stroke, and help prevent osteoporosis. Hydrogen peroxide, methylglyoxal, and peptide defensin-1 were identified as antibacterial substances responsible for the antibacterial activity of honeys [3]. It was observed that the concentration of the defensine-1 is apparently higher in pine honey as a result of the bottom-up MRM-based proteomic survey. Our novel findings suggested that pine honey may also be the potential for being medical-grade honey with elevated levels of defensine-1. Furthermore, HR-MS analysis revealed that pine honey inherently provides oligosaccharides. Oligosaccharides' ability to act as prebiotics may offer a wide range of health benefits, including a stronger immune system, and improved gut health. Additionally, D-Pinitol as cyclitol was also measured in pine honey. Quantification of the purposed cyclitol was performed by utilizing HILIC-UPLC-ESI-MS/MS analysis after GC-MS annotation. The antidiabetic and cardioprotective properties of D-Pinitol are the notable health benefits it adds to pine honey. Detection of this content revealed that pine honey offers alternative health benefits owing to its rich bioactive components and can be consumed as a promising apitherapy product.

Key Words: Pine honey, Peptide, Oligosaccharides, Mineral, Cylitol, Phenolic

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ORAL PRESENTATION ABSTRACT

POPULATION STRUCTURE ANALYSIS BASED ON ISSR MOLECULAR DATA REVEALED TWO SUBPOPULATIONS AMONG IRANIAN PURSLANE (*PORTULACA OLERACEA* L.) ACCESSIONS

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Purslane (*Portulaca oleracea* L.), which belongs to the Portulacaceae family containing valuable vitamins and nutrients, has high tolerance for salt and drought stress growing under different environmental conditions. In this study, the population structure of Iranian purslane accessions was investigated using 25 molecular Inter Simple Sequence Repeat markers. The 25 primers yielded a total of 92 bands, of which 62 were polymorphic bands. Population structure analysis was performed using bioinformatics tools based on the molecular data. In this regard, the number of possible K was calculated and a two-dimensional plot was generated. The number of subpopulations for maximum likelihood was considered as the optimal number of subpopulations. The initial values of K ranged from one to ten, and in order to increase the accuracy, three repetitions were used for each of the subpopulations. Burn-in duration (10000) and MCMC (100000) were selected to obtain the maximum likelihood plot. Finally, the obtained results divided the Iranian purslane populations into two subpopulations. The results of this study can be useful and effective in identifying genetic diversity and preserving Iranian purslane germplasm.

Key Words: Inter Simple Sequence Repeat, likelihood, Population structure, Purslane, Subpopulation

ORAL PRESENTATION ABSTRACT

UNLOCKING THE BIOACTIVE POTENTIAL OF POLYPHENOLIC COMPOUNDS AS ANTICANCER AGENT FROM MALAYSIAN *ARTOCARPUS* SPECIES

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Artocarpus is a genus belonging to the mulberry family (Moraceae). Several members of this genus are ethnomedicinally used in the treatment of malaria fever, diarrhea, tuberculosis, wound, fever and anti-inflammatory. In this study, phytochemical analyses were conducted on five Artocarpus species native to Malaysia (*A. obtusus*, *A. elasticus*, *A. kemando*, *A. melinoxylus* and *A. rigidus*), along with bioactivity assessments. The phytochemical study involved chromatographic works and the structural elucidations of the isolated compounds were carried out using spectroscopic methods (NMR, HRMS, FTIR, UV). This study has led to the isolation and identification of several intriguing polyphenolic compounds, with certain compounds displayed notable bioactive properties. Two potential bioactive compounds, namely artonin E (AE) and pyranocycloartobiloxanthone A (PA) were identified and further studied for anticancer activity against selected ovarian cancer cell lines (SKOV-3 and CAOV3). The MTT assay, clonogenic assay, acridine orange and propidium iodide double staining, cell cycle and annexin V analyses were performed to explore the mode of action for AE an PA induce cell death. The DNA laddering, activation of caspases-3, -8, and -9, multi-parametric cytotoxicity-3 analysis by high-content screening, measurement of reactive oxygen species generation and Western blot were employed to study the pathways involved in the apoptosis. The MTT results showed that AE and PA inhibited the growth of SKOV-3 and CAOV3 cells, with IC_{50} values of 6.5 ±0.5µg/mL (14.8±0.2µM), 7.0±0.5 (15.4±0.2µM) and 1.0±0.25µg/mL (2.3±0.5µM), 2.0±0.25 (4.4±0.5 µM), respectively after 72 h treatment and showed less toxicity toward normal human ovarian cells (T1074), with IC_{50} value more than 30µg/mL. Results of this study showed that AE and PA inhibited the cell viability and induced cell apoptosis in SKOV-3 and CAOV3 cells in both 2D monolayer and 3D spheroid cultures. In conclusion, the current study demonstrated AE and PA possess strong anticancer activity against ovarian cancer cell lines
.

Key Words: *Artocarpus obtusus*, *Artocarpus elasticus*, artonin E, pyranocycloartobiloxanthone A, apoptosis, ovarian cancer.

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ORAL PRESENTATION ABSTRACT

THE APPLICATIONS OF AROMATIC OILS FOR MANAGING THE COMPLICATION OF PSYCHOLOGICAL DISEASES IN ANCIENT PERSIA

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Today, aromatherapy is considered a holistic healing practice, aiming to improve both mental and physical well-being, but the history of aromatherapy is as fascinating as it is interesting. It has been utilized for centuries across various cultures for its therapeutic properties. Although the first usage of aromatic plants comes back to the neolithic era according to archeological findings, it seems that the first conscious human uses of aromatic plants for treatment were about the 3rd millennium BCE in Persia, India, and China.

In ancient Persia, aromatic oils which are known as the first medicinal plant products that carry the scent of aromatic plants had a variety of applications; applied topically and inhaled, used in cleaning, and sanitizing household products, and also believed to help decrease anxiety, ease headache, and help improve to sleep, massage and managing psychosomatic disorders (millennia before we are familiar with the modern concept of Psycho-aromatherapy). The use of plants and their medicinal products was one of the duties of a type of "Ourva Baeshaza" (almost equivalent to a pharmacist), but when the patient had a mental illness, a type of physician called "Mantru Baeshaza" (almost equivalent to a psychiatrist) treated him. In most of the treatments the physician first rubs aromatic oils such as frankincense oil, sandalwood oil, and chamomile oil on the patient's body and treats the patient along by singing a song that has a musical style. They believed that the effect of the scent of the oil along with singing directly affects the patient's nervous and psychological system and makes him well. They have also used aromatic oils such as Lilyus oil and Iris oil to treat psychosomatic stress and as a relaxant, which can be a guide for the development of common aromatherapy.

Key Words: Persian medicine, history of pharmacy, history of medicine, aromatic plants, psychological disease

ORAL PRESENTATION ABSTRACT

THE EFFECT OF POMEGRANATE (*Punica granatum* L.) PEEL EXTRACT ON OSTEOARTHRITIS

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Osteoarthritis (OA) is a disease characterized by focal (initially nonuniform) loss of articular cartilage accompanied by hypertrophic reaction (sclerosis) in the subchondral bone and new bone formations (osteophytes) on the joint surface. In the early stage of osteoarthritis, chondrocytes proliferate and increase the synthesis of DNA, RNA, proteoglycan, prostaglandin, and collagen. This is the compensation period since protective factors are in effect. If the strain and load on the joints continue, damage-causing factors are active instead of repair mechanisms. Matrix metalloproteinases (MMPs) and proinflammatory stoichins secreted by synovial cells and chondrocytes are essential mediators of cartilage damage. Many studies investigate the beneficial properties of pomegranate in the prevention and treatment of osteoarthritis. The activities of pomegranate peel can be attributed to high amounts of phenolics and tannins (especially punicalagin). Punicalagin is one of the main active compounds in *Punica granatum* peel. This compound has multiple bioactivities, such as antioxidant, antibacterial, and antitumor activities. Rafrah et al. (2017) found a significant reduction in OA pain levels when using pomegranate peel hydroalcoholic extracts orally for eight weeks compared to the control group. In a different study, *P. granatum* peel had an antiosteoarthritic effect *in vivo*. In the survey by Shivnath et al. (2019), OA mice were given *Punica granatum* L. peel extract orally for 30 days. As a result of the study, it was determined that the experimental group had a significant decrease in serum alkaline phosphatase, MMP-3, and COX-2 levels compared to the control group. Pomegranate (*Punica granatum* L.) peel extract has a solid antiosteoarthritic effect and improved disease symptoms.

Key Words: Osteoarthritis, Pomegranate, Metalloproteinases, Extract

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ORAL PRESENTATION ABSTRACT

EVALUATION OF ANTIFUNGAL ACTIVITIES OF TUNISIAN FOREST RESOURCES

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Agriculture, essential to our global food supply, is currently facing a major challenge posed by plant-pathogenic fungi. These fungal organisms cause enormous damage to crops, leading to significant economic losses. In addition, faced with the rise of resistance to chemical fungicides, the high costs of treatments, and the associated environmental threats, the agricultural sector is now forced to seek sustainable solutions. In this context, this study aims to search for alternative and sustainable methods of control by using essential oils extracted from forest resources as a promising solution for the management of fungal diseases in agriculture. The antifungal effects of the essential oils obtained from *Eucalyptus citriodora*, *Cymbopogon citratus*, *Lavandula stoechas*, *Rosmarinus officinalis* and *Thymus capitatus* were evaluated *in vitro* aginst eight phytopathogenic fungal strains (*Alternaria alternata*, *Fusarium culmorum*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Fusarium solani*, *Phoma sp.*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*). Essential oils were incorporated at increasing concentrations in PDA medium (0, 2, 4, 6, 8, 10 and 12 µl/ml). The results obtained allowed to classify the essential oils studied in order of increasing antifungal activity: Thyme and lemongrass essential oils being the most powerful (at 2 µl/ml with 100 % inhibition of fungal growth), followed by eucalyptus (from 2 to 8 µl/ml) and rosemary (from 4 to 8 µl/ml) with moderate antifungal activities while lavender (from 8 to 12 µl/ml) has the lowest activity. For the fungal strains studied, the results showed that the *Rhizoctonia solani* strain is the most sensitive while *Phoma sp.* is the most resistant strain.

Key Words: Essential oils, antifungal activity

ORAL PRESENTATION ABSTRACT

PREPARATION OF GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *DUABANGA GRANDIFLORA* LEAF EXTRACT AND EVALUATION OF THEIR THERAPEUTIC APPLICATIONS

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The current research focused on the green synthesis of silver nanoparticles (AgNPs) using *Duabanga grandiflora* leaf extract. The green synthesis of AgNPs was confirmed by the surface plasmon resonance band at 453 nm in a UV-visible analysis. The formulated AgNPs had a diameter of around 99.72 nm with a spherical shape. Fourier transform infrared (FTIR) spectrum revealed the bio-reducing potential of phytochemicals present in *D. grandiflora*, which fundamentally influenced the synthesis of AgNPs. Zeta potential, dynamic light scattering (DLS), scanning electron microscopic (SEM), energy-dispersive X-ray spectroscopic (EDX), X-ray diffraction (XRD), and transmission electron microscopic (TEM) analyses were executed to reveal the physicochemical attributes of the AgNPs. The AgNPs were further investigated for their antioxidant, antidiabetic, anticancer, and antibacterial potential. The DPPH free radical assay revealed the potential radical scavenging capacity (IC_{50} = 76.73 µg/ml) of green synthesized AgNPs. α-Amylase inhibitory assay displayed significant inhibitory potential (IC_{50} = 162.11 µg/ml) of this starch-breaking enzyme by AgNPs, revealing the anti-diabetic potential of AgNPs. AgNPs exhibited potential cytotoxic activity (IC_{50} = 244.57 µg/ml) against malignant human kidney cells. In addition, AgNPs showed outstanding antibacterial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacterial strains. AgNPs showed cytotoxic and antimicrobial activities at much higher concentrations than radical scavenging and α-amylase inhibitory concentrations. Thus, our finding elaborated the scope of green synthesized AgNPs for diverse therapeutic applications (dose-dependent) for further clinical translation.

Key Words: *Duabanga grandiflora*; silver nanoparticles; antioxidant; antidiabetic; cytotoxic; antibacterial

ORAL PRESENTATION ABSTRACT

ENHANCED ANTIOXIDANT ACTIVITY THROUGH THE SYNERGISTIC EFFECT OF ESSENTIAL OILS IN NANOEMULSION FORMULATION

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Nanoemulsions loaded with essential oils (EOs) are garnering significant interest due to their potential in various applications. EOs are recognized for their antioxidant properties, but their direct use is often hindered by issues such as high volatility, instability, and poor water solubility. The advent of nanoemulsion technology offers a solution to these challenges, enhancing the delivery and efficacy of EOs. In this study, we aimed to identify an optimal mixture of nanoemulsions loaded with EOs from Rosmarinus *officinalis*, *Salvia officinalis*, and Thymus *satureioides* to counteract oxidative stress. The chemical compositions of the EOs were determined using gas chromatography and mass spectrometry (GC/MS). Subsequently, oil-in-water (O/W) nanoemulsions were formulated and characterized. The antioxidant activities of the individual nanoemulsions and their combinations were evaluated. The optimal mixture was identified using Design Expert software. GC/MS analysis revealed that 1,8-cineole (39.48%) was the predominant compound in R. *officinalis EO*. *T. satureioides* EO was primarily composed of endo-borneol (20.73%) and thymol (15.16%), while caryophyllene (30.49%) was the main compound in *S. officinalis EO*. The combination that exhibited the highest antioxidant activity consisted of 34.11% rosemary nanoemulsion, 24.11% sage nanoemulsion, and 41.77% thyme nanoemulsion. These findings suggest that these EO-loaded nanoemulsions could serve as potent antioxidant agents, indicating potential applications in various fields.

Key Words: Medicinal plants, essential oils, nanoemulsion, antioxidant activity, combination

ORAL PRESENTATION ABSTRACT

THE EFFECT OF 12 LOCAL MEDICINAL PLANT EXTRACTS AGAINST UROPATHOGENIC ESCHERICHIA COLI

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Urinary tract infections are among the most serious public health issues in all age groups. Thus, the empirical therapy should base on local levels of resistance, as indicated in several studies from different countries, to effectively avoid the emergence of multidrug-resistant bacterial strains and recurrent infections. Numerous effective antibiotic treatments are available, but would be ineffective for treating recurrent cystitis caused by a urinary tract infection, as well as the emergence of drug resistance. That is why the aim of this study was to highlight the antibacterial and the antioxidant activity of 11 medicinal plants used traditionally in Algeria, against E. coli, the most responsible urinary tract infections. First, the extraction of total polyphenols with aqueous acetone showed variable yields. The highest yield was obtained by *Asplenium trichomanes* with 27%, followed by *Petroselinum crispum* and *Ciannamomum cassia* with an equal yield of 21%. Artemisia herba-alba gave the lowest yield (9%).

The extracts of different plants showed variable contents of phenolic compounds. Reducing power and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity revealed that most of the extracts studied had significant activity. The anti-free radical activity was very high in the extract of *Asplenium adiantumnigrum* compared with the other extracts studied but *Petroselinum crispum* and *Parietaria officinalis* had the lowest reducing activity; antibacterial activity was determined on E. coli strains using the diffusion, MICs (Minimum Inhibitory Concentrations) and MBCs (Minimum Bactericidal concentrations) methods. The strains tested were sensitive to most extracts studied, except Asplenium adiantum-nigrum extract, for which both strainsshowed resistance.

Key Words: E. coli, Medicinal plants, phenolic compounds, urinary infections

ORAL PRESENTATION ABSTRACT

GASTRO PROTECTIVE EFFECT OF JUNIPERUS *PHOENICIA* EXTRACT ON THE ULCER INDUCED BY NSAIDS

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Juniperus *Phoenicia* is a medicinal plant, family of Cupressaceae, has been widely used in traditional medicine in the treatment of various diseases, among these diseases Gastric ulcer is one of the most serious diseases. The objective of this work is to evaluate the gastro protective effect of the *Juniperus phoenicia* extract on the ulcer induced by NSAIDs: Diclofenac sodium 50 mg [Votrex®]. For this fact, we adopted research experimental in female NMRI strain mice. Macroscopic and microscopic evaluation at the stomach levels shows that oral administration of diclofenac has caused gastric lesions in the glandular part of the stomach and the presence of edema. Because these lesions are less important in the poisons treated with *Juniperus phoenicia* extract, these results suggest that the Juniperus *Phoenicia* extract has a gastro protective effect which is due to its ability to prevent the formation of lesions, and to induce the protective mechanisms of the gastric mucosa that would be due to its gastro intestinal effect potential. - protective, anti-ulcerative and anti-inflammatory.

Key Words: Gastric ulcer, Juniperus *phoenicia*, NSAIDs, sodium diclofenac, gastro protective effect, NMRI mice

ORAL PRESENTATION ABSTRACT

THE USE OF DATES BY-PRODUCT IN TRADITIONAL MEDICINE BY THE RURAL PEOPLE OF BISKRA (SOUTHERN EAST OF ALGERIA)

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The fruit of the date palm (*Phoenix dactelyfera* L.), date is the main food for people of Sahara and arid zona in Algeria, this fruit is ranged in varieties, either used fresh (the best varieties like Daglet Nour) or after processing (the varieties of 2nd and 3rd rank), the date and its by –product surround the saharian people used it as food, sweet, fuel, cosmetics products and treatment of several ailment and diseases. The objective of this study is to investigate the knowledge of the local people of Biskra in processing dates and the use of those products in traditional medicine, we led a survey using a questionnaire prepared in Arabic language among 30 holders of small factories of date processing, during the period from February- April 2021. The interview allowed to collecte as much information as possible concerning the processing methods, the choice of the raw material, its treatments, its therapeutical uses. We enumerate 10 by products of a dry date variety called Mech Deguela and one product the date molasses or syrup (dabs el tamer) is used in treatment of anemia, diarrhea, constipation bronchitis, osteoarthritis and low cholesterol alone or with other ingrdiants. Moreover, there are 4 by products (date paste, date flour, date honey, pollen grain) that are used to treat 11 diseases. There is no doubt that dates are not only a food but also a medicine because this is confirmed in Islamic culture, the obtained results clarify that the by-product are also useful for the healthcare of human as the fruit does.

Key Words: date fruit, syrop of date, anemia, bronchitis, Mech Deguela

ORAL PRESENTATION ABSTRACT

EFFECT OF SEAWEED EXTRACT (*ULVA RIGIDA* AND *FUCUS SPIRALIS*) ON ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *LAVANDULA DENTATA*

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Lavandula dentata, from Lamiacae family, is intensively exploited as source of the essential oil from the wild. In order to preserve this valuable medicinal plant from risk of extinction, the cultivation (with and without seaweed extract fertilizer) of two lavender populations (*L. dentata* from Asni and Essaouira) and its consequent effect on yield, antioxidant and antimicrobial activities of essential oils were studied. The Yield of essential oils decreased slightly in cultivated lavender compared to wild plants. However, fertilization by seaweed extract significantly increased the content of essential oils, with values ranging from 2.13±0.05 % to 2.25±0.06 % and from 1.83±0.05 % to 2.12±0.08 % for wild and cultivated lavender with seaweed extract harvested from Asni and Essaouira respectively. Similarly, the antioxidant activity of essential oils decreased slightly in cultivated lavender compared to wild plants. However, fertilization by seaweed extract increased this activity in all assays as measured by DPPH free radical scavenging ability, reductive potential and β-carotene assays. Specifically, the IC50 values of *L. dentata* from Asni ranged from 405.33±4.19 µg/mL to 509.20±5.16 µg/mL for wild and from 351.44±4.09 µg/mL to 489.14±5.69 µg/mL for cultivated with seaweed extract respectively. Additionally, the IC50 values for *L. dentata* from Essaouira ranged from 418.86±4.21 µg/mL to 485.55±4.12 µg/mL for wild and from 384.65±4.91 µg/mL to 467.87±5.46 µg/mL for cultivated with seaweed extract respectively. Regarding antimicrobial activity, essential oils from cultivated plants with seaweed extract showed a strong inhibitory effect comparable to that of essential oils from wild and cultivated plants without seaweed against most tested strains. From this work, it can be concluded that cultivation with seaweed extract fertilizer could be a promising solution to ensure the sustainable utilization of *L. dentata* species.

Key Words: Cultivation, Essential oil, Antimicrobial; Antioxidant, *Lavandula dentata*; Seaweed extract.

ORAL PRESENTATION ABSTRACT

INVESTIGATION OF THE CONTENT AND PANCREATIC LIPASE INHIBITORY EFFECT OF EXTRACTS OBTAINED FROM ISABELLA GRAPE WASTE THROUGH EXPERIMENTAL DESIGN

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With the increased awareness of the side effects associated with synthetic components, consumers have been encouraged to prefer natural and healthy ingredients. Various factors such as the economical valorization of waste generated in the food industry, potential positive effects on health, and low toxicity levels have accelerated research into the utilization of these wastes in different areas such as the food, paint, pharmaceutical, and cosmetic industries. These wastes are thought to potentially serve as natural agents that could substitute synthetic components [1]. In particular, grape pomace, a by-product of grape processing known to possess a rich content, is noteworthy. This study aims to prepare extracts from the pomace of the Isabella grape (*Vitis labrusca*), a value endemic to the Black Sea Region, using an experimental design, and to examine its content as well as its pancreatic lipase inhibitor effects. *V. labrusca* L., which grows in the Black Sea region, is popularly called "kokulu üzüm, izabella üzümü, çilek üzümü, Amerikan üzümü, siyah üzüm". It is consumed as food by the local people [2]. It has been reported that *V. labrusca* is rich in phenolic compounds, organic acids, vitamins and minerals, enzymes, monosaccharides and their derivatives, nitrogenous compounds, terpenic compounds and lipids [2]. It has been shown in the literature that grape extracts have many activities such as anticarcinogenic, antibacterial, antifungal, antiviral, antioxidant, antiallergic, wound healing, and antiedema [3]. The extraction procedure was optimized using the Design Expert 11.1.2. The phenolic contents of the extracts has been determined by RP-HPLC [4]. Total phenolic, anthocyanin contents and pancreatic lipase enzyme inhibitory activities of the extracts were also evaluated spectrophotometrically [5,6]. The most influential parameters on the extraction process were determined as solvent volüme (ethanol %), microvawe power and temperature. It was determined that the phenolic and anthocyanoside contents of the extracts showed a positive correlation with their lipase inhibitory effects. In this way, the study aims to contribute to the increasing demand for natural and healthy ingredients and a sustainable future.

Key Words: Isabella, anthocynanoside, pancreatic lipase, design expert

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ORAL PRESENTATION ABSTRACT

ESSENTIAL OILS OF TUNISIAN TETRACLINIS ARTICULATA (VAHL) MAST.: CHEMICAL COMPOSITIONS AND BIOLOGICAL INSIGHTS

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Tetraclinis articulata (Vahl) Mast. is native to the Mediterranean area and belongs to Cupressaceae family. It is an evergreen tree that is able to endure unfavourable climatic conditions. The aim of this study were: i) to determine the chemical composition of essential oils (EOs) of *T. articulata* obtained from its stems, leaves, and cones using GC coupled to GC/MS; II) to evaluate their antioxidant activity using non enzymatic (DPPH, ABTS and FRAP) and enzymatic methods (catalase activity); III) to evaluated their anti-enzymatic activity on enzyme involved in metabolism (α- amylase, α-glucosidase) and central nervous system (acetyl- and butyryl- cholinesterase and tyrosinase). α-Pinene, limonene, and bornyl acetate were the main components of the three EOs. Moreover, the EO derived from cones showed the best antioxidant activity and was also able to increase of catalase activity. All EOs were active against α-amylase in similar way, instead EO from leaves was more active against α glucosidase and EO from cones was more active against cholinesterase respect to other EOs. This will highlight the potential uses of *T. articulata* EOs in different sectors, promoting innovation and sustainability in the fields of health and agriculture.

Key Words: *Tetraclinis articulata*, essential oil, chemical composition, antioxidant activity, anti-enzymatic activity

ORAL PRESENTATION ABSTRACT

THERAPEUTIC POTENTIAL AND CHEMICAL COMPOSITION OF *TANACETUM POLYCEPHALUM* SUBSP. *ARGYROPHYLLUM*

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This study aimed to investigate the tyrosinase, elastase and cyclooxygenase enzyme inhibitory activities and chemical content of *Tanacetum polycephalum* subsp. *argyrophyllum* collected from Van in June 2021. The plant extracts were prepared with *n*-hexane, ethyl acetate and ethanol using the maceration method. The essential oil was obtained by hydrodistillation using the Clevenger apparatus. The chemical content of the extracts was analyzed by LC-MS/MS and the essential oil content by GC and GC-MS. Tyrosinase inhibitory activity of the samples was tested at concentrations from 100 to 500 µg/mL, and the extracts showed higher inhibition activity than the essential oil. While the extracts did not exhibit any inhibition on the elastase, the essential oil had a weak effect at 500 µg/mL. None of the samples had a significant inhibitory effect on the cyclooxygenase enzymes at 100 µg/mL. The yield of the essential oil was found as 1%. Thirty-five compounds in the essential oil were identified. The main component of the essential oil was found to be camphor (29.68%). More compounds were detected in the ethanol extract, where the main compound was cyronoside (10.71 mg/g extract), compared to other extracts.

Key Words: *Tanacetum polycephalum* subsp. *argyrophyllum,* anti-tyrosinase, anti-elastase, anti-cyclooxygenase, LC-MS/MS, GS-MS

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ORAL PRESENTATION ABSTRACT

IN SILICO, IN VITRO EXPERIMENTATION ON RARE HERBAL PLANTS FROM WESTERN GHATS OF INDIA IN MITIGATION OF ALZHEIMER THROUGH MANAGEMENT OF BACE1 ENZYME ACTIVITY AND TAU HYPERPHOSPHORYLATION

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

Key Words: Rare plants, bioactive compounds, Phenolics, cell line, molecular docking.

ORAL PRESENTATION ABSTRACT

MORPHOLOGICAL FEATURES OF THE SECTION *MESOCENTRON* (ASTERACEAE) OF GENUS *CENTAUREA* IN TURKIYE

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The genus *Centaurea* L. is a member of the Asteraceae family which includes medicinal and aromatic plants. Section *Mesocentron* (Cass.) DC. is represented by *Centaurea solstitialis* L. species in the Flora of Turkiye. With the addition of the *C. verutum* L. species, which was later published as a new record, the number of species of the section in Turkiye increased to two and the number of taxa to four. *C. solstitialis* spp. subsp. *solstitialis*, subsp. *carneola* (Boiss.) Wagenitz and subsp. *pyracantha* subsp. (Boiss.) Wagenitz, including three subspecies. *C. solstitialis* subsp. *carneola* and *C. solstitialis* subsp. *pyracantha* are endemic taxa for Türkiye and the endemism rate of the *Mesocentron* section is 50%. Among these, there are reports of the use of *C. solstitialis* subsp. *solstitialis* against different diseases. Detailed studies have been conducted on the morphology of the *Mesocentron* section within the genus *Centaurea*. Within this framework, measurements were taken of the stem, leaves, involucre, appendages, achenes, and pappus. Floral and involucral characteristics were sampled from the terminal capitula, and the measurements were conducted using dried herbarium materials. The results indicate that *C. verutum* distinguishes itself from other taxa based on stem indumentum, basal leaf shape, involucre size, and spine length. *C. solstitialis* subsp. *carneola* stands out from other taxa due to differences in spine color, flower measurements, and coloration. *C. solstitialis* subsp. *solstitialis* differs from other taxa in possessing dimorphic achenes and *C. solstitialis* subsp. *pyracantha* differs from other taxa based on pappus measurements.

Key Words: Compositae, endemism, knapweed, taxonomy

ORAL PRESENTATION ABSTRACT

FLUORESCENT CHARACTERISTICS AND METAL ION BINDING PROPERTIES OF 1,3-DIHYDROXY SUBSTITUTED UROLITHIN DERIVATIVE

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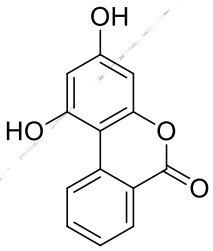
Urolithins, the bioavailable metabolites of ellagitannins, attract pretty much attention in the last two decades. Considering the research studies, many aspects of biological activities of ellagitannins and ellagitannin-rich diets have been attributed to urolithins, since these macromolecules are subject to gastrointestinal microflora-based biotransformation reactions. Our research group has long been involved in the discovery of diverse properties of urolithins (i.e., hydroxy-substituted benzo[c]chromen-6-one derivatives). Previously, we have defined the metal interaction properties with respect to fluorescence measurements of major urolithins. Within this work, we have synthesized 1,3-dihydroxy-6H-benzo[c]chromen-6-one employing resorcinol. Our investigations on the molecule pointed out its interaction characteristics with Fe(III) yielding out a fluorescence quenching property. This outcome clearly demonstrates the compound available to be employed as fluorescence on-off probe in the presence of the metal.



ORAL PRESENTATION ABSTRACT

EXTRACTION OF VARIOUS MULBERRY FRUITS BY NOVEL METHODS AND DETERMINATION OF BIOACTIVE COMPONENTS AND BIOLOGICAL ACTIVITIES OF THE EXTRACTS

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Mulberry fruits are consumed widely throughout the world, particularly in Asia. Mulberry trees are deciduous plants of the genus Morus (family Moraceae), which includes more than 17 species that grow in tropical climates. These include Morus alba L., Morus rubra, Morus nigra, Morus australis, *Morus atropurpurea*, *Morus cathayana*, *Morus notabilis* and *Morus mesozygia*. The most common varieties are Morus alba (white mulberry), M. rubra L. (purple mulberry) and Morus nigra (blackberry). In this review, the similarities and differences between the different mulberry varieties and the effects of different extraction methods on the extract quality of these species and their health potentials were elucidated due to current literature. Mulberry berries have been studied more often in relation to their immunological effects on the human body. In the current literature, supercritical extraction, ultrasound-assisted extraction, and microwave-extraction of different parts of Morus alba, Morus rubra, and Morus nigra which are the most common species of mulberry fruits, have been studied as novel extraction techniques. These techniques are widely used for the extraction of phenolic compounds in mulberry fruits and have been in increasing demand because they are much faster and environmentally friendly. The purpose of this study is to review the literature on the novel extraction methods and potential therapeutic effects of different kinds of mulberry fruits against essential health problems such as cancer and inflammation.

Key Words: Mulberry fruit, extraction, ultrasound-assisted, maceration, supercritical, bioactive properties

ORAL PRESENTATION ABSTRACT

AN INVESTIGATION ON POSSIBLE ANALGESIC EFFECTS OF AVENANTHRAMIDE-C

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New drug research and development studies continue due to problems such as side effects and tolerability of existing drugs in acute pain. For this purpose, natural products are examined as potential alternatives. Avenanthramides are soluble phenolic compounds that have attracted attention in this context in recent years. They are abundant in oats (*Avena sativa L.*) and have been shown to have different bioactivities such as anti-oxidative, antiinflammatory, anti-histaminic, anti-cancer activities. Form C (caffeic acid ester) is the most common form in oats and it is stated that this form has higher biological activities than the others. However, in vivo pharmacological activity studies are still insufficient. In this study, it was aimed to investigate the central and peripheral antinociceptive effects of Avenanthramide-C at doses of 10, 20 and 40 mg/kg, p.o., in acute pain in mice in hot plate, tail immersion and writhing tests. The efficacy of avenanthramide-C has been compared with the effects of 300 mg/kg dipyrone and 10 mg/kg diclofenac. Since the opening of K+ channels leads to hyperpolarization of the cell membrane and a consequent decrease in cell excitability, leading to a potential analgesic effect, participation of K^+ currents to the observed effects was assessed by Kv7 channel blocker XE991 pre-applications to the lowest dose, 20 mg/kg, which exhibited a significant level of statistical significance. Among all tested doses in various degrees across all test methods, statistical significance was observed ($p<0.05$ – $p<0.001$). A return effect has been observed with XE991, however, the return effect observed in the hot plate test was not a full reversal. These results both demonstrate the potential effect of avenanthramide C in acute pain and provide a report on the mechanism of this effect. The necessity of evaluating natural substances in pain research is once again highlighted.

Key Words: Avenanthramide-C, acute pain, K^+ channels, *Avena sativa L.*

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ORAL PRESENTATION ABSTRACT

INVESTIGATION OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID SUBSTANCE AMOUNTS OF *Filipendula ulmaria* (L.)

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Filipendula ulmaria (L.) Maxim. is a perennial plant belonging to the Rosaceae family. Meadowsweet plant grows widely throughout Western Asia, Europe, North America and Siberia. In our country; It has a natural distribution in the Western, Central and Eastern Black Sea, Upper Sakarya, Upper Euphrates, Erzurum-Kars, Upper Murat-Van and Hakkari sub-regions. FU contains flavonoids, tannins, phenolic glycosides (salicylate), essential oils (salicylaldehyde), minerals and vitamin C. It is a plant with anti-ulcer, anti-rheumatic, immunomodulatory and cytotoxic properties because it contains various polyphenol compounds. The aboveground parts of the plants were collected from Van Yüzüncü Yıl University, Medicinal and Aromatic Plants Garden in 2020. In this study; antioxidant activity, total phenolic and flavonoid substance amounts were examined. In the results of working; The amount of antioxidant substance was determined as 136.59 µmol TE/g, the total phenolic substance (198.08 mg GAE/g) and the total flavonoid substance amount was determined as 24.71 mg QE/100 g.

Key Words: *Filipendula ulmaria*, medicinal plant, antioxidant, phenolic, flavonoid

ORAL PRESENTATION ABSTRACT

INVESTIGATION OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID SUBSTANCE AMOUNTS AND DUALEX VALUES OF BURDOCK (*Arctium lappa* L.) PLANT

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Burdock (*Arctium lappa* L. = *Lappus officinalis* L.) is a commercially an important plant often used in traditional medicine. Burdock (*Arctium lappa*) is an edible plant from a member of the *Asteraceace* family. Burdock is widely used as a traditional medicinal herb due to its beneficial effects on inflammatory disorders such as arteriosclerosis, gout, and hepatitis. Additionally, it has anti-inflammatory, antimutagenic and anti-aging properties due to its rich antioxidant content. The plants were collected from Van Yüzüncü Yıl University, Medicinal and Aromatic Plants Garden in 2020. In this study; antioxidant activity, total phenolic and flavonoid substance amounts and dualex values (nitrogen balance index (NBI), chlorophyll content, flavonol and anthocyanin content) were examined. In the results of working; The amount of antioxidant substance was determined as 124.87 µmol TE/g, total phenolic substance (211.50 mg GAE/g) and total flavonoid substance amount was determined as 11.87 mg QE/100 g. The data obtained in terms of Dualex values (dx) such as Nitrogen Balance Index (NBI), chlorophyll content, flavonol and anthocyanin content are respectively; It was determined as 13.73 mg/g, 25.80 mg/cm^2, 1.88 dx and 0.07 dx.

Key Words: *Arctium lappa* L., medicinal plant, antioxidant, phenolic, flavonoid, dualex value

ORAL PRESENTATION ABSTRACT

THE EFFECT OF STORAGE TEMPERATURE ON THE ESSENTIAL OIL COMPOSITION OF *LAURUS NOBILIS* L.

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Laurus nobilis L (bay leaf: BL)., a member of the Lauraceae family, occurs naturally in the southern Mediterranean climate. Extracts of this plant can be used in a wide range of applications, from medical applications to foods, cosmetics and sustainable pesticides. It has various beneficial effects such as anti-bacterial, antioxidant, anti-tumoral and acetylcholinesterase inhibition. All these properties can be attributed to the plant essential oil components. In the realm of medicinal and aromatic plants, the conditions under which they are stored play a pivotal role in determining the quantity and quality of their active substances. However, few studies have delved into the alterations of secondary metabolites post-extraction. This study employed a factorial experiment based on a completely randomized design with three replications. The variables included three storage temperature levels: freezer temperature (–20 °C), refrigerator temperature (4 °C), and room temperature (23 ± 2 °C), along with four durations of storage time (fresh distillation, 3 months, 6 months, 9 months, and 12 months). The essential oils extracted via hydro-distillation were subjected to analysis using gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). A total of 27 components were detected in the leaves of LEO. The dominant compounds included 1,8-cineole, followed by linalool, α-pinene, α-terpinyl acetate, methyl eugenol, β-pinene and sabinene. Findings indicated that at each storage temperature, certain compounds like 1,8-cineole, nerol, and cis-ocimene experienced a decline. Conversely, the primary components of *L. nobilis* essential oil, such as linalool, linalyl acetate, and α-terpinyl acetate saw an increase across all temperatures, with the most significant rise at freezer temperature and the least at room temperature. Storing the *L. nobilis* essential oil in the freezer was found to enhance its quality.

Key Words: Laurel, oil quality, phytochemical compounds, shelf life

ORAL PRESENTATION ABSTRACT

ALLEVIATION OF SALT STRESS CONDITION OF THE SELENIUM APPLICATIONS ON MORPHOLOGY AND YIELD PROPERTIES OF OREGANO

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Turkish oregano (*Origanum onites*) is an aromatic plant belonging to the Lamiaceae family, which is cultured economically in Türkiye. The essential oil of oregano is preferred as a raw material in the perfume and cosmetic industries due to its distinct and characteristic aroma. At the same time, its essential oil is used for medical, antimicrobial, antiviral and antifungal purposes in the industrial sector. In sustainable agriculture, abiotic environmental stresses such as extreme temperatures, drought and salinity negatively affect agriculture. Selenium (Se) is known to have positive effects on plants under salt stress, such as preventing lipid peroxidation processes, increasing free proline accumulation, and decreasing the number of chloride ion in shoot problems. The present study was addressed to assay the effect of different Se dose fertilization on Turkish oregano under salt (NaCl) stresses. The results showed that Se treatments caused significant improvements in the fresh (3.78-6.00 g) and dry (1.30-2.18 g) weight of oregano under salt stress. The highest mean fresh and dry weight was observed in the combined treatment of 2 ppm of Se and 100 mM/L of NaCl, followed by 6 ppm of Se and 50 mM/L of NaCl respectively. Totally 31 correlations were found as positive, and negative, and PCA analysis revealed 65.7% total variation. The cluster analysis clustered into two main groups (A and B) based on the applications of different dose Se and NaCl over 63% of the applications took place in the group B and 75% of the Se applications fell into the same group. Therefore, applying Se can be effective way of improving oregano yield under salt stress conditions.

Key Words: *Origanum onites*, Salinity, Salt stress, Selenium, Growth parameters.

ORAL PRESENTATION ABSTRACT

EFFECT OF GROWING CONDITIONS ON EMULSION CAPACITY, EMULSION STABILITY, AND PROTEIN CONTENTS OF DIFFERENT FENUGREEK GENOTYPES

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Fenugreek (*Trigonella foenum-graecum* L.) is both a legume and medicinal and aromatic plants cultivated in different countries as Türkiye, Ethiopia, Egypt of the World. It is an annual plant and used for the medicinal, food, cosmetic industries because of including many important chemical properties such as carbohydrate, oil, protein and polysaccharide. In this study, the emulsion capacity (EC), emulsion stability (ES), and protein content (PC) of the different origin fenugreek genotypes were determined grown under different growing conditions (irrigated and dryland). The experiment was conducted according to randomized complete block design with three replications. The gums of the three local cultivars (Berkem, Çiftçi and Güraslan) with 18 different origin fenugreek genotypes were used for the determination of the examined properties. The EC, ES and PC of the genotypes under irrigated condition changed between 78.57-92.86%, 62.50-92.31% and 10.52-17.63%, respectively. Under dryland conditions, EC, ES and PC values varied between 76.92-93.75%, 78.13-90.91%, and 10.40-15.11% among the fenugreek genotypes. The highest EC values were found from Berkem cultivar under dryland condition, and PI 215615 genotype under irrigated condition. The maximum ES and PC were obtained from PI 613633 genotype and Berkem cultivar under irrigated condition, respectively. The cluster analysis clustered into two main groups and the PCA analysis showed over 79.4% of total variations for the EC, ES, and PC values.

The obtained result showed that the examined properties showed high variability among the fenugreek genotypes. In conclusion, the means of the EC and PC values were found higher in irrigated condition, however, the genotypes showed variability. So, the obtained results may be used for the fenugreek breeding programs and developing new cultivars.

Key Words: Fenugreek, Gum content, Protein content, Emulsion factors

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ORAL PRESENTATION ABSTRACT

EXAMINATION OF TOXIC METALS (CO, CR, MO, NI, PB) IN SOME COMMONLY USED SPICES

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In recent years, research on monitoring the heavy metal content of edible plants has continued at an increasing rate. In this samples, the cobalt (Co), chrome (Cr), molybdenum (Mo), nickel (Ni) and lead (Pb) element contents of the spices were determined by ICP-OES. It was found that there was a wide variation among the heavy metal contents of spices that 28 different spices had a very wide range of Co content 0.04-1.72, Cr content 0.01-0.97, Mo content 0.05-4.98, Ni content 0.16-16.98 and Pb content 0.04-6.93 mg kg^{-1}. The wide variation in the heavy metal content of spices collected from different regions has been effective in the high accumulation of heavy metals in some spices, especially in areas close to highways and areas with high vehicle traffic, where gases from vehicle exhausts affect plants. Although the lowest and highest contents of Co, Cr, Mo, Ni and Pb elements of 28 different spices were in a wide range, they were found to be below the maximum permissible limit set by WHO/FAO (2002). The results of this study revealed that the heavy metal contents of some selected spice plants commonly grown in Turkey were in the low values

Key Words: Spices, Heavy Metals, Critical limit, ICP-OES

ORAL PRESENTATION ABSTRACT

ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLES USING *CYDONIA OBLONGA* LEAF EXTRACT AND THEIR ANTIOXIDANT ACTIVITY

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The aim of the present study was to prepare silver nanoparticles (AgNPs) using *Cydonia oblonga* leaf extract at room temperature via green biosynthesis route and to evaluate their antioxidant activity. Particle analyses such as size, shape, surface charge, and components were performed using versatile characterization techniques. Total flavonoid and phenolic contents were also calculated. Biosynthesized AgNPs were spherical in shape. Their surface charge was negative. Fourier-transform infrared spectroscopy results indicated that plant extract acted as a capping agent. Based on the antioxidant assay, the nanoparticles exhibited high antioxidant activity, which was attributed to their flavonoid and phenolic content. In conclusion, the biosynthesis of AgNPs from *Cydonia oblonga* leaf extract, which is a natural source, can enhance the therapeutic efficiency of biomedical applications due to their potent antioxidant capacity and phytochemicals on their surface.

Key Words: *Cydonia oblonga*, silver nanoparticles, antioxidant activity, green synthesis

ORAL PRESENTATION ABSTRACT

DETERMINATION OF CHROMIUM (Cr), NICKEL (Ni) AND LEAD (Pb) IN MEDICINAL PLANTS WIDELY GROWN AND USED IN THE SOUTHERN REGION OF TURKEY

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The bioaccumulation of Cr, Ni and Pb in medicinal plants raises concerns about the safety and efficiency of herbal medicines obtained from these plants. It is crucial to understand the mechanisms of metal uptake, transport, and accumulation in medicinal plants to assess the potential health risks associated with their consumption. In this study, it was determined that the lowest and highest contents of Cr, Ni and Pb of 23 different medicinal plants were more than 100-fold different. Among 23 medicinal plants, the lowest Cr and Ni content were 0.02 and 0.06 mg kg^{-1} in Boswellia serrata (*Gummi olibanum* Hunk.) and the highest Cr and Ni content were found as 2.17 and 11.48 in mg kg^{-1} Thymbra (*Thymbra spicata* L.). In this research, it was determined that Cr and nickel Ni contents in some commonly grown and used medicinal plants are in a very wide range. While the lowest Pb content was 0.13 mg kg^{-1} in Senna (*Cassia acutifolia* L.), the highest Pb content was found in Chamomile (*Matricaria chamomilla* L.) with 7.71 mg kg^{-1}. These results show that although there is a wide variation among the Cr, Ni and Pb contents in these medicinal plants, they are below toxic limits.

Key Words: Herbs, Medicinal Plants, Heavy Metals

ORAL PRESENTATION ABSTRACT

EFFECT OF HARVEST TIMES ON ESSENTIAL OIL COMPONENTS AND ANTIMICROBIAL ACTIVITY IN PLANT ORGANS OF *FERULA LYCIA* BOISS.

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Pharmaceutical active ingredients, which vary in concentration across a range of medicinal plants, serve as potent natural anti-microbial agents in medical applications. These oils were extracted through hydrodistillation from samples that had been air-dried. The extraction yield of the essential oils, expressed as a percentage of weight to weight (w/w%), varied across different developmental stages, ranking as follows: floral budding stage (1.1%) surpassed immature fruit stage (0.9%), which was followed by both vegetative and flowering stages at an equal yield (0.8%), and finally, the ripening fruit stage (0.5%). The composition of the essential oils was determined using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). Across the different stages, a total of 27, 38, 41, 28, and 37 components were identified and quantified, respectively. In this study, composition and antimicrobial effects of essential oils of *Ferula lycia* Boiss. root-stem, leaf, flower, flower head samples were evaluated periodically and daily. Major components of the essential oils were α-pinene, β-pinene, and limonene. Significant differences in α-pinene, β-pinene, and limonene ratios were determined depending on the phenological periods, plant parts and harvest time. The highest major component rates were detected in camphor obtained from root-stem samples harvested at full flowering period-1 pm, in α-pinene obtained from flower head samples harvested before seed maturation stage, and in borneol obtained from root-stem samples harvested at pre-flowering stage-6 am. A total of 14 new compounds were first detected in *F. lycia*. When all plant parts are evaluated, it was determined that flower head samples harvested in the evening at seed maturation stage and leaf samples harvested in the evening at post flowering stage showed strong antimicrobial activities against microorganisms. Study results showed that the harvest time of compounds that can be used as antimicrobial agents in the treatment of infectious diseases varies depending on the harvest time.

Key Words: Diurnal variation, microorganisms' minimum inhibitory concentration, Umbelliferae

ORAL PRESENTATION ABSTRACT

BEHAVIORAL PREFERENCE OF SILVERLEAF WHITEFLY (*BEMISIA TABACI*) ON BASIL (*OCIMUM BASILICUM*) GENOTYPES WITH DIFFERENT MAIN COMPONENTS OF ESSENTIAL OILS

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Silverleaf whitefly (*Bemisia tabaci* (Gennadius), (Hemiptera: Aleyrodidae)) is an important pest of crops worldwide. It is among the most harmful species, especially in subtropical and tropical regions. Chemical control methods are commonly used in the control against this very significant pest. However, essential oils, composed of various components, can act against insect pests through complex mechanisms. This study investigated the behavioral preferences of silverleaf whiteflies on basil genotypes with different main components, including eucalyptol, estragole, geraniol, methyleugenol, methyl cinnamate, citral, and linalool. In the genotypes, during the initial observation on July 2, 2022, the density of silverleaf whiteflies recorded was 12.58 individuals per leaf, whereas in the subsequent observation on July 19, 2022, the density decreased to 4.66 individuals per leaf. Across both observations, the highest mean number of silverleaf whiteflies per leaf was found in genotype PI 172998 (Estragole chemotype) with a mean observation of 20.08 individuals, while the lowest mean was in genotype PI 652070 (Linalol-Estragole chemotype) with a mean observation of 1.43 individuals. Since the Linalool-Methyleugenol and Linalool-Estragole chemotypes exhibited the lowest frequency of occurrence in both sets of observations, it is advisable to investigate the potential of these components as insect repellents. This study highlights the varying behavioral responses of silverleaf whiteflies to distinct basil chemotypes, offering valuable insights for pest management approaches.

Key Words: Basil, silverleaf whitefly, chemotype, essential oil components, hemiptera.

ORAL PRESENTATION ABSTRACT

COMPARATIVE STUDY OF *HEDERA* SPECIES AS A PHYTOPHARMACEUTICAL RESOURCE

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Hedera helix L. and *Hedera colchica* K. Koch are commonly used as painkillers and for bronchitis, whooping cough, arthritis, and rheumatism [1,2]. The medicinal effects of *H. helix* on cough and bronchitis have been proven and its use in the treatment of mild inflammatory conditions of the mucous membranes of the upper respiratory tract and chronic inflammatory bronchial diseases has been approved by the German E Commission [3]. Due to these effects, standardised dry extracts obtained from the leaves of *H. helix* are used as pharmaceutical raw materials in mono products as well as in combined products developed by different companies [2]. In our study, it aims to evaluate to antiinflammatory activities of *H. helix* and *H. colchica* species in two different vegetation periods, obtained extracts rich in saponin and phenolic substances. Compared to *H. helix*, *H. colchica* has a higher total phenolic and total saponin content. *H. colchica* fruit extract (C3S), with the highest saponin content (468.19 ± 16.01 mg HE/g dry weight), and *H. colchica* early vegetative stage leaf extract (C1F), with the highest total phenolic content (108.60 ± 5.61 mg GAE/g dry weight) were tested for antiinflammatory effects via COX-1 and COX-2. According to the results, C1F showed the highest selective COX-2 inhibition. The experimental results show that besides saponins, various secondary metabolites present in *Hedera* species may also contribute to the efficacy of the extract; by using appropriate extraction techniques, more potent raw materials can be provided to the market.

Key Words: antiinflammatory, *Hedera helix, Hedera colchica,* medicinal product

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ORAL PRESENTATION ABSTRACT

EFFECT OF COMMERCIAL ESSENTIAL OILS OF TANGERINE, SWEET ORANGE, LEMON AND CLOVE ON OXIDATIVE STABILITY AND FRYING OF SOYBEAN OIL

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This study was designed to investigate and discuss the effect of commercial essential oils (EsO) from Tangerine, Sweet Orange, Lemon and Clove on oxidative stability of soybean oil (SBO) and during deep fat frying process. Chemical profile of the analyzed EsO showed the occurrence of limonene in *Citrus* (82.64-98.77%) and eugenol (98.30%) in Clove as major terpene components. These EsO demonstrated their ability to act as free radical scavenging entities. On this basis, they were added at a concentration of 50 ppm and tested towards accelerated oxidation (Schaal oven aging and Rancimat tests) and polar compounds production during frying. Tangerine and Lemon EsO significantly lowered the hydroperoxides compared to control SBO ($p < 0.05$). However, Clove EsO showed the highest resistance to drastic oxidation conditions (17.79 h) compared to SBO and other samples ($p < 0.05$). Overall, EsO had no significant effect on polar compounds production compared to control SBO ($p < 0.05$) except for Clove EsO which produced more polar compounds ($p > 0.05$). The obtained data highlighted the possibility to use these commercial EsO as antioxidants in food items against lipid oxidation. The use of EsO in food-agric industries as safer additives in foods, in replacement of synthetic ones, opens the door for boosting eco-friendly exploitation of plant resources in local circular economy.

Key Words: essential oils, soybean oil, oxidation, oxidative stability, antioxidants

ORAL PRESENTATION ABSTRACT

ISOLATION OF THE MAJOR SECONDARY METABOLITES AND BIOLOGICAL ACTIVITIES OF THE AERIAL PARTS OF *TRACHYSTEMON ORIENTALIS*

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Trachystemon orientalis D. Don (Boraginaceae) is a perennial plant, which is used as food and for treatment of the various diseases [1,2]. Studies have shown that its phytochemical content includes anthocyanins, proanthocyanidin, phenolic acids, essential oil, tannins, mucilage, saponin, minerals, and vitamins [3]. In this study, it was aimed to performed isolation and biological activity (antioxidant and lipase inhibitory activities) studies on the extracts of the aerial parts of *T. orientalis*. The structures of the isolated compounds were characterized using ^{1}H-NMR, ^{13}C-NMR, and 2D-NMR spectral methods. Ferric reducing antioxidant power (FRAP) assay to determine antioxidant activity, and pancreatic lipase enzyme inhibitory activities of the extracts were evaluated. *β*-sitosterol (**1**) was isolated from the chloroform subextract. Besides, rosmarinic acid (**2**) from the ethyl acetate subextract, the mixture of rosmarinic acid and danshensu (**3**) from the remaining aqueous subextract were isolated. The compounds were obtained for the first time from the aerial parts of the plant with the study. According to the results of biological activity assays, the ethyl acetate subextract showed the highest FRAP value (TEAC = 177 ± 4.5825 µM) and pancreatic lipase inhibitory activity (IC_{50} = 47.577 ± 0.931 µg/mL). Rosmarinic acid, the main component of the ethyl acetate subextract, may be responsible for the high activities. According to the results of this study, *T. orientalis* has high antioxidant capacity and lipase inhibitory activity and may be a promising potential natural agent for obesity.

Key Words: Antioxidant, Boraginaceae, *β*-sitosterol, danshensu, pancreatic lipase, rosmarinic acid

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ORAL PRESENTATION ABSTRACT

PRODUCTION OF NATURAL FOOD COLORANT FROM PLANT EXTRACTS

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Food coloring agents are important substances used in the food industry because of more make appealling foods or recovery the color of products. Due to the negative effects of synthetic colorants on human health, the use of natural food colorants has become increasingly important in recent years.
Safflower (Carthamus tinctorius L.) grows in different geographical regions of Asia, including China and India, belongs to the Asteraceae family and it is an important oilseed plant. However, safflower is also used to color foods due to the yellow (carthamidin) and red (carthamin) pigments in its flower petals. In this study the yellow and red color pigments of the Olein and Zircon varieties of the safflower plant has an important cultivation area in Turkey were extracted. The yellow colors of both varieties of the plant were suspended in distilled water and the red colors were extracted using sodium carbonate solution. Total phenolic substance content, total flavonoid substance content and DPPH free radical scavenging activity of the obtained extracts were determined. To determine the total phenolic substance content was used the Folin-Ciocalteu method. The total content of flavonoid substance was determined with quercetin standard solution, and the antioxidant activity was determined using the DPPH free radical scavenging method. As a result of the in-vitro analysis, the highest total phenolic substance content (mg/gGAE) was determined as 2229.8 mg/gGAE in the yellow color extraction of the Zircon variety, the lowest was determined as 669.3 mg/gGAE in the red color extraction of the Olein variety. In the total flavonoid substance content analysis, the highest value was determined as 185.12 mgQE/g in the red color extraction of the Zircon variety, and the lowest value was determined as 64.77 mgQE/g in the red color extraction of the Olein variety. The highest %DPPH free radical scavenging activity values were determined in the yellow color extraction of the Zircon variety with 69.59%, while the lowest value was determined in the red color extraction of the Olein variety with 43.37%.

Key Words: Safflower, Food colorants, Extraction, Antioxidant activity

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ORAL PRESENTATION ABSTRACT

THERAPEUTIC POTENCY OF *Pistacia Terebinthus*: FROM TRADITIONAL APPLICATIONS TO MODERN INSIGHTS

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Pistacia terebinthus, commonly known as the terebinth or turpentine tree, has been a subject of growing interest due to its extensive medicinal properties. This paper explores the multifaceted therapeutic potential of *P. terebinthus*, integrating traditional knowledge with contemporary scientific evidence. Through a comprehensive analysis of its phytochemical composition and pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, this paper elucidates the diverse therapeutic benefits offered by *P. terebinthus*. Furthermore, recent studies have highlighted its potential role in specific medical conditions. Investigations suggest that *P. terebinthus* resin may induce cell death in MDA-MB-231 cells through caspase-independent apoptosis pathways, indicating its potential as a supportive treatment for breast cancer. Additionally, observations reveal that terebinth oil exhibits healing and protective effects in the treatment of ovarian ischemia/reperfusion injury, attributed to its modulation of radixin protein expression. Moreover, *P. terebinthus* extract has shown promising results in reducing bacterial load and accelerating wound healing processes. The chemical composition of *P. terebinthus*, rich in organic acids, sugars, essential oils, resins, proteins, tannins, and flavonoids, contributes to its diverse therapeutic effects. Traditional uses of *P. terebinthus* in treating peptic ulcers, asthma, gastralgia, rheumatism, skin inflammation, and other ailments are supported by scientific evidence. However, its potential applications extend beyond medicine, including its use in food, beverage production, and biodiesel production. The utilization of *P. terebinthus* resin as a yeast immobilization support in alcoholic fermentations demonstrates its potential in the beverage industry. Additionally, the genetic diversity of *P. terebinthus* populations and its suitability for biodiesel production highlight its significance in agriculture and environmental sustainability. Overall, this paper provides a comprehensive overview of the medicinal properties of *P. terebinthus*, emphasizing its potential as a valuable resource for healthcare, agriculture, and industry.

Key Words: Terebinth, Folk Medicine, Phytochemical Composition, Pharmacological Activities

ORAL PRESENTATION ABSTRACT

MORPHOLOGICAL DIVERSITY AMONG MEDICINAL WILD BARBERRY (*BERBERIS VULGARIS* L.) FRUITS

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Berberis vulgaris L., is a shrub which produces berries. In the present study, 9 wild individuals of this fruit species from different regions of Isfahan province from the center of Iran were evaluated using 18 morphological characters. For some morphological characters, variability found ranged from 0.5 to 1.6 cm in fruit length, 0.4 to 0.9 cm in fruit width and 0.12 to 0.44 g in fruit weight. In addition, color of mature fruit were varied from light yellow to dark red. Barberry fruit weight has a positive and significant correlation with seed length($r = 0.5$). Barberry color showed negative correlation with fruit length ($r = -0.79$) and positive correlation with leave color ($r= 0.74$). Furthermore, Number of fruits per panicle showed negative correlation with seed width($r = -0.73$). Also fresh fruit weight has positive correlation ($r= 0.79$) with blade length. The findings from the study highlight the necessity of conserving the individuals under examination as precious genetic assets. Moreover, enriching the genetic diversity of wild barberry is crucial for identifying numerous valuable, well-adapted specimens suitable for production.

Key Words: Diversity, Morphological traits, Barberry

POSTER PRESENTATION ABSTRACT

POSTER PRESENTATION ABSTRACTS

POSTER PRESENTATION ABSTRACT

EFFECT OF THE CULTIVATION SYSTEM OF *SARCOCORNIA PERENNIS* ON ITS NUTRITIONAL PROPERTIES

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Sarcocornia A. J. Scott, an edible halophyte in the Amaranthaceae family, is valued for its nutritional, sensory, and medicinal attributes and thrives in high-salinity environments. This study sought to enhance the growth of *Sarcocornia perennis* using semi-hydroponic (cocopeat) and conventional (peat, coco peat, and perlite) methods, assessing their impacts on nutrient and mineral profiles. Cultivated in an integrated multi-trophic aquaculture (IMTA) system with 35 mS/cm diluted effluent, the conventional approach outperformed semi-hydroponic in yield (1093 g FW/m^2 with 90% survival, compared to 685 g FW/m^2 with 97% survival) and in chlorophyll and carotenoid content, as well as plant growth. Both cultivation methods resulted in biomass proved microbiologically safe. Fiber content was similar across methods, albeit with variations in cellulose (7.88 and 8.86 g/100g DW for semi-hydroponic and conventional, respectively). The conventional method yielded higher ash content, possibly due to a greater mineral retention capacity, as reflected in the elevated levels of sodium, calcium, magnesium, aluminum, and vanadium. The findings indicate that conventional cultivation within an IMTA system leads to high productivity and robust nutritional quality of *S. perennis*, making it suitable for human consumption as confirmed by microbiological testing.

Key Words: Halophytes, *Sarcocornia perennis*, semi-hydroponic cultivation, conventional cultivation, nutritional properties

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POSTER PRESENTATION ABSTRACT

ABILITY OF *SALVIA HISPANICA* (CHIA) *IN VITRO* CULTURES TO BIOACCUMULATE HEALTH-PROMOTING METAL IONS

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Salvia hispanica L. (Lamiaceae), is a species that provides chia seeds, which, due to their rich chemical composition and valuable biological activities are classified as so-called "healthy food". Fortification with bio-elements is a modern technique for enriching foods with essential macro- and micro-nutrients [1,2]. The purpose of this study was to investigate the bioaccumulation capacity of the health-promoting metal ions magnesium (Mg), calcium (Ca), zinc (Zn), iron (Fe), chromium (Cr), selenium (Se), and lithium (Li) using a model of microshoot *in vitro* cultures of *S. hispanica* and to propose them as a source of therapeutic raw material with high nutritional value.

Microshoot cultures were grown on medium according to Murashige-Skoog [3] without the use of plant growth regulators. Salts of macronutrients - calcium ($CaCl_2$ x $6H_2O$) and magnesium ($MgSO_4$ x $7H_2O$) and micronutrients - iron (FeNaEDTA x $2H_2O$), zinc ($ZnSO_4$ x $7H_2O$), selenium (Na_2O_3Se), chromium ($K_2Cr_2O_7$), and lithium (Li_2SO_4 x H_2O) were added to the media in concentrations: 1, 5, 10, 25, 50 (mg per element per liter of medium). The results obtained were compared with the control samples (culture grown without the addition of elemental salts). The culture cycle lasted 14 days. Cultures were conducted under continuous white light (LED). Three culture series were carried out. Analysis of bioelement content was performed using inductively coupled plasma excitation mass spectrometry (ICP-MS) with an Agilent Technologies 7700 series instrument. The conducted experiments proved the high bioaccumulation capacity of elemental ions in the biomass of in vitro cultures of *S. hispanica*. The highest bioaccumulation (mg/100 g d.m.) was found for Cr (9.69), Zn (5.81), Se (2.24) and Li (0.49) ions supplemented at a concentration of 50 mg/L. *In vitro* cultures of *S. hispanica* can be proposed as an "innovative food" that can be used to enrich a wide range of food products with bio-elements that are valuable from a pharmaceutical nad nutritional point of view.

Key Words: *Salvia hispanica*, chia, in vitro, plant biotechnology, fortification

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POSTER PRESENTATION ABSTRACT

DISCOVERY AND CHARACTERIZATION OF GS27, A NOVEL ATP CITRATE LYASE INHIBITOR SHOWING IN VIVO ACTIVITY

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Oleocanthal, a key ingredient of EVOO, has garnered considerable scientific attention in recent years because of its biological activities and contribution to various aspects of human health. Prompted by the significant interest in this high-value natural compound, we describe herein the development of a concise and scalable procedure for the synthesis of various Oleocanthal analogues. This synthesis is achieved through a convenient biomimetic and stereo-controlled approach, starting from oleuropein, an abundant raw material found in olive leaves [1].

All synthesized compounds were evaluated for their anticancer activity against nine cancer cell lines with interesting activities. Among these, GS27 exhibited significant activity against all tested cancer cell lines, while also demonstrating appropriate ADME properties. GS27 altered the phosphorylation profile of ACLY, AMPK, and p70S6 in different cell types. ACLY is the "gate"-keeping enzyme that controls the de novo liposynthesis in cells, thus, targeting ACLY activity is a promising strategy to tackle the needful glucose dependent lipid synthesis for the propagating cancer cells. Furthermore, GS27 not only decreases the phosphorylation of ACLY but also p70S6, thereby inhibiting two major metabolic pathways: de novo lipogenesis and protein synthesis.

Key Words: Oleocanthal, oleuropein, anticancer activity, ACLY, AMPK

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POSTER PRESENTATION ABSTRACT

IMMUNOMODULATOR ACTIVITY POTENTIALS of ADJUVANT CANDIDATE SAPONINS ISOLATED from *CEPHALARIA* SPECIES

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It is known that pandemics pose a great threat to public health in the past, present and future. In the last century, it has become clear that the most effective way to protect against many infectious diseases is vaccination. For an effective vaccine, adjuvant is as important as antigen. Improvements in new vaccines can only be achieved with newly developed adjuvants and adjuvant systems. In this way, to create a stronger effect on the immune system is intended[1-2]. Saponins' one of the effects on the immune system is that they increase the level of specific immune response against the substance used in vaccine applications[3].
Within the scope of this project, it is aimed to develop vaccine combinations containing the potential saponin adjuvant obtained from *Cephalaria elmaliensis*. The immunomodulatory activity potentials as an adjuvant of saponins isolated from *Cephalaria elmaliensis* were examined in detail.

Key Words: Vaccine adjuvant, saponin, elmalienosides A-C, immunomodulatory effect, *Cephalaria*

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POSTER PRESENTATION ABSTRACT

ANTIOXIDANT, ANTIBACTERIAL, AND ANTIBIOFILM ACTIVITIES OF AQUEOUS CRUDE *GYMNEMA INODORUM* LEAF EXTRACT AGAINST VANCOMYCIN-RESISTANT *ENTEROCOCCUS FAECIUM*

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Vancomycin-resistant *Enterococcus faecium* causes nosocomial infections with high mortality and morbidity rates. Considering the urgent need for novel medicines or therapeutic options, we conducted this study to evaluate the antibacterial and antibiofilm activities of aqueous crude *Gymnema inodorum* leaf extract (GIE) against vancomycin-resistant *E. faecium* ATCC 700221.

We investigated GIE's antimicrobial and antibiofilm activities against the pathogen using the disk diffusion method and broth microdilution. The morphology of *E. faecium* ATCC 700221 after GIE treatment was observed by scanning electron microscopy. The antioxidant potential was evaluated by measuring the total phenolic and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^+$) radical scavenging activities. GIE exhibited antibacterial activities against vancomycin-resistant *E. faecium* ATCC 700221 with minimum inhibitory and minimum bactericidal concentration values of 125 and ≥250 mg/mL, respectively. GIE also inhibited biofilm formation in this pathogen and eradicated the biofilm formed by *E. faecium* ATCC 700221. GIE contained total phenols and exhibited antioxidant activities in the DPPH and $ABTS^+$ radical scavenging assays. This is the first report of GIE demonstrating its antimicrobial and antibiofilm activities against *E. faecium*. GIE can be considered a promising alternative treatment or preventive agent for bacterial diseases. However, further research might be necessary to identify the phytoconstituents responsible for potentiation or antagonism.

Key Words: *Gymnema inodorum*, biofilms, antibacterial activity, antioxidant activity, VRE

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POSTER PRESENTATION ABSTRACT

EXPLORING THE ANTICANCER POTENTIALS OF *LAVANDULA ANGUSTIFOLIA* MILLER AND *LAVANDULA* X *INTERMEDIA* EMERIC EX LOISEL

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The pharmaceutical industry is focusing on the research and development of medicinal and aromatic plant-based drugs due to their potential for enhanced efficacy and fewer side effects, especially in conditions like cancer treatment that significantly impact patients' quality of life. The genus Lavandula L. (Lamiaceae) possesses over 39 species and 79 intraspecific taxa and hybrids [1]. Recent studies have highlighted the promising anticancer potentials of various Lavandula species [2, 3]. This study aims to evaluate the anticancer potentials of L. angustifolia Miller and L. x intermedia Emeric ex Loisel against various cancer cell lines. Plant material was collected from Istanbul, Turkiye, during the flowering season. The MTT method [4] was employed to assess the extracts' impact on cancer cell viability, with 5-fluorouracil serving as the positive control, and experiments were triplicated.

Results revealed significant bioactivities in both Lavandula species tested. L. angustifolia extract demonstrated superior efficacy, particularly against A549 and MCF-7 cell lines, with IC50 values of 5.55 ± 0.21 µg/mL and 41.54 ± 0.26 µg/mL, respectively. Notably, its potency was greater than the positive control (5.75 ± 0.05 µg/mL) against A549 cells. Conversely, L. x intermedia exhibited moderate inhibition on cells, with IC50 values ranging from 51.90 ± 0.43 µg/mL to 77.37 ± 0.34 µg/mL. Due to the promising inhibition of L. angustifolia on the A549 cell line, ongoing investigations in our laboratories focus on the plant's phytochemical characterization and its molecular mechanism on lung cancer.

Key Words: *Lavandula angustifolia, Lavandula x intermedia,* cancer, cytotoxicity

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POSTER PRESENTATION ABSTRACT

EXAMINATIONS ON THE DRUG MELISSAE FOLIUM

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Lemon balm, scientifically known as *Melissa officinalis* L., has a long history of use in folk medicine as a sedative, carminative, sudorific, and stomachic (1,2). These medicinal properties have also been verified through experimental research (1,3). Rosmarinic acid, a hydroxycinnamic acid derivative, is one of the main secondary metabolites present in lemon balm leaves and is used as a marker compound for the plant (3,4). The European Pharmacopoeia monograph calculates the percentage of total hydroxycinnamic acid derivatives of the drug based on rosmarinic acid (4).

This study evaluated the compliance of the 9 purchased samples of *M. officinalis* with Turkish Pharmacopoeia-European Pharmacopoeia Adaptation (2016) standards. Of the 9 samples, 7 were purchased from herbalists in the Spice Bazaar and 2 were purchased from websites selling herbal medicinal tea. Macroscopic and microscopic examinations, loss on drying and total ash determination, TLC, and UV spectroscopy experiments were conducted, and it was concluded that all samples except for 4 herbalist samples were found to suitable for the pharmacopeia standards. The examination revealed that 5 samples were identified as *Melissa officinalis*. The study also conducted a morphologic, microscopic, and chromatographic comparison of *M. officinalis* with *Aloysia triphylla*, a drug frequently sold as 'melisa' in herbalists, and the results were discussed (3,4). It is recommended to obtain the herbs from reliable sources such as certified online stores specializing in herbal teas or certified herbalists, where the herbs have undergone active ingredient controls and have been checked for compliance with pharmacopoeia standards. This ensures a more reliable and trustworthy supply of herbal products.

Key Words: *Melissa officinalis*, European Pharmacopoeia, Rosmarinic acid, Citral

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POSTER PRESENTATION ABSTRACT

INVESTIGATION ON THE TRADITIONAL EXTRACTION METHOD OF BLACK RADISH (*RAPHANUS SATIVUS* VAR. *NIGER*): CHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY SCREENING

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Raphanus sativus var. *niger* (black radish) is an agricultural plant in the Brassicaceae family which grows in temperate climates[1,2,3]. Radishes have miscellanous properties such as anti-cancer, antimicrobial, antiviral and antioxidant[3,4]. Radishes have been used in traditional medicine and homeopathy for cough, gallstones, nausea, indegtion and etc[5]. Black radish has been approved by Commission E for cough, bronchitis and dyspeptic complaints[5]. In our country, black radish has been used in the treatment of cough, bronchitis and as an expectorant with a traditional preparation method[6]. In the traditional preparation method, a cavity is made in the root of the black radish and the root is placed in this cavity and waited for 24 hours[6]. The mixture is collected from the lower parts of the plant through the holes and used in the treatment of cough by drinking[6]. There are various studies on the black radish[4,7,8,9]. One of these studies is on the phenolic compounds and antioxidant activity contained in the plant[4,9]. The use of products with antioxidant activity is very important for reducing oxidative stress, which is seen as the cause of many diseases today, and thus preventing many oxidative damage-based diseases that may occur in the human body. In this study, black radish was extracted with different solvents such as honey, carob extract and juniper molasses by using both the traditional method and as an alternative to this method, slicing and direct mixing of black radish extract with solvents. Some of the liquid extracts obtained were lyophilized and some were preserved in liquid form. The antioxidant activity of both lyophilized and liquid extracts was determined by DPPH method. The best activity was observed in the extracts obtained using carob solvent. Thus, LC/HRMS analyses were performed on the carob extract and black raddish juice for comparison of the phenolic compounds.

Key Words: Antioxidant, traditional, black radish, cough, bronchitis

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POSTER PRESENTATION ABSTRACT

ETHNOBOTANY OF *TARAXACUM* SP. IN TURKIYE AND PHARMACOPOEIA ANALYSIS OF *TARAXACUM HELLENICUM* DAHLST. AND *TARAXACUM ALEPPICUM* DAHLST.

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Taraxacum is a genus belonging to the Asteraceae family, is represented with 54 taxa in Turkiye [1]. Ethnopharmacological, ethnobotany, chemical composition, biological activity and animal studies on the genus have shown that *Taraxacum* sp. has a value in drug development [2]. *Taraxacum officinale* F. H. Wigg is also included in the Turkish Pharmacopoeia as an official medicinal plant [4]. In this study, we aimed to collect the ethnobotanical uses of *Taraxacum* sp. in Turkiye for possible future studies and to investigate the standardisation potential of two species commonly found in Turkiye, according to the Turkish Pharmacopoeia. Website of the National Thesis Center of Council of Higher Education and Google Scholar were used to search for ethnobotanical uses in Turkey. Both *Taraxacum hellenicum* and *Taraxacum aleppicum* were collected from Turkiye. Macroscopical and microscopical evaluation, thin layer chromatography, ash and water contents determination analyses have conducted according to the requeriments under the "Taraxaci officinalis herba cum radice" title in Turkish Pharmacopoeia [4]. Some additional phytochemical tests have applied to the water extracts of both roots and aerial parts of two plants to understand the presence of chemical groups in the plants as described in Baytop's book [3]. Of the 94 master's/doctoral theses in the database that contain both *Taraxacum* and ethnobotanical concepts, only 68 theses contain information on the ethnobotanical use of *Taraxacum* sp. While food use is one of the most common uses, medical and ethnoveterinary uses are also found throughout the country. When all Pharmacopoeial analyses were evaluated, similar results were found between *Taraxacum officinale* and both *T. hellenicum* and *T. aleppicum* were found similar according to the requeriments in the Pharmacopoeia. Considered to the phytochemical tests, all extracts contain flavones, tannins and saponins.

Key Words: Taraxacum, pharmacopoeia analysis, phytochemical tests.

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POSTER PRESENTATION ABSTRACT

ONOSMA AMBIGENS LACAITA: EVALUATION OF CHOLINESTERASE INHIBITORY ACTIVITY

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Alzheimer's disease presents cognitive decline and memory loss challenges. Cholinesterase inhibitors promise symptom alleviation by enhancing cholinergic neurotransmission. Natural products, crucial in Alzheimer's research, offer diverse structures for potent inhibitors, with multifaceted pharmacological properties including antioxidant and neuroprotective effects, potentially leading to safer and more effective treatments, thus improving patient outcomes.*Onosma ambigens* Lacaita (Boraginaceae), exhibits over 40% endemism in our country. With 230 species globally and 104 taxa in Turkey, including 55 endemics, Anatolia emerges as a significant gene center for this genus. *Onosma* species are valued for medicinal and culinary purposes, with uses ranging from wound healing to neurological disorders. Despite numerous studies exploring the biological activities of various *Onosma* species, none have investigated the specific impact of *Onosma ambigens* on Alzheimer's. This study aims to address this gap and determine the potential of the plant in Alzheimer's treatment.*Onosma ambigens* were collected in August 2021, from Tokat at an altitude of 2000 m. A voucher specimen was prepared with GOPU 8614 code in the Herbarium of Tokat Gaziosmanpaşa University. The aerial and subaerial parts were separately air-dried in the shadow and then milled. Methanol extract was prepared from aerial and subaerial parts of the plant and alkaloid-containing chloroform extract was prepared from this methanol extract. The extracts of *Onosma ambigens* were evaluated for their inhibition of Acetylcholinesterase (AChE) and Butrylcholinesterase (BChE) enzymes using the Ellman method, with Galantamine employed as a positive control for in vitro testing. All the reactions were performed in triplicate. As a result of the study, only alkaloid-containing chloroform extract of aerial parts of *Onosma ambigens* showed a butyrylcholinesterase inhibitory effect, while other extracts did not exhibit the same performance.

Key Words: Onosma ambigens, Boraginaceae, Alkaloids, Cholinesterase inhibitor, Ellman

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POSTER PRESENTATION ABSTRACT

POLAR EXTRACTS PREPARATION OF *PLATANUS ORIENTALIS* AND DETERMINATION OF THEIR PHYTOCHEMICAL CONTENT BY LC-HRMS ANALYSIS

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Platanus orientalis (Eastern Plane) is a member of the *Platanaceae* family. It is common in Asian Countries, Middle Eastern Countries, Eastern Europe, and especially Turkiye. In traditional Turkish medicine, different parts of plants are used for pain relief, rheumatism, and wounds (1,2). There are numerous studies prove various activities of different extracts of Platanus orientalis (3-5). For determining the contents of *Platanus orientalis* four extracts with different solvent rates and methods were prepared (6-7). Extracts obtained; *P. orientalis* EtOH- Soxhlet extract (P1) *P. orientalis* (70%) EtOH/Water-Soxhlet extract (P2), *P. orientalis* (70%) EtOH/Water- Maceration extract (P3), *P. orientalis* EtOH- Maceration extract (P4). Analyzed separately with LC-HRMS. After evaluating and calculating the LC-HRMS results, the analysis report was generated. Major components and phenolic contents of the extracts were revealed. As a result of LC-HRMS analysis of the four polar extracts prepared, the major components of the P1 extract are chlorogenic acid, 6-OH-luteolin-7-glucoside, hyperoside and quercitrin, respectively; chlorogenic acid, quercitrin, fumaric acid, hispidulin-7-glucoside, rhamnocitrin, quercetin and ascorbic acid are the major components of P2 extract; hyperoside, quercitrin, rutin and hispidulin- 7-glucoside are the major components of P3 extract, 6-OH-luteolin-7-glucoside, chlorogenic acid, quercetrin, hispidulin-7-glucoside, rhamnocitrin and ascorbic acid were identified as the major components of P4 extract. Thus, as a result of this study, the analysis and determination of the major compounds that may be responsible for the activities of the *P. orientalis* plant, which is active in terms of different activities, were carried out.

Key Words: *Platanuns orientalis*, Soxhlet Extraction, Maceracion Extraction, LC-HRMS, Phenolic content

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POSTER PRESENTATION ABSTRACT

VIRTUAL SCREENING OF GALANTAMINE DERIVATIVES AS NOVEL ACETYLCHOLINESTERASE INHIBITORS

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Alzheimer's disease is a condition that causes memory loss and cognitive disorders as a result of cholinergic impairment. Galantamine, an alkaloid extracted from *Amaryllidaceae* species, is an acetylcholinesterase inhibitor used in the treatment of mild to moderate Alzheimer's disease which increases the acetylcholine amount in the synapses. Considering the severity of Alzheimer's disease, exploring novel drug candidates is crucial for developing future treatment options. In this study, 1843 new galantamine derivatives substituted from the hydroxyl group were examined using R-group library and the virtual screening workflow of Schrodinger Suites. Galantamine is substituted with the R- groups in the Schrodinger Suites' library and the derivatives obtained are docked by high throughput virtual screening, standard docking and extra precision docking methods to acetylcholine esterase enzyme, respectively, to determine the most potent molecule. As a result, (3-(aminomethyl)azetidine-1-yl)methyl substituted derivative showed a -13.563 kcal/mol docking score with an MM-GBSA ΔG binding affinity score of -46,03 kcal/mol. Additionally, to investigate protein-ligand stability molecular dynamics studies were performed utilizing D.E. Shaw Research Desmond. Trajectories obtained from dynamics simulation were analyzed and RMSD value of ligand fit on protein were measured as 1.83 Å, while ligand fit on ligand RMSD is 0.26 Å and RMSD of C-alpha atoms of protein residues is 2.07 Å. Consequently, upon discovery of new acetylcholinesterase inhibitors, galantamine provides promising core with substituents bearing aliphatic amine groups providing hydrogen bond and cationic interactions.

Key Words: Alzheimer's disease, galantamine, acetylcholinesterase, virtual screening, molecular dynamics.

POSTER PRESENTATION ABSTRACT

ISOLATION AND PURIFICATION OF VACCINE ADJUVANT CANDIDATE SAPONINS FROM *C. SCOPARIA*

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Vaccine adjuvants play a critical role in enhancing the immunogenicity of vaccines, thereby bolstering the immune response and increasing vaccine efficacy. Saponins, natural compounds derived from various plant sources, have garnered attention as potent adjuvant candidates due to their ability to stimulate both innate and adaptive immune responses [1]. When they used as adjuvants, saponins can enhance the bioavailability and absorption of co-administered drugs [2]. *Cephalaria scoparia* is an endemic plant of Turkiye, occurs in southwestern Anatolia [3]. Previous studies have observed that *C. scoparia* is rich in saponins, particularly scoposides A-C, suggesting great potential as adjuvants [4-5]. Promising preliminary results obtained from the study entitled "*In vitro* Investigation of the Potential for Use of Saponins as Adjuvants in Seasonal Influenza (H3N2) Vaccine Formulations" showed that these saponins used with inactive H3N2 stimulate the IFN-γ cytokine response, which is important in the formation of a Th1 response and the development of an immune response against viruses [5].
Therefore, this study focused on the isolation and purification of adjuvant candidate saponins namely scoposides A-C in high yield. This process outlines a comprehensive methodology for the isolation and purification of vaccine adjuvant candidate saponins from *C. scoparia*. It involves solvent extraction followed by further chromatographic techniques such as column chromatography and medium-performance liquid chromatography (MPLC). Characterization techniques such as infrared, nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry are employed to confirm the identity and structural integrity of the isolated saponins. In conclusion, the isolation and purification methodology outlined in this study provides a promising approach for obtaining high yields of scoposides A-C from *Cephalaria scoparia*.
Key Words: Vaccine adjuvant, *Cephalaria scoparia*, Caprifoliaceae, scoposides A-C, saponin.

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POSTER PRESENTATION ABSTRACT

LC-HRMS ANALYSES OF *SALVIA VENERIS* HEDGE ROOT EXTRACTS WITH ANTI-ALZHEIMER ACTIVITY

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Salvia L., a genus with around 1000 species, has a long history of use in traditional folk medicine. The *Salvia* genus is a member of the Lamiaceae family, and 10 species of the *Salvia* genus grow in the Cyprus flora. *Salvia veneris* Hedge, an endemic species in Cyprus, is commonly used as herbal tea instead of *S. fruticosa*. The root parts of *S. veneris* were collected from Northern Cyprus in 2014 and were extracted with dichloromethane and methanol, respectively. The anti-Alzheimer's activity of dichloromethane and methanol extracts from *S. veneris* was studied for their inhibitory effects on acetylcholinesterase and butyrylcholinesterase enzymes using the Ellman's method. The results were compared with galantamine, a standard compound.
The dichloromethane and methanol extracts from *S. veneris* inhibited BChE activity in a dose dependent-manner and dichloromethane extract was found to be the most active extract. Whereas all extracts did not show any observable inhibitor activity on acetylcholinesterase. The LC-HRMS analysis was conducted to identify polar compounds present in the methanol extract. The extract contains chlorogenic acid (6803.25 mg/kg), p-coumaric acid (2291.48 mg/kg), hyperoside (18558.70 mg/kg), myricetin (2647.80 mg/kg) and quercetin (4246.06 mg/kg) as the main compounds, as well as some phenolic acids and flavonoids. Previous research indicates that *Salvia* species' terpenoid components are responsible for their inhibitory effects on acetylcholinesterase and butyrylcholinesterase. Accordingly, a bioactivity-directed isolation investigation is proposed to identify the anticholinesterase inhibitor compounds of *S. veneris* root dichlorometane extracts.

Key Words: *Salvia veneris*, LC-HRMS, anti-Alzheimer activity, phenolics, flavonoids

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POSTER PRESENTATION ABSTRACT

EVALUATION OF ETHNOBOTANICAL CHARACTERISTICS OF URTICA GENUS

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The aim of this study was to determine of *Urtica* species with ethnobotanical usages in Turkey. For this purpose, ethnobotanical researches conducted so far were reviewed. The genus *Urtica* in the Urticaceae family is represented by 5 species in Turkey: *U. dioica*, *U. haussknechtii*, *U. membranacea*, *U. pilulifera* and *U. urens*. Stinging nettle is found in almost every region of Turkey and has a very wide distribution. *Urtica* species are popularly known as Isırgan, Isırgan otu, Deli ısırgan, Sırgan, Sıgırgan, Dalağan, Dalağaz otu, Dalan, Dılan, Dızlağan, Cızlağan, Çincar, Gezerek Yığınç, Kopriva and Kupriva. All Urtica species are used as traditional folk medicines. Of these *U. dioica*, *U. pilulifera* and *U. urens* species are the most widely used. Stinging nettle is used as a traditional folk medicine for shortness of breath, cough, expectorant, colds, flu, lung diseases, bronchitis, digestive system diseases, cancer, insomnia, gynecological diseases, diabetes, pain relief, rheumatism and skin diseases. Especially of the aerial parts of *U. dioica* and *U. urens* are also consumed as a food and it is cooked as a meal, soup and borek. As a result, stinging nettle is a plant that is both used in treatment and consumed as food by the people and has a wide distribution in our country.

Key Words: Urtica, Ethnobotany, Medicinal plants, Edible plant, Folk medicine, Turkey

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POSTER PRESENTATION ABSTRACT

ISOLATION, PURIFICATION and STRUCTURAL DETERMINATION of IMMUNOMODULATOR ACTIVE SAPONINS from *C. ELMALIENSIS*

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Immunomodulators play a pivotal role in regulating the immune system, offering promising avenues for therapeutic intervention in various diseases [1]. Saponins, naturally occurring compounds abundant in plant sources, have emerged as potent immunomodulators. Renowned for their diverse biological activities, low toxicity, and widespread availability, they represent a promising area of research [2]. Among these saponins, elmalienosides A-C derived from *Cephalaria elmalienis*, exhibit remarkable immunomodulatory properties [3-4]. It has been determined that elmalienosides A-C saponins increase IL1-beta production and do not have any cytotoxic effects on cells. Therefore, improving the isolation process to obtain these saponins in high quality and yield are crucial for future studies and commercial applications. This study focusing on the isolation, purification of saponins from *C. elmaliensis*. The process entails solvent extractions (MeOH, BuOH (1:1), *n*-hexane), followed by chromatographic techniques such as RP-VLC, column chromatography and medium-performance liquid chromatography (MPLC). Structural determination is accomplished through advanced analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy and high resolution mass spectrometry (HR-MS). This study highlights the significance of isolating, purifying, and structurally characterizing elmalienosides A-C from *C. elmalienis*, contributing to the exploration of novel immunomodulatory agents for disease management and drug development.

Key Words: Vaccine adjuvant, *Cephalaria elmaliensis*, Caprifoliaceae, elmalienosides A-C, saponin.

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POSTER PRESENTATION ABSTRACT

THE EVALUATION OF ANTIOXIDANT EFFECTS OF CHRYSIN AND ITS BETA-CYCODEXTRIN COMPLEX

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Chrysin is a polyphenolic compound included in flavones group. It is present in honey, propolis and medicinal plants (*Momordica charantia*, *the* flowers of *Juglans regia*, the leaves and fruits of *Hyphaene thebaica*, the peel of *Passiflora edulis* fruit). In some medicinal plants, chrysin is present as glycosides. The reduced number of hydroxyl groups, especially in B and C ring explains the low water solubility of chrysin. The aim of our study was the evaluation of antioxidant properties of chrysin and its complex with beta-cyclodextrin. By including chrysin in beta-cyclodextrin we improved the water solubility of this. In addition, the bioavailability increases and the transformation of chrysin into the digestive system is reduced. We evaluated the antioxidant properties by the next tests: the DPPH scavenging test, the iron-chelating test, the hydroxyl-scavenging test, and the lipoxygenase inhibition test. All tests were carried out by spectrophotometric methods. For each tests we determined the inhibition or the scavenging ability of compounds and the value of EC50 (effective concentration 50%). The antioxidant effects of chrysin depend on the dose used. In the same time, by beta-cyclodextrin complexation we improved the antioxidant properties. The antioxidant properties of chrysin and its complex with beta-cyclodextrin are important to ensure the body's protection in diseases that are determined or aggravated by oxidative stress.

Key Words: chrysin, antioxidant test, iron chelating test, lipoxygenase

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POSTER PRESENTATION ABSTRACT

EFFECT OF MYCORRHIZAL FUNGI ON THE CHEMICAL PROFILE OF *MENTHA SUAVEOLENS* EHRH. SUBSP. *TIMIJA* FROM MOROCCO

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Aromatic and medicinal plants (MAP) have always attracted growing interest due to their potential therapeutic, cosmetic, and culinary properties. Regarding socio-economic importance, Morocco is the world's 12th largest exporter of these plants. However, abusive and uncontrolled exploitation of these MAPs, exacerbated by global warming, may lead to genetic erosion and extinction of these resources. It is currently accepted that plants can associate with microorganisms as endophytes or symbionts in different parts of the plant. In the soil, arbuscular mycorrhizal fungi (AMF) can interact synergistically to stimulate the growth and development of all MAPs. This study aimed to evaluate the effectiveness of AMF in enhancing the qualitative and quantitative production of bioactive molecules in Mentha *suaveolens Ehrh. subsp. Timija*, under different microenvironments (or sites). We studied the physico-chemical parameters of rhizospheric soil, microbial profile parameters, and, finally, the phytochemical parameters of spontaneous plants of this species at the two sites. The results show that the microenvironment significantly affects mycorrhization intensity, frequency, and AMF spore density. The rhizospheric soil from site 1 showed higher mycorrhization intensity (10%) and spore density (819 spores/100 g) than site 2. AMF improves the quantity and quality of total polyphenols and essential oils (in terms of quality) in *Mentha suaveolens Ehrh. subsp. Timija* by promoting the solubilization of total phosphorus into a form more assimilable by plants.

Key Words: Aromatic and Medicinal Plants, *Mentha suaveolens Ehrh. subsp. Timija*, Arbuscular Mycorrhizal Fungi, Physicochemical analysis, Biochemical analysis.

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POSTER PRESENTATION ABSTRACT

EXPLORING THE CARDIOPROTECTIVE EFFECTS OF MELILOTUS OFFICINALIS AND MELILOTUS ALBUS EXTRACTS: INSIGHTS INTO TRADITIONAL ROMANIAN HERBAL MEDICINE

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This study explores the cardioprotective properties of hydroalcoholic extracts from the aerial parts of two species of *Melilotus*, namely *Melilotus officinalis* and *Melilotus albus*, which are traditionally utilized in Romanian folk medicine for their diuretic, anti-inflammatory, anti-edematous, and antispasmodic benefits. Despite their widespread use, their impacts on heart health remain under-researched. Our investigation focused on assessing the protective effects of these extracts against isoprenaline-induced myocardial infarction in rats, monitored through electrocardiograms, evaluation of serum markers of oxidative stress, and cardiac injury indicators. The total phenolic, flavonoid, and coumarin contents of the *Melilotus* sp. extracts were analyzed, alongside specific polyphenols via HPLC-MS. Both extracts demonstrated notable antioxidant activities in vivo; specifically, *Melilotus officinalis* and *Melilotus albus* extracts significantly lowered oxidative stress indices (OSI and TOS) and nitric oxide levels, respectively, with the former also elevating sulfhydryl (SH) group levels. LC-MS analysis revealed several polyphenolic compounds in both plants, including catechin, syringic acid, protocatechuic acid, and vanillic acid, with gallic acid and rosmarinic acid exclusive to *Melilotus officinalis*. These findings suggest the potential of *Melilotus officinalis* and *Melilotus albus* extracts in offering cardioprotection by mitigating oxidative stress during myocardial ischemia.

Key Words: *Melilotus* species, cardioprotective properties, preclinical trial, LC-MS analysis.

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POSTER PRESENTATION ABSTRACT

GEUM URBANUM L. RHIZOME EXTRACTS DERIVED NOBLE METAL NANOPARTICLES: SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT POTENTIAL

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This study aimed to develop an innovative and eco-friendly method for the synthesis of silver (AgNPs) and gold nanoparticles (AuNPs) by using aqueous and ethanolic *Geum urbanum* L. rhizome extracts. The noble metal nanoparticles were characterized by UV-Vis, FTIR and EDX spectroscopies, dynamic light scattering technique (DLS) and transmission (TEM) and scanning (SEM) electron microscopies. The antioxidant potential of these products was also evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and lipoxygenase inhibition (LOX) assays. The bio-synthesis process of AgNPs and AuNPs was confirmed by the presence on the UV-Vis spectra for the specific peaks corresponding to the surface plasmon effect of metallic Ag (400 – 450 nm) and Au (500 – 550 nm). FTIR spectroscopy revealed the involvement of *G. urbanum* rhizome extracts' phytochemicals in the nanoparticle synthesis process. As indicated by TEM and SEM, AgNPs were majorly spherical in shape, while AuNPs showed a variety of shapes, including quasi-spherical, polygonal and triangular; all dimensions were under 25 nm. EDX analysis confirmed the presence of Ag and Au by the detection of strong signals at 3 (AgNPs) and 2.2 keW (AuNPs). In DLS analysis, all samples showed negative values for zeta potential, between - 21 and - 13 mV, suggesting the stability of the colloid system in dispersion. AgNPs and AuNPs were also characterized by an important antioxidant potential, significant activity being obtained for the aqueous *G. urbanum* rhizome extracts derived AgNPs (EC50 = 34.2 ± 1.86 mg/mL in LOX assay). In conclusion, this study reports a rapid and simply route for the synthesis of AgNPs and AuNPs having a promising antioxidant potential.

Key Words: green synthesis, plant extract, silver nanoparticles, gold nanoparticles, antioxidant potential.

Acknowledgements
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POSTER PRESENTATION ABSTRACT

ISOLATION AND SEMI-SYNTHETIC DERIVATIVES OF CHIOS MASTIC GUM TRITERPENIC ACIDS AND EVALUATION OF THEIR ANTI-INFLAMMATORY ACTIVITY

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Chios Mastic Gum (CMG) is a resin derived from the evergreen shrub *Pistacia lentiscus* var. *Chia* L. of Anacardiaceae family. It is a rich natural resin with approximately 120 constituents described so far, categorized into three groups: the polymer fraction, the essential oil and the fraction of terpenoids. Terpenes constitute the major chemical group and is devided into two groups the neutral and the acidic fraction of triterpenoids. Notably, the characteristic triterpenic acids found in CMG are the 24*Z*-masticadienonic acid (MNA) and the 24*Z*-isomasticadienonic acid (IMNA) belonging to the group of tirucallane-type triterpenes. Additionally, oleanonic and moronic acid, which are pentacyclic triterpenes, are abundant as well [1] . Many studies highlight the beneficial biological properties of pentacyclic triterpenes such as anticancer and anti-inflammatory activities. However, regarding the biological profile of the two isomers MNA and IMNA, there is limited information available due to the challenging task of isolating them as pure compounds. Thus, the aim of this work was to establish an alternative approach for the isolation of the major triterpenic acids, the production of MNA and/or IMNA and the evaluation of their anti-inflammatory activity. Prior to any chromatographic method, the removal of cis-1,4-poly-β-myrcene from CMG was performed, followed by a liquid-liquid extraction involving a gradual pH adjustment from basic to acidic, to separate neutral from acidic triterpenes. Then, a preparative HPLC-PDA method was developed and applied to the acidic fraction of triterpenes, so oleanonic, moronic and the mixture of MNA/IMNA were isolated in pure form. Then a method for obtaining the two isomers of IMNA (24*Z*/*E*) in high-yield was developed using BBr_3. Remarkably, the mixture of MNA/IMNA, along with 24*Z*-IMNA and 24*E*-IMNA, exhibited substantial anti-inflammatory properties. Among these, 24*E*-IMNA, a non-previously described compound, showed a significant reduction in TNF-α, IL-6, and NF-kB mRNA levels in RAW 264.7 macrophage cells.

Key Words: Chios Mastic Gum, resin, 24*Z*-masticadienonic acid, 24*Z*-isomasticadienonic acid, anti-inflammatory properties

Acknowledgements
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POSTER PRESENTATION ABSTRACT

PHYTOCHEMICAL COMPOSITIONS, ANTIOXIDANT ACTIVITIES AND ANTI-INFLAMMATORY ACTIVITIES OF *ARTEMISIA LACTIFLORA* EXTRACT

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Artemisia lactiflora, a Chinese-origin medicinal plant, has been reported to have unique phytochemicals responsible for its activities. However, scientific literature regarding the biological activities of *A. lactiflora* remains unclear. Investigation into its composition, activities, and mechanisms may provide insight into its therapeutic potential in addressing various health conditions. In this study, we conducted an extraction and fractionation of *A. lactiflora* leaves using solvents with varying polarities. The evaluation encompassed total phenolics, total flavonoids, DPPH and ABTS scavenging activities, as well as cytotoxicity. Additionally, anti-inflammatory properties were assessed by pre-treating macrophages with the extract or fractions followed by induction of an inflammatory response using lipopolysaccharide (LPS). Inflammatory responses were assessed by measuring expression of pro-inflammatory genes and the secretion of pro-inflammatory cytokines. Among all the extracts and fractions of *A. lactiflora*, the butanol fraction exhibited the highest levels of phenolics, flavonoids, and DPPH scavenging activity. Furthermore, all extracts and fractions significantly suppressed the expression of pro-inflammatory genes (*RelA*, *TNF*, *IL6*) and reduced the secretion of pro-inflammatory cytokines (TNF-α, IL-6) ($p < 0.0001$) compared to LPS-induced macrophages. Notably, the ethyl acetate fraction demonstrated the most pronounced anti-inflammatory activity by decreasing the secretion of pro-inflammatory cytokines. These findings suggest the potential of *A. lactiflora* in mitigating inflammatory responses possibly through inhibition of the NF-κB-dependent pathway. Collectively, these results highlight the prospective potential of this plant in both the prevention and treatment of inflammatory reactions triggered by bacterial infections.

Key Words: Artemisia lactiflora, Antioxidant, Anti-inflammatory.

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POSTER PRESENTATION ABSTRACT

VARIATION IN TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *OPUNTIA FICUS-INDICA* L. SEEDS EXTRACT OF TUNISIAN CULTIVARS

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Opuntia ficus-indica L is one of the most important cacti for agronomic and medicinal uses. In recent years, research have focused on the Opuntia seeds which contain bioactive molecules with antioxidants proprieties. In this context, the objective of our work is to demonstrate the richness of prickly pear seeds collected from three regions grown in Tunisia (Kasserine, Gafsa, and Sidi Bouzid) in total phenolic content and to determine their antioxidant potential. Total phenolic and flavonoids contents (TPC and TFC) were determined by the Folin-Ciocalteu and Quercetin methods, respectively. Antioxidant activity was evaluated using the DPPH radical scavenging assay. The TPC of prickly pear seeds ethanolic extract of three regions ranged from 178 mg to 333 mg gallic acid equivalent/g dry extract (mg GAE/100g DE), and the TFC varied from 21.6 mg to 28.5 mg quercetin equivalent/g dry extract (mg QE/100g DE). It can be inferred that the population of Kasserine is the richest in antioxidants with a value of 490 mg EAA/100g of extract, followed by the population of Sidi Bouzid with a value of 420 mg EAA/100g DW and 350 mg EAA/100g DW for the population of Gafsa. These results confirm that the phenolic compounds in our seed extracts significantly contribute to their antioxidant capacity, and the quantity of these compounds depend on the botanical origin of the sample. Cactus pear can indeed be exploited as a good and cheap source of natural antioxidants.

Key words: *Opuntia ficus-indica* L., Total phenolic content, Antioxidant activity, correlation.

POSTER PRESENTATION ABSTRACT

IN VITRO EVALUATION OF THE ANTIFUNGAL EFFECT OF THE ESSENTIAL OIL OF *ORIGANUM COMPACTUM*

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Aromatic and medicinal plants contain active molecules with significant properties in different fields. The use, the function and the importance of them are variable. Produced by different extraction technics from aromatic plant; the essential oils constitute a complex natural mixture of secondary metabolites with rich source of molecule having multiple properties of fight against pathogens. Some fungi are human pathogens; *Penicillium* is one of them, many species belonging to the genus have been well studied; it is an organism that has demonstrated extraordinary survival capabilities; cases of *Penicillium* infections in humans have been described and involved both immunocompetent and immunocompromised individuals. The objective of this work was to *in vitro* investigation of the antifungal properties of the essential oil of *Origanum compactium* against *Penicillium*. By quantitative and qualitative estimation of the power of the oil extracted by steam entrainment of *Origanum* leaves against a clinically isolated mycelial train in comparison to an antiseptic as a positive control and this following the methods of disk diffusion, disk volatilisation and estimation of minimal concentration effect. Following the results obtained, the *Origanum compactum* essential oil tested showed a strong inhibitory potential on the growth of *Penicillium*. The qualitative and quantitative with only 10 microlitre and 0.03 % respectively, demonstrat fungicidal effect. *Origanum compactum* essential oil demonstrated an antifungal effect on *Penicillium.* These results must be pursued by *in vivo* tests to reveal the applications of the product; in order to valorize the Algerian aromatic plants and exploit multiple scientific knowledge. These volatile molecules will find each their application in prescribed dose in the various fields that it is pharmaceutical, food and many other.

Key Words: aromatic, essential oil, *Origanum compactum*, *in vitro*, *Penicillium*

POSTER PRESENTATION ABSTRACT

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF *TWO MOROCCAN THYME SPECIES* METHANOLIC EXTRACTS

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Natural bioactive compounds have been gathering the interest of researchers due to their potential effect and their uses in a plethora of industries. Polyphenols one of the most abundant secondary metabolites of plants have garnered growing interest owing to their powerful antioxidant attributes and their significant impact on averting diverse diseases linked to oxidative stress, including cancer.

The aim of the present study was to assess the antioxidant and antibacterial activities of phenolic extracts (*Thymus pallidus, Thymus satureioides*). In order to accomplish the objective, the phenolic extracts were obtained using methanol maceration then the phenolic content was determined. While the antioxidant ability of the phenolic extracts was evaluated using TAC, DPPH and FRAP assays the antibacterial potential was assessed using microdilution method.

The phenolic content of *T.satureioides* was significantly higher than *T.pallidus* .Regarding the antioxidant activity ,the phenolic extract of *Thymus satureioides* exhibited an important IC_{50} (DPPH 0,032 ± 0,001 mg/mL), (TAC 0,012 ± 0,001 mg/mL) and EC_{50} (Frap 1,21± 0,05 mg/mL), compared to *Thymus pallidus* that showed an IC_{50} (TAC 0,483 ± 0,001 mg/ml), and an inactivity for both DPPH and FRAP assays. Concerning the antibacterial potential, results showed that phenolic extracts of *T.satureioides* and *T.pallidus* demonstrated an inihibitory effect against tested human pathogenic strains.

These finding results suggested that the phenolic extracts may be an efficient source of bio-active compounds, which could be used at the pharmaceutical and cosmetic scale.

Key Words: Phenolic extract, *Thymus satureioides, Thymus pallidus,* antioxidant activity, human pathogenic strains

POSTER PRESENTATION ABSTRACT

AROMATHERAPY IN DERMATOLOGY

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Aromatherapy is based on the use of essential oils (EOs) that are obtained from plants to be applied through inhalation or skin for various therapeutic purposes. The application throughout the skin can be useful in dermatology which is defined as the study or practice of therapeutic products or cosmetics to reach disease-free skin. Aromatherapy can serve as anti-acne, photoprotective, anti-fungal, anti-aging, anti-viral, anti-psoriatic, and anti-cellulite action in skin care. Nevertheless, as essential oils can lead to allergic reactions, especially due to components like alcohol and monoterpenes, they should be diluted in carrier vegetable oils and used carefully. The EOs of *Rosmarinus officinalis* L., *Lavandula angustifolia* Mill., *Melaleuca alternifolia* Cheel, *Melissa officinalis* L., and *Citrus bergamia* Risso & Poit. have been included in this study. The most abundant EOs in these plants and their therapeutic effects are 1,8 cineole and camphor in Rosemary oil as antioxidant and antibacterial while linalool and linalyl acetate in Lavender oil are responsible for the same activity. On the other hand, terpinen-4-ol is the major component that provides antimicrobial activity to Tea tree oil. Meanwhile, Lemon balm oil has an antiviral effect thanks to geranial and citronellal basically in its chemical composition; whereas D-limonene is responsible for the anti-inflammatory and anti-microbial activity of Bergamot oil. In terms of treatment for skin disorders, Rosemary oil is used as an anti-acne; Tea tree oil as anti-fungal and anti-psoriatic; and Lavandula and Bergamot oils as anti-psoriatic. The current study aims to express the role of aromatherapy in dermatology and highlight the essential oils that are used in dermatological disorders.

Key Words: aromatherapy, dermatology, essential oils, dermatological disorders.

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POSTER PRESENTATION ABSTRACT

2-METHYLOXOLANE (2-MEOX), A BIO-BASED SOLVENT FOR THE EXTRACTION OF NATURAL PRODUCTS AND FOOD INGREDIENTS

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2-methyloxolane (2-MeOx), also known as 2-methyltetrahydrofuran, is a very promising "green" solvent for the extraction of the lipophilic components from natural sources. 2-MeOx is 100% produced from renewable biomass by hydrogenation of carbohydrate fractions, obtained by acidic hydrolysis of hemicellulose from various feedstock. In recent years, 2-MeOx has emerged as a valid solvent in oil extraction, thanks to his high affinity for lipophilic compounds. This solvent has a boiling point of 80°C that is compatible with the current extraction procedures, while also providing a safe toxicological profile. In 2022, EFSA assessed the safety of 2-MeOx for oil and food additive extraction, as an alternative to Hexane, which poses severe toxicity and environmental hazards and is the most common used petroleum-based solvent.

The aim of this study was to establish a method for the extraction of fatty acids (saturated and unsaturated) and sterols using "green" solvents, from natural and food sources. Olive pomace (OP), an already known lipophilic natural product, was used as starting material. OP is one of the main by-products generated during olive oil production.The extraction was performed in a Soxhelt apparatus using three different solvents (n-hexane, a mixture of n-Hexane:*i*-PrOH/3:2 and 2-MeOx). The analysis of the produced extracts was performed with Gas Chromatography coupled with a Flame Ionization Detector (GC-FID). The results showed that 2-MeOx exhibited the highest extraction yield and the fatty acids and sterols were quantified in similar levels, regardless of the extraction solvent. Overall, a "green" methodology for the extraction of fatty acids and sterols from lipophilic sources using 2-MeOx was proposed with potential application in natural products and food ingredients.

Key Words: 2-methyloxolane (2-MeOx), fatty acids, sterols, Soxhlet, olive pomace

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POSTER PRESENTATION ABSTRACT

ARTEMISIA UMBELLIFORMIS SUBSP. *ERIANTHA* PHYTOCHEMISTRY AND ANTILEISHMANIAL ACTIVITY

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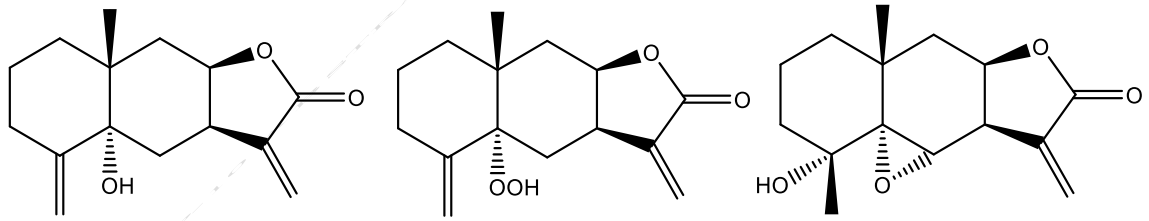
An anstileishmanial screening of different *Artemisia* species extracts from Greece, showed that the ethyl acetate extract of *Artemisia umberlliformis* subsp. *eriantha* strongly inhibited the growth of the parasite. The extract demonstrated an IC50 of 12 μg/mL against *Leishmania infantum* promastigotes and a low inhibition on THP-1 macrophages (IC50 =80 μg/mL). Since to the best of our knowledge, there are only a few articles concerning this rare *Artemisia* species, which belongs to the génépi group [1], so we sought to investigate its chemistry and leishmanicidal activity. The plant material was extracted in ethyl acetate by ultrasonication. Fractionation of the extract using a variety of techniques such as Countercurrent partition chromatography, Column chromatography, preparative-HPLC, preparative-TLC led to the isolation of 2 polyacetylenes, 2 methoxyflavones and 8 C-8 eudesmane type sesquiterpene lactones. The elucidation of all the structures and the configurations was carried out by 1-D and 2-D NMR spectroscopic analysis, MS experiments and bibliography data. 11 of the compounds are known substances, while 4-hydroxy-5(6)-α-epoxyalantolactone represents a novel natural product reported for the first time. Tests of the isolated compounds against *Leishmania infantum* promastigotes showed that telekin and 5-deoxy-5-hydroperoxy-telekin were the most active with an EC50 of 5.1 μM and 4.5 μM respectively, while remaining non-toxic for THP-1 macrophages. 5-deoxy-5-hydroperoxy-telekin was further tested against *Leishmania infantum* amastigotes to give an EC50 of 2.5 μM, making it a promising antileishmanial agent.



telekin | 5-deoxy-5-hydroperoxy-telekin | 4-hydroxy-5(6)-a-epoxyalantolactone

Key Words: *Artemisia umbelliformis*; *Asteraceae*; Sesquiterpene Lactones; *Leishmania infantum*

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POSTER PRESENTATION ABSTRACT

DETERMINATION OF *TRANS*-RESVERATROL CONTENT IN INDUSTRIAL WASTE OF GRAPE POMACE AND HARVESTED *VITIS LABRUSCA* CV. ISABELLA GRAPES

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Resveratrol is a phytoalexin compound that is synthesized in plants as a defense against microbial threats or abiotic stress [1]. Studies revealed that resveratrol has cardioprotective, neuroprotective, anti-inflammatory, anticancer, antidiabetic, antioxidant, and antiaging properties [2]. Due to such therapeutic effects, resveratrol is widely used in cosmetic products and dietary supplements [3]. This phenolic compound, which is a stilbenoid type, is present in grape, blueberry, peanut, and Japanese knotweed plants [4]. As a result of the zero waste approach, current researches focus on recovering resveratrol from industrial grape pomace [5,6].

In this scope, industrial waste of grape pomace and different parts of the harvested *Vitis labrusca* cv. Isabella grapes (fruits, stalks, and leaves) were extracted in an orbital shaker with methanol and liquid extracts were evaporated under low pressure. Dry extracts were subjected to TLC and HPLC-DAD analysis for qualitative and quantitative determination of resveratrol. TLC analysis displayed *trans*-resveratrol presence in harvested grape leaf extract which is observed as a weak spot on the plate. *Trans*-resveratrol could be detected in harvested grape stalk extract (<LOD) and leaf extract (196,48 mg *trans*-resveratrol/kg dry plant) via HPLC-DAD analysis. However, industrial grape pomace and harvested grape fruit extracts didn't show any peak in HPLC chromatograms or spots on the TLC plate as evidence of *trans*-resveratrol presence.

According to the results, we concluded that more extraction studies are needed for recovering *trans*-resveratrol from the industrial waste of grape pomace. Nevertheless, grape leaves seem to be an unprocessed alternative to grape pomace which is generated as a by-product of the beverage industry.

Key Words: *Vitis labrusca* cv. Isabella, *trans*-resveratrol, HPLC-DAD, TLC, industrial waste

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POSTER PRESENTATION ABSTRACT

ENCHANTING FRAGRANCES OF MARCESCENT LEAVES

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Fragrances in winter are opaque compared to those induced in other seasons. The winter fragrances are designed to match the beauty of cold environment. The objective of the study was to find out the winter fragrances of *Parrotia persica*, Hamamelidaceae and *Quercus alba*, Fagaceae leaves. Supercritical carbon dioxide extraction of *P. persica* and *Q. alba* leaves was done at the of pressure 30 MPa, temperature of 40°C and CO_2 flow rate of 2 $kg \cdot h^{-1}$. The obtained extracts were analyzed by GC-MS. The chromatograms obtained revealed the compounds influencing the design of winter fragrances. The main compounds detected in *P. persica* leaves extract were: phytol, neophydiene and hexahydrofarnesyl acetone indicating that the *P. persica* winter leaves fragrance consists of a delicate floral type odor. The main compounds detected in *Q. alba* leaves extract were: α-pinene, limonene, anethole, α-eudesmol and β-eudesmol indicating that the *Q. alba* winter leaves fragrance consists of a pines odor with camphoraceous nuances along with lemon-like and herbal-spice licorice odor. The marcescent leaves capture the essence of winter beauty.

Key Words: fragrances, marcescent leaves, supercritical carbon dioxide extraction

POSTER PRESENTATION ABSTRACT

BIOLOGICAL ACTIVITY OF NEW PURINE ISOSTERS WITH STRUCTURAL ANALOGY TO NATURAL COMPOUNDS

Panagiotis Marakos[1], Maria Georgiou[1], Nikolaos Sakalis[1], Nikolaos Lougiakis[1] Nicole Pouli1, Eleni Mavrogonatou[2], Harris Pratsinis[2], Dimitris Kletsas[2]

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Purine analogues are important therapeutic tools due to their affinity to enzymes or receptors that are involved in critical biological processes. With the aim to discover novel derivatives with potential cytotoxic activity, our research group is actively involved in the synthesis of numerous purine isosters and the subsequent evaluation of their cytotoxic activity. We have thus discovered compounds possessing IC50 values in the nM, or low μM concentration, against a variety of cancer cell lines of human origin. As a continuation of this project, we present here the design and synthesis of some new 6-azaindole derivatives, substituted with aryl groups at positions -3 and -7 of the scaffold. Commercially available 2-amino-3-nitro-4-methylpyridine was used as the starting material for the preparation of the target compounds. In total, 20 novel derivatives were synthesized and were evaluated for their potential to inhibit the proliferation of four cancer cell lines (A431, HT-1080, MCF-7, MDA-MB-231). Additionally, their effect on the proliferation of normal, non-cancer cells was examined. The cytotoxicity results revealed interesting SARs concerning the effect of the substitution pattern of the novel compounds on the antiproliferative activity. The analogues bearing the 2,4-dimethoxyphenyl group at position -7 of the 6-azaindole core proved to be the most potent, with IC50 values in the range 12-18 nM against the HT-1080 and MDA-MB-231 cancer cell lines. Notably, all analogues showed insignificant effect on the normal cell line AG01523, thus possessing great selectivity indices. In order to investigate the possible mechanism of action of the novel derivatives, a cell cycle analysis was performed for the most active compounds. The novel derivatives proved to cause a significant cycle arrest at phase G2/M when tested at the breast cancer cell line MDA-MB-231.

Key Words: Purine, Pyrrolopyridine, Suzuki-type coupling, Antiproliferative, Cell cycle arrest

Acknowledgements
This work has been funded by the Special Research Account (ELKE) of the National and Kapodistrian University of Athens.

POSTER PRESENTATION ABSTRACT

ANTIPROLIFERATIVE ACTIVITY EVALUATION OF NEW NATURAL PRODUCT INSPIRED PURINE ANALOGUES

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Fused pyridine and pyrimidine derivatives constitute an interesting medicinal chemistry scaffold, since their structural resemble to purines results in their involvement in crucial biological processes. Numerous purine or purine-like compounds have already reported as cytotoxic agents, through a variety of investigated mechanisms. As part of a program aiming to discover novel derivatives with potential cytotoxic activity, our research group has previously synthesized a number of nitrogen containing heterocyclic compounds, that exhibited promising in vitro and/or in vivo anticancer activity. In this work, we have designed and synthesized a number of new, suitably substituted pyrrolo[2,3-*c*]pyridines, using as lead compound a hit, recently identified by our group. For the synthesis of the target derivatives 2-amino-3-nitro-4-methylpyridine was used as the starting material, which was initially converted to the key intermediate 7-chloropyrrolo[2,3-*c*]pyridine, followed by the introduction of appropriate substituents to this scaffold. The new derivatives were subsequently evaluated for their potential to inhibit the proliferation of human origin cancer cell lines. The evaluation of the cytotoxicity results revealed interesting SARs since certain compounds possessed strong antiproliferative activity, that could assist to the design of the next generation of derivatives. Interestingly, the new compounds proved to be more effective against the A431 cancer cell line, which expresses abnormally high levels of the epidermal growth factor receptor (EGFR) and contains no functional p53, a potent tumor suppressor gene.

Key Words: purine derivatives, pyrrolo[2,3-c]pyridine, azaindole, cytotoxicity

Acknowledgements
This work has been funded by the Special Research Account (ELKE) of the National and Kapodistrian University of Athens.

POSTER PRESENTATION ABSTRACT

ANTIOXIDANT ACTIVITY OF *LAVANDULA DENTATA'S* ESSENTIAL OIL USING FRAP, TAC AND DPPH ASSAYS

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Over the past few years, there has been a growing recognition of the powerful efficacy of herbal antioxidant products in treating a variety of diseases. In fact, the abundance of antioxidant-rich essential oils (EOs), have sparked significant interest in fields such as food science and medicine due to their potential as natural antioxidants. Terpenes, which are abundant in essential oils derived from medicinal plants, particularly aromatic species of the Lamiaceae family, are known as powerful natural antioxidants. Their potential makes them viable candidates for use as dietary supplement additives, providing a preventive measure against oxidative stress, which is a major contributor to the onset of degenerative diseases. The main objective of the present work was to evaluate the antioxidant potential of the essential oil extracted from *Lavandula dentata* aerial parts, using FRAP, TAC, and DPPH assay. Results showed that essential oil exhibited an overall significant antioxidant activity, as measured by DPPH, TAC and FRAP assays, with IC_{50} (0,53 ±0,02 mg/mL DPPH, 0,07 ±0,001 mg/mL TAC) and an EC_{50} value of 4,39 ±0,05 mg/mL for FRAP assay. Overall, essential oil derived from *Lavandula dentata* show promise as a potential agent against damage caused by free radicals and as an efficient source of bioactive antioxidants.

Key Words: *Lavandula dentata*, essential oil, FRAP, DPPH, TAC

POSTER PRESENTATION ABSTRACT

ANTILEISHMANIAL ACTIVITY AND *IN SILICO* MOLECULAR DOCKING STUDIES OF *ZINGIBER CASSUMUNAR* ROXB. EXTRACT AGAINST *LEISHMANIA CHAGASI*

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Leishmaniasis, caused by protozoan parasites of the genus *Leishmania*, remains a significant public health concern globally. With limited treatment options and emerging resistance, there is a need for novel therapeutic agents. *Zingiber cassumunar* Roxb. (Zingiberaceae) has been traditionally used in medicine for its diverse pharmacological properties. In this study, we investigated the antileishmanial activity of *Z. cassumunar* extract against *Leishmania chagasi*, the causative agent of visceral leishmaniasis. The crude extract of *Z. cassumunar* was assessed for antileishmanial activity using *in vitro* assays. Our results demonstrate potent antileishmanial activity of *Z. cassumunar* extract, with IC_{50} values of 11.38 ± 1.02 µg/mL and 7.34 ± 0.63 µg/mL for promastigotes and amastigotes, respectively. The crude extract was not toxic to host cells, with a CC_{50} value of 24.76 ± 1.07 µg/mL. Interestingly, it presents a selectivity index value of 3.37. We also employed *in silico* molecular docking studies to explore potential interactions between bioactive compounds present in *Z. cassumunar* extract and key molecular targets in *L. chagasi*. Molecular docking simulations revealed promising binding affinities between the active constituents of *Z. cassumunar* and crucial protein targets, providing valuable information regarding molecular interactions underlying the observed antileishmanial activity. In summary, our findings suggest that *Z. cassumunar* extract possesses significant antileishmanial activity against *L. chagasi*. This investigation will be beneficial for further development of therapeutic agents for leishmaniasis.

Key Words: *Leishmania*, *Zingiber cassumunar*, Molecular docking, Drug, Cytotoxicity

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POSTER PRESENTATION ABSTRACT

CONTRIBUTION TO THE ESTABLISHMENT OF A HEALTH AND SAFETY MANAGEMENT SYSTEM ACCORDING TO THE DRAFT INTERNATIONAL STANDARD ISO 45001: 2018 WITHIN AN AGRI-FOOD COMPANY.

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In their search for the acquisition of a sustainable competitive advantage, agri-food companies opt for a strategic approach to sustainable development which integrates the social component. The latter includes, among other things, the health and safety at work of employees who constitute very important capital. within any company. This internal resource constitutes, according to various experts in the field of organizational management, a resource enabling the development of a sustainable competitive advantage. Health and safety management systems occupy an important place and make it possible to respond to various challenges for companies operating in the agri-food sector.
It is in this context that our industrial bakery and pastry shop has chosen to launch a strategic project entitled: “Implementation of the ISO 45001 version 2018 standard”. This work consists of preparing for the implementation of the OH&S SM through the response to certain project requirements of the international standard ISO45001 version 2018 which are essentially related to the “Plan” phase.

During our approach, the professional risk assessment procedure to help our industrial bakery control all units was detailed. It is nevertheless necessary to emphasize that this approach has limits:

- It requires monitoring over time to be able to last and be effective.
- Part of the evaluation is subjective because it is based on the risk perception of the agents and the working group as well as on the experience and knowledge of each person.
- It is not necessarily exhaustive and the fact of dividing the activity can obscure the risks of interference between the different activities of the community.

In all cases, the aim of risk assessment is to initiate a global prevention approach involving as many stakeholders as possible. This approach should be based on a principle of continuous improvement. Indeed, for organizational, technical or financial reasons, not all prevention measures can be implemented within the year following the initial risk assessment. It will therefore be appropriate to plan actions over several years.
Thus, the professional risk assessment was carried out according to the following stages:

- ✓ Preparing the evaluation with the composition of the working groups, the choice of methodology and communication.
- ✓ The division of the company into activities
- ✓ The inventory of activities
- ✓ Risk identification
- ✓ The classification of risks according to factors and a precise scale
- ✓ Determination of existing technological, organizational and human prevention measures.
- ✓ Determination of technical, organizational and human corrective actions.
- ✓ Updating the professional risk assessment grid.

The assessment of occupational risks and its application in an industrial environment requires a lot of communication, diplomacy and rigor. The operational working group must convince the company's agents of the value of this assessment, which will make it possible to better understand professional risks and identify the dangers encountered within the company. Once the agents were made aware, they volunteered and participated fully in the working groups, through their proposals and their feedback. This occupational risk assessment has proven to be an essential tool for management to identify risks and avoid them.

POSTER PRESENTATION ABSTRACT

ANTIBACTERIAL POTENTIAL OF *OLEA EUROPEA* LEAVES EXTRACT TARGETING *MULTIDRUG RESISTANT ESKAPEE HUMAN PATHOGEN*

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The purpose of this study was to invetigate the antimicrobial potency of ethanolic extract obtained from olive leaves (*Olea europaea* L.) of a Tunisian olive tree variety against multidrug-resistant (MDR)-ESKAPEE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp. and *Escherichia coli*) *human* most critical pathogen causing nosocomial infectious complications globally,which remains a widely unresolved problem and a heavy burden to health service worldwide. The method of disc diffusion in agar (aromatogram) was used for susceptibility testing. Diffusion on broth media was used to determine the minimum inhibitory (MIC) and bactericidal concentrations (MBC). The tests were carried out on a varied range of clinical strains included four representatives references strains (ATCC). The (MDR) pathogenic bacteria were chosen on the basis of WHO priority list based on their threat to human such as Carbapenem-resistant *A.baumanii*, (CRAB) and *K. pneumoiae* (CRK) including two typed strains producing (OXA 48 and NDM 5), Extended spectrum β-lactamase (ESBL) producing *Enterobacterales;* methicillin- resistant *S. aureus* (MRSA), Glycopepetide-resistant *Enterococcus* (GRE) , and VIM producer *Psd. aeroginosa*. The ethanolic extract tested showed a remarkable antibacterial effect against all tested strains, with more efficient activity than first-line antibiotics. All strains seem to be sensitive, with zones of inhibition ranging from moderate (9.7 ± 0.6) to high (23.3 mm) Diameter; (MIC) and (MBC) concentrations ranged respectively from 1.90 to 7.63 mg/mL and 3.82 to15.25 mg/mL , showing a general bactericidal effect. These findings could represent a basis for possible further use of (*Olea europaea* L.) active biomolecules extracts in the treatment of human microbial infections and/or hospital material disinfection. These promising antimicrobial properties highlighted that regarding the search for new natural compounds fighting multiresistance scourge, exploration of beneficial agents based on alternative products such as medicinal plant extract deserves to be deepened.

Key Words: *Olea europeae Leave*, *Ethanolic Extract*, *Antimicrobial activity*, *Multidrug resistance*, ESKAPEE pathogens

POSTER PRESENTATION ABSTRACT

A GC-MS STUDY OF THE VOLATILOME OF PROPOLIS FROM VARIOUS ORIGINS

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In this study, five samples of bee propolis from different origins and one commercial propolis extract were evaluated regarding volatile components. The volatilome was analyzed using HS-SPME and GC-MS. The profile of volatile compounds of the tested samples was complex and varied. Over 170 different volatile components were identified, representing different classes of organic compounds, of which 27 were common to all samples studied. These compounds included, among others, benzaldehyde, sulcatone, *p*-cymene, phenyl ethyl alcohol, camphor, α-copaene, *cis*-calamenene, and eudesmol isomers. The dominant volatile from Polish, Turkish, and Romanian propolis was benzoic acid, found in volatilome at levels of 25.1%, 10.6%, and 31%, respectively. Additionally, these samples contained benzyl alcohol at levels of 4.4–9.4% and benzaldehyde at levels of 2.4-5.9%. In contrast, propolis from Australia and Uruguay either did not contain or had lower levels of benzoic acid and its derivatives. α-Pinene was one of the major volatile compounds found in the volatile profile of Uruguay (28.9%) and Australian propolis (12.1%), the latter being accompanied by α-copaene at 12.92%. These and the other differences in the volatilome indicate distinctions between propolis from Eurasia and other continents. There was also variability observed within all samples studied.The volatile profile of ethanolic extract from Uruguayan propolis correlated with its raw counterpart, though some variations were observed. Apart from the ethanol, the solvent of the extract, the dominant volatile was hydrocinnamic acid ethyl ester, which was found at trace levels in crude propolis.

Key Words: propolis, volatile compounds, ethanolic and acid ethyl ester extracts

POSTER PRESENTATION ABSTRACT

PHYOCHEMICAL PROFILE OF *ERICA ARBOREA* L. EXTRACTS OBTAINED BY DIFFERENT EXTRACTION TECHNIQUES

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Erica arborea, the tree heath or tree heather, is a species of flowering plant (Angiosperms) in the heather family *Ericaceae* and native to the Mediterranean Basin. It is commonly found in Algeria and used in traditional medicine as antiseptic, diuretic, astringent as depurative. In this work, three different extraction methods: maceration (MAC), Soxhlet (SOE), ultrasound-assisted extraction (UAE) were used to compare total phenolic compounds, flavonoids, flavanols, and condensed tannins from *Erica arborea* leaves extracts grown in two regions of Western Algeria (Saida and Oran). The total contents of phenolic compounds, flavonoids, flavanols and condensed tannins were in the range of: MAC > UAE > SOE in both studied regions. It has been found that Ultrasound-assisted (UAE) was the best method for extraction of flavonoids in comparison to the other methods. While, *MAC* was the *best extraction method* for the other *phenolic compounds*. According to the obtained results, it has been shown that *Erica arborea* hydroalcoholic extracts from Oran contain more phenolic compounds than those from Saida. These methods could contribute to the good recovery of natural antioxidants from *Erica arborea* in the pharmaceutical industries.

Key Words: *Erica arborea* L., extraction methods, hydroalcoholic extracts, phenolic compounds, Western Algeria.

POSTER PRESENTATION ABSTRACT

CHEMICAL PROFILE AND ANTIMICROBIAL ACTIVITY OF *ESSENTIAL OIL OF CITRUS SINENSIS* KOMPOST PRODUCTS

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Citrus plants belonging to the Rutaceae family are cultivated in tropical and sub-tropical regions. *Citrus* peels, flowers and leaves are rich in essential oils and the drugs also contain secondary metabolites such as carotenoids, alkaloids and flavonoids. Thus, *Citrus* species are frequently used in phytotherapy, aromatherapy, food, cleaning and cosmetic industries. Various biological activities have been reported regarding the *Citrus* plants such as antibacterial, antifungal, anthelmintic. In order to obtain more productive products from cultivated *Citrus* species, the branch tips of the trees are pruned at certain periods of the year. In this study, the phytochemical contents of *C. sinensis* oils obtained by different extraction methods were compared by GC-MS. Essential oils were extracted from the pruning material which are the waste products, leaves and branch tips of *Citrus sinensis* (CB) by hydrodistillation and supercritical CO_2 methods. For the comparison leaves and branch tips were also collected from the fruiting period (CM). The yield of CM hydrodistillation sample (CMH) was recorded as 0.1%; the yield of CM supercritical CO_2 sample (CMS) was 0.5%. Hydrodistillation sample of CB (CBH) was 0.1%, and supercritical CO_2 sample of CB (CBS) was 0.2%. According to GC-MS analysis results, the main components of the CMH were spathulenol, phytol, β-caryophyllene and linalool, while containing of the CMS hentriacontane, phytol, α-tochopherol and linoleic acid. The volatile oil of CBH contain sabinene and limonene while containing CBS linoleic and hexadecanoic acid. The antibacterial activity of the four samples was also tested. The anticandidal and antibacterial effects of the four samples were compared with ketaconazole and chloramphenicol. According to the minimum inhibitory concentration analysis results the samples collected during the fruiting period and obtained by both methods showed the highest effect. In this study, it was aimed to evaluate the *Citrus* pruning parts separated as waste and it was determined that these products may have the potential to be used in the medical, industrial or food sector considering the essential oil yield and content.

POSTER PRESENTATION ABSTRACT

ANTIOXIDANT AND ANTI-UREASE ACTIVITIES OF *ORIGANUM MAJORANA* L.

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The genus *Origanum* in the Lamiaceae family is very important among aromatic and medicinal plants. Most of the species in this genus are scattered around the Mediterranean region, Eurasia and north of African. The species in this genus gains economic and medical importance in terms of the essential oil they contain. These species have traditionally been used for gastrointestinal diseases (such as diarrhea, stomach pain, colic and peptic ulcers), respiratory diseases (asthma, cough and chest pain), abnormal menstrual cycles, kidney and liver diseases, metabolic, hormonal and neuronal disorders, skin and urogenital disorders. Additionally, *Origanum* species are used to treat nausea, rheumatism, arthritis, hemorrhoids, sexual diseases, animal bites and poisoning, and to control diabetes and obesity. These species are also known to be used as a source of carminative, diaphoretic, tonic and antimicrobial compounds. Our study plant, *Origanum majorana* L. (Syn: *Origanum dubium*) is known as one of the economically important thyme species grown wild in Turkey. The most important feature of this type of thyme is its high essential oil yield content (6%-8%). It is also known to contain high levels of carvacrol and linalool compounds. Since the species in this genus have antimicrobial effects and are traditionally used against peptic ulcers, the aim of our study is to examine the in vitro anti-urease activity of the methanol extract obtained from the *Origanum majorana* by the maceration method, according to the indophenol method. According to the findings, it was observed that the methanol extract of the *Origanum majorana* could be used as a potential therapeutic agent, as it showed urease enzyme inhibition, with scientific studies that will support this study in the future.

Keywords; *Origanum majorana* (Syn: *Origanum dubium*), antioxidant activity, anti-urease activity, enzyme inhibition

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POSTER PRESENTATION ABSTRACT

EXPLORING THE IMPACT OF TEMPERATURE STRESS ON CAROB TREE, *CERATONIA SILIQUA*; ECOANATOMICAL STUDY, SECONDARY METABOLITE ANALYSIS AND ANTIMICROBIAL ACTIVITY

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The carob tree, *Ceratonia siliqua* L. (*Fabaceae*) is an emblematic species thriving among evergreen sclerophyll formations. The species has developed remarkable adaptations to successfully escape the overwhelmingly high temperatures and drought, during summer months, and the chilling temperatures, during winter. Commonly, biosynthesis of secondary metabolites, as a response to temperature stress, is a major reaction of the plants thriving in the Mediterranean. Considering the climate crisis and the extreme temperature stress, the purpose of the current research was to study the impact on stressed young carob seedlings (aged 3 months) compared to control ones. Biomass production is reduced in stressed plants, mainly in the heat treated, the leaves of which appear more xeromorphic. Chlorophyll-a content is inferior in cold-stressed leaves while heat-stressed leaves accumulate more phenolics and experience higher oxidative stress. Carob leaves were investigated for being a valuable and abundant source of bioactive compounds. Different extracts obtained from stressed carob leaves were characterized for their phytochemical profile by a multiplatform approach utilizing LC-HRMS and GC-MS analytical platforms, to gain insight into metabolites produced under stress. Moreover, LC-HRMS/MS metabolomic workflow was utilized for the discovery of biomarkers, over- or under-regulated in the stressed conditions.
Furthermore, the antimicrobial activity of carob extracts-fractions was assessed against six human pathogen strains (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Staphylococcus aureus, Saccharomyces cerevisiae*) and three phytopathogen bacterial strains (*Xanthomonas campestris pv. campestris, Pseudomonas syrigae pv. syringae, Erwinia amylovora*) using broth microdilution technique. The results indicated that MeOH-H_2O and DCM extracts showed notable activity against *C.albicans* and *S.cerevisiae*, while DCM extracts inhibited the growth of *E.amylovora*. This work suggests that plant exposure to temperature stress does not have significant influence on secondary metabolic pathways indicating that carob is a resilient species. Finally, it has been demonstrated that carob leaf extracts exhibit important antimicrobial properties.

Key Words: *Ceratonia siliqua* L., leaf extracts, environmental stress, ecophysiology, antimicrobial activity

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POSTER PRESENTATION ABSTRACT

CURCUMINOID QUANTIFICATION AND ANTIOXIDANT PROPERTIES OF THE EXTRACTS FROM TURMERIC (*CURCUMA LONGA*) PLANTS GROWN IN TÜRKIYE

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Curcuma longa L. is a medicinal plant used in miscellaneous diseases such as rheumatism, flu, skin, gall bladder, liver and stomach disorders. Although turmeric doesn't grow naturally in Türkiye, it has a very common usage area. Today, oxidative stress, which is seen as the main cause of degenerative diseases such as multiple sclerosis, Alzheimer's, diabetes, and cancer, is recommended to be used in the prophylaxis and treatment of such diseases by reducing it in the body with the antioxidative preperations. According to scientific research on turmeric, it's been determined that the antioxidant activity of the plant is quite high[1]. It's been noted by researchers that this activity of turmeric is due to the phenolic components it contains, and the most basic component is curcumin. There are various scientific studies on curcuminoids especially curcumin and turmeric extracts[2]. Within the scope of the study, curcumin-rich extracts were obtained from cultivated samples of turmeric from Antalya, Yalova and Istanbul using various extraction methods, which are soxhlet apparatus, maceration in shaking incubator, and supercritical CO_2. Acetone, ethanol: acetone (1:1), ethanol, ethanol: distilled water (7:3), ethanol: distilled water: isopropyl alcohol (35:35:30) solvents were used for maceration and soxhlet. For supercritical CO_2, ethanol was used[3]. The curcuminoid ratios of them were obtained by analytical HPLC analyses. Additionally, antioxidant activity assays were performed on each extract. According to the HPLC results, Antalya sample has the highest curcuminoid content with the method maceration in shaking incubator with ethanol: distilled water: isopropyl alcohol (35:35:30) solvent. Also, the sample which is prepared with maceration in shaking incubator with acetone: ethanol (1:1) has the most antioxidant activity. This study shows that cultivating turmeric species in Antalya, and choosing maceration as the extraction method might be preferable for high curcuminoid value and antioxidant activity. More studies should be done on this topic.

Key Words: *Curcuma longa*, curcumin, antioxidant, turmeric, HPLC

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POSTER PRESENTATION ABSTRACT

COMPOSITION OF THE VOLATILE OIL OF ALGERIAN *ELAEOSELINUM FONTANESII AND* THEIR PHYTOTOXIC ACTIVITY

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The aim of the study is to evaluate the antigerminative effects of the essential oil from *Elaeoselinum fontanesii* Boiss. (Apiaceae) grow spontaneously in Algeria, against the germination and the radicle growth of seeds from cultivated plant *Triticum durum* Desf. The volatile oil obtained by hydrodistillation from the aerial parts of *E. fontanesii* was analyzed by gas chromatography–mass spectrometry. 41 constituents were identified, representing 92% of the total oil, monoterpenes were the major chemical groups (75.9%) and high content of myrcene that was a characteristic compound. The germination and radicle growth of seeds of durum wheat were controlled in different concentrations of the plant extract. The results showed high inhibition against the growth of radicle and the germination of the seeds.

Key Words: Volatile oil, *Elaeoselinum fontanesii*, Allelopathic activity, Monoterpenes, Myrcene.

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POSTER PRESENTATION ABSTRACT

THE BIOLOGICAL ACTIVITIES OF *ECHIUM PLANTAGINEUM*

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Echium plantagineum of Mediterranean origin and a member of the Boraginaceae family, is widely used across Africa, America, Asia, Europe and Oceania to treat many diseases including cough, urinary tract infection, fever, inflammation and muscle strain. Scientific findings have indicated that the various parts of Echium species can be used for antibacterial, anti-inflammatory, anti-proliferative, antidepressant, antioxidant, antiviral, anxiolytic and cytotoxic properties. Therefore, the aim of this study was to obtain different extracts (petroleum ether, dichloromethane and methanol) from the aerial parts of the plant using maceration and evaluate their antioxidant and antidiabetic activities. Antioxidant activity of the extracts was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) methods, while total phenolic contents were determined by using the Folin–Ciocâlteu reagent (FCR) method. In this study, the antidiabetic activity of the extracts was evaluated with α-glucosidase inhibition assay. According to the findings, the dichloromethane extract of *E. plantagineum* exhibited stronger CUPRAC reduction activity and total phenolic contents compared to other extracts. The petroleum ether extract demonstrated the highest iron (III) reduction activity and DPPH activity potential. Furthermore, both petroleum ether and dichloromethane extracts of *E. plantagineum* exhibited stronger α-glucosidase inhibition potential than the methanol extract.

Key Words: *Echium plantagineum*, antioxidant activity, antidiabetic activity, enzyme inhibition

POSTER PRESENTATION ABSTRACT

INVESTIGATION OF THE ANTI-INFLAMMATORY EFFICACY OF THE OPTIMIZED EPILOBIUM DODONAEI EXTRACT IN CARRAGENAN-INDUCED PAW INFLAMMATION IN WISTAR RATS

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The genus *Epilobium*, with 17 species native to Romania, is traditionally used in folk medicine to alleviate symptoms of benign prostate hyperplasia, inflammation, and gastrointestinal issues. This investigation focuses on *Epilobium dodonaei* to assess its anti-inflammatory potential utilizing an optimized extract in a carrageenan-induced acute paw inflammation model in white male Wistar rats. The experimental animals were divided into three distinct groups: a negative control, a positive control treated with indomethacin, and a third group receiving the optimized extract of *E. dodonaei*. Following the induction of acute inflammation, the animals were euthanized at two specific time points: 2 and 24 hours. Biochemical analyses of paw tissue homogenates included measurements of oxidative stress markers such as malondialdehyde, reduced and oxidized glutathione (and their ratio), catalase, and glutathione peroxidase. Additionally, the levels of pro-inflammatory cytokines IL-6 and TNF-α, along with Western Blot analysis for COX-2, IL-10, NRF2, and GAPDH, were conducted. The findings demonstrated that the *Epilobium dodonaei* extract exhibited anti-inflammatory properties, with biomarker and cytokine levels comparable to those in the indomethacin-treated control, suggesting a significant potential for this species in anti-inflammatory treatments.

Key Words: *Epilobium dodonaei*, preclinical trial, anti-inflammatory potential, Western Blot.

Acknowledgements
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POSTER PRESENTATION ABSTRACT

CLINICAL AROMATHERAPY: WHAT IS THE ROLE OF NATURAL PRODUCTS IN THE MANAGEMENT OF CHEMOTHERAPY-INDUCED ADVERSE EFFECTS?

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Aromatherapy is a field of phytotherapy using essential oils (EO) at many forms as an adjuvant therapy in hospitals, especially in oncology [1]. The main objective of this study is to evaluate the efficacy of essential oils in the management of chemo-induced side effects including nausea-vomiting (NV), anxiety, depression and insomnia as well as its impact in the improvement of the general condition and well-being of cancer patients. The study was conducted from March to May 2021, the international standardized scales of anxiety, depression [2], nausea-vomiting [3] and insomnia [4] (HADS, PUQE and ISI) respectively, were used before the beginning of aromatherapy and 20 days after. Four essential oil formulas were prepared, two for atmospheric diffusions during the chemotherapy treatment and two others for aromatic sticks, one against NV and a second against anxiety, stress, and insomnia. The safety of use of the EO was estimated in all patients through a safety scale. In this study; 35 patients were included, 20 patients were on a highly emetogenic chemotherapy protocol and 15 patients on a moderately emetogenic protocol. As results ,we noted a significant decrease in NV scores (P=0.003) and for insomnia (P=0.012) in half of the patients. On the other hand, we did not observe a significant decrease in anxiety and depression scores. This work demonstrates the value of aromatherapy in the complementary management of chemo-induced side effects, in particular nausea vomiting and insomnia.

Key Words : Clinical aromatherapy, natural products, oncology, chemo-induced side effects.

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POSTER PRESENTATION ABSTRACT

CHOLINESTERASE INHIBITORY ACTIVITIES of *VERONICA CHAMAEDRYS* L.

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Veronica, a genus in the Plantaginaceae family, comprises 450 species utilized in European and Asian folk medicine for various ailments including those related to the nervous and respiratory systems, wound healing, and diuresis. Limited scientific evidence supports their traditional medicinal uses but suggests neuroprotective, antioxidant, and anti-inflammatory properties in select species. *Veronica chamaedrys* L. is admired for its blue-violet flowers, adding aesthetic value. In Turkish traditional medicine, it's used for respiratory and digestive issues, yet lacks substantial scientific validation. Alzheimer's disease (AD) involves diminished levels of acetylcholine and butyrylcholine targeting cholinesterase enzymes is a promising AD therapy. Natural products, like Galanthamine from plants, offer potential AD treatments. They provide diverse structures for potent enzyme inhibitors with antioxidative and neuroprotective effects, promising safer and more effective therapies. Research aims to evaluate Veronica's potential in AD treatment by studying cholinesterase inhibition, particularly due to the lack of studies on *Veronica chamaedrys*, despite promising findings in other species regarding cholinesterase inhibition and antioxidant anti-inflammatory activities. *Veronica chamaedrys* were collected in May 2022, from Sakarya at an altitude of 91 m. The plant was authenticated by Çağla Kızılarslan Hançer from the Pharmaceutical Botany Department of Bezmialem Vakif University. The aerial parts were air-dried in the shadow and then milled. Hexane and methanol extracts were prepared from aerial parts by percolation method. The extracts of *Veronica chamaedrys* were evaluated for their inhibition of acetylcholinesterase and butrylcholinesterase enzymes using the Ellman method, with Galantamine employed as a positive control for in vitro testing. All the reactions were performed in triplicate. The study revealed that *Veronica chamaedrys* methanol extract can inhibit the butyrylcholinesterase enzyme, suggesting its potential as a neuroprotective agent against Alzheimer's disease. Further research is necessary to validate these findings and explore the plant's effects in more depth.

Key Words: Veronica chamaedrys, Plantaginaceae, Cholinesterase inhibitor, Ellman, Alzheimer's disease

POSTER PRESENTATION ABSTRACT

FIBRINOLYTIC AND THROMBOLYTIC POTENTIAL OF INDIGENOUS VEGETABLES FROM SOUTHERN THAILAND

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Recent evidence suggests that regular consumption of functional fruits and vegetables could decrease the risk of thromboembolism. This study examined the fibrinolytic and thrombolytic potential of indigenous vegetables from Southern Thailand, known for their high total phenolic content and antioxidant activity. Six varieties of indigenous vegetables were sourced from Southern Thailand. Their aqueous and methanolic extracts were screened for *in vitro* fibrinolytic activity using the fibrin-agar-plate method. Extracts showing fibrinolytic efficacy were further evaluated for *in vitro* thrombolytic activity using a whole blood clot lysis assay. Among the selected vegetables, extracts from young Mon-pu (*Glochidion perakense* Hook.f.) and cashew (*Anacardium occidentale* L.) leaves demonstrated antithrombotic properties. The highest observed activity in terms of clear lysis area was 20.72 ± 1.54 mm^2 at a concentration of 10 µg/µL for Mon-pu methanolic extract. Mon-pu and cashew extracts displayed significant ($P < 0.05$) thrombolytic activity compared to the negative control (normal saline solution). The highest clot lysis for Mon-pu methanolic extract was 43.52 ± 1.62%, while for standard streptokinase, it was 65.37 ± 2.18%. A dose-dependent clot lytic effect was observed in all experimental approaches. These findings demonstrate the promising fibrinolytic and thrombolytic activity of Mon-pu, suggesting their potential as alternative nutraceutical sources of thrombolytic agents.

Key Words: Southern Thai indigenous vegetables, fibrinolytic activity, thrombolytic activity, *in vitro* assay

POSTER PRESENTATION ABSTRACT

BIODIVERSITY OF GENUS *OPHRYS* L. (ORCHIDACAE) IN ALGERIA EVIDENCED BY MORPHOMETRIC AND ECOLOGIAL DATA

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The Orchidaceae family is a key group within petaloid monocots, particularly their Mediterranean subfamily Orchidoideae with several rare and endemic taxa. Within the Orchidinae tribe the genus *Ophrys* L. is morphologically distinct group including perennial species that form a single few-flowered inflorescence. In recent years, their tubers were intensively collected by the local population for its ethnobotanical properties, especially against sterility difficulties. This anthropogenic menace, habitat degradation and climate change, seriously threaten their survival. Despite the increasing fragility of these plants, systematic evolutionary studies are almost absent in Algeria. We therefore carried out a systematic inventory with updating of the nomenclature and plant material sampled from various bioclimatic sites was subjected to macro-and micro-morphometric measurements of vegetative and floral characters. Data matrix was subjected to multivariate statistical analyses (Principal component analysis). Results **revealed distinctive** groups structured in a different bioclimatic gradient emphasizing adaptations and suggesting genome plasticity. This work aimed to clarify ecological status of *Ophrys* Algerian taxa and prepares systematic revisions with a particular focus on threatened endemics. Molecular phylogenetic inferences are currently carried out.

Key Words: Orchidaceae, Systematic, Algeria, Morphology, Ecology, Evolution.

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POSTER PRESENTATION ABSTRACT

ANASTATICA HIEROCHUNTICA AND *SELAGINELLA LEPIDOPHYLLA:* CHEMISTRY AND ANTIPROLIFERATIVE ACTIVITY OF TRUE AND FALSE "ROSE OF JERICHO" RESURRECTION PLANTS

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The Rose of Jericho name is used for two distinct plants, unrelated, yet notable for their remarkable ability to survive extreme drought. The true Rose of Jericho (*Anastatica hierochuntica*) is native to western Asia and is the only species of the genus *Anastatica* (Brassicaceae). Its religious connotations are evident in its alternative names such as the "leaf of Maryam" (mother of Jesus) and the "hand of Fatima" (daughter of the Prophet). Conversely, *Selaginella lepidophylla*, the false Rose of Jericho, also called the resurrection fern, is native to the Chihuahuan Desert (southwestern USA and northern Mexico). Belonging to the Selaginellaceae family, it bears no genetic relation to the true Rose of Jericho [1]. Glucosinolates (GSLs) from *A. hierochuntica* were identified and quantified by their desulfo counterparts using LC-MS/MS. 3-(Methylsulfinyl)propyl GSL (glucoiberin), methionine derived GSL, was predominant in both the aerial part and root (35.46 and 1.49 μmol/ g DW, respectively), while its deoxygenated counterpart, 3-(methylsulfanyl)propyl GSL (glucoibervirin), was present as the minor one. 3-(Methylsulfinyl)propyl GSL was isolated as desulfoglucosinolate and confirmed by NMR analysis. In order to investigate antiproliferative potential, essential oils (EOs) were isolated from the whole plant material of *A. hierochuntica* and *S. lepidophylla* using hydrodistillation in Clevenger-type apparatus and analysed by GC-MS. EO derived from *A. hierochuntica* was notably abundant in 3-methylsulfanylpropyl isothiocyanate, a degradation product of glucoibervirin. However, 3-methylsulfinylpropyl isothiocyanate, originating from the major GSL, glucoiberin, was not detected, likely due to its water solubility. EO of *Selaginella lepidophylla* was rich in different volatiles (benzaldehyde, 1,2-diphenoxyethane, hexanal, benzeneacetaldehyde, etc.). The EOs were tested for antiproliferative activity on human cervical (SiHa) and ovarian (SK-OV-3) cancer cells using MTT assay. The best cytotoxic activity against SiHa cell line was observed for *S. lepidophylla* EO after 48h incubation time (IC_{50}=54.33 μg/mL), and against SK-OV-3 cell line (IC_{50}= 71.01 μg/mL).

Key Words: *Anastatica hierochuntica*, *Selaginella lepidophylla*, glucoiberin, essential oil, NMR, MTT.

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POSTER PRESENTATION ABSTRACT

NUTRITIONAL AND ANTINUTRITIONAL PROFILES, VOLATILES AND PHENOLIC COMPOSITION OF *ATRIPLEX HALIMUS* L. SEEDS

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Atriplex halimus L., commonly known as Mediterranean saltbush, is an edible halophyte whose leaves have been utilized for thousands of years in the treatment of heart conditions and diabetes through decoctions, and for rheumatism via infusions used in bathwater [1]. However, the nutritional and functional potential of *A. halimus* L. seeds remain largely unexplored. In this study, we analyzed the nutritional composition of *A. halimus* L. seeds and prepared an infusion, decoction, and tincture to assess their antinutritional factors, phenolic content, and volatile compounds. The seeds exhibited a high content of neutral (67.7 g/100g DW) and acid detergent fiber (37.2 g/100g DW). Linoleic acid was the most abundant fatty acid, present at 38.8%, followed by oleic (17.1%) and palmitic (10.6%) acids. Significant amounts of calcium and potassium, along with trace elements like iron and manganese, were found, while toxic metals such as cadmium and lead were absent. Antinutritional analysis revealed increased amylase inhibition and condensed tannins in the tincture extract, whereas phytic acid predominated in the infusion and decoction extracts without any detection of trypsin inhibition. The decoction extract exhibited the highest levels of total phenolics and flavonoids. Volatile analysis identified annulene, hexanal, and nonanal as major components across all extracts. Overall, this study highlights the *A. halimus* seeds' potential as a nutritious food source with valuable fiber and minerals and beneficial functional properties across all extracts.

Key Words: Mediterranean saltbush, *Atriplex halimus* L. seed, volatile compound, nutritional composition, antinutritional profile

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POSTER PRESENTATION ABSTRACT

EVALUATING THE INHALATION OF BLACK PEPPER PLUS LAVENDER ESSENTIAL OILS AS A PROMISING APPROACH TO SUPPRESS NICOTINE CRAVINGS

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Cigarette smoking poses a global health concern that affects individuals, governments, and the environment on a wide scale [1]. The existing conventional pharmacotherapy have certain drawbacks, such as the high relapse rates, that require more attention [2]. Essential oils such as black pepper, lavender, and others have been studied for their potential therapeutic effects on reducing nicotine craving [3]. This study comprises two distinct components: The first part focuses solely on observation and explores smoking patterns among residents of Oran (Algeria). In contrast, the second part aims to examine the impact of inhaling a combination of black pepper and lavender essential oils on nicotine cravings in current smokers. This is a pretest/posttest experimental study to evaluate the reduction of nicotine craving among fifteen smokers who inhale mixture of black pepper and lavender EO. Before the inhalation session, participants completed questionnaires assessing instant smoking urges (QSU-brief), individual characteristics, nicotine dependence level scores, and willingness to quit smoking. Following the session, participants completed the QSU-brief again to assess post-inhalation nicotine craving levels. The Fagerström Test for Nicotine Dependence (FTND) scores revealed different levels of dependence among participants, ranging from highly dependent to minimally dependent. The results showed a reduction in smoking urges scores after inhaling the essential oil mixture. In conclusion, inhaling a mixture of black pepper and lavender essential oils showed potential in reducing nicotine cravings among current smokers. Further research with a larger sample size and control group is recommended to validate these findings and explore the underlying mechanisms of this effect.

Key Words: Smoking cessation, nicotine craving, Fagerström Test, Black pepper essential EO, Lavender essential EO.

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POSTER PRESENTATION ABSTRACT

VARIATION IN CHEMICAL COMPOSITION OF ESSENTIAL OILS FROM SEA FENNEL (*CRITHMUM MARITIMUM* L.) COLLECTED ALONG DIFFERENT LOCATIONS OF THE ADRIATIC COAST

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Sea fennel (*Crithmum maritimum* L.) is a wild-growing halophyte used in cuisine, traditional medicine, and cosmetics due its nutritional richness as well as appealing taste. It can be found along the coasts of the Mediterranean and Black Seas and Atlantic Ocean [1]. The aim of this study was to analyze the chemical composition of essential oils isolated from the flowers of sea fennel plants gathered from various locations along the Adriatic coast. Essential oils were obtained from dry plant specimens of sea fennel originating from distinct Adriatic coast locations, spanning from north to south, including Krk, Senj, Pag, Šibenik, Split, Drašnice, Korčula, Pelješac, Neretva, and Cavtat. The essential oil was isolated using hydrodistillation in a Clevenger type apparatus for 3 hours. The separation and analysis of the essential oils isolated from sea fennel flowers were performed using GC-MS. The essential oil yields from dried flowers varied from 0.19% to 1.87%. Sixteen compounds were identified in the analyzed samples. Monoterpenes, specifically limonene and γ-terpinene, alongside bicyclic terpenes such as sabinene and α-pinene, represented molecular tags of all essential oils. Limonene was the most abundant, constituting from 50.82% to 97.31% of the essential oil, followed by sabinene, which ranged from 0.32% to 31.73%. The highest limonene levels were found in oils from plants along the southern part of Adriatic coast, from Split to Cavtat, while sabinene levels decreased. Conversely, samples collected further north along the Adriatic coast, from Split to Krk, exhibited increased sabinene content (ranging from 16.62% to 31.73%). Additionally, monoterpenoids and a non-terpene compound, octanal, were identified, albeit in low quantities.

Key Words: sea fennel, halophyte, Adriatic coast, essential oils, limonene, sabinene.

Acknowledgements
This work is part of the PRIMA programme supported by the European Union. Project title: "Innovative sustainable organic sea fennel (*Crithmum maritimum* L.)-based cropping systems to boost agrobiodiversity, profitability, circularity, and resilience to climate changes in Mediterranean small farms" (acronym: SEAFENNEL4MED). We are also thankful for the scientific-research equipment financed by EU grant "Functional in-tegration of the University of Split, PMF-ST, PFST and KTFST through the development of the scientific and research infrastructure" (KK.01.1.1.02.0018).

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POSTER PRESENTATION ABSTRACT

ASSESSMENT OF SAFETY FOR KERRA FORMULA CAPSULES THROUGH ORAL ADMINISTRATION IN HEALTHY VOLUNTEERS: PHASE I CLINICAL TRIAL

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The Kerra Formula, an herbal medicine rooted in the ancient Thai wisdom of Tak-Ka-Si-La, combines nine medicinal plants and is recognized for its efficacy in reducing fever, duly approved by the Thai Food and Drug Administration (FDA). Despite some individuals using it for fever relief during COVID-19 infections, comprehensive research on its safety for human consumption is lacking. This phase I clinical trial aimed to assess the safety and acute adverse effects of orally administering Kerra Formula capsules to healthy volunteers. Eleven volunteers (5 males, 6 females) received two capsules (500 mg/cap) orally four times a day, three times before meals and once before bedtime, for 14 days. Safety evaluation encompassed symptom observation, medical history recording, physical examinations, and laboratory tests analyzing parameters like complete blood count, urine analysis, coagulation profiles, fasting blood sugar levels, lipid profiles, inflammation markers, liver function, and kidney function. Comparison of baseline results before capsule administration and results after 1, 7, and 14 days, and 14 days post-washout period revealed no abnormalities during physical examinations. Additionally, no adverse reactions or statistically significant disparities were observed in laboratory findings. These findings suggest that consuming Kerra capsules at a total daily dosage of 4,000 mg (1,000 mg four times a day) for 14 consecutive days is considered safe. However, further studies are crucial to evaluate the herb's long-term effects.

Key Words: Kerra Formula, Oral Administration, Safety, A Phase I Clinical Trial

POSTER PRESENTATION ABSTRACT

INVESTIGATION OF ANTIOXIDANT ACTIVITIES OF DIFFERENT EXTRACTS FROM ENDEMIC SPECIES: *ONOSMA BORNMUELLERI* HAUSSKN.

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The *Onosma* L. is the largest genera of Boraginaceae family, with about more than 102 taxa in Türkiye and the rate of endemism among native species is about 50%. The members of many *Onosma* are used in food and medical industries due to the chemicals they contain. These species are also used as coloring agent in many foodstuffs, especially powdered pepper, due to their distinctive red color. Some *Onosma* species have also been used as anthelmintic and laxative agents by the people for many years. Traditionally, taxa of the genus *Onosma* L. are used as stimulants in rheumatism, bladder pain, kidney irritation, heart and heart palpitations due to their diuretic, cooling, astringent and sedative effects. In India, it is used to treat hypertension, fever and nervous disorders. In Turkey, these plants are used in the treatment of inflammatory diseases and pain such as tonsillitis, hemorrhoids and bronchitis. The main components of *Onosma* taxa are alkaloids, naphthoquinones, polyphenols, phytosterols, terpenoids and fatty acids. *Onosma bornmuelleri* Hausskn. is an endemic species and known as ‘’Amasya Şincarı’’ in our country. *O. bornmuelleri* is very rich in flavonoid content compared to other *Onosma* species. The aim of this study is to examine the antioxidant activities of different extracts (n-heptane, chloroform, methanol) from the plant. The findings showed that the methanol extract exhibited higher antioxidant activity than the other extracts.

Key Words; *Onosma*, *Onosma bornmuelleri*, Antioxidant activity

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POSTER PRESENTATION ABSTRACT

SYNTHESIS, CHARACTERIZATION AND ANTIOXIDANT EVALUATION OF NANOEMULSIONS DERIVED FROM *ROSMARINUS OFFICINALIS, SALVIA OFFICINALIS* AND *THYMUS SATUREIOIDES* OILS*

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Essential oils (EOs) from *Rosmarinus officinalis, Salvia officinalis* and *Thymus satureioides* are renowned for their health-promoting properties and diverse applications. However, their integration into water-based products presents a significant challenge due to their low stability and insolubility, particularly when exposed to light, oxygen, and heat. This obstacle can be overcome by formulating nano-sized EOs to enhance their chemical stability, water solubility, and biological properties *via* nanoemulsion systems. The objective of this study was to assess the antioxidant activity of nanoemulsions derived from *R. officinalis, S. officinalis* and *T. satureioides*. Oil-in-water (O/W) nanoemulsions were synthesized and characterized. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method and the ferric-reducing antioxidant power (FRAP) assay. The mean droplet sizes of the prepared nanoemulsions ranged from 30.99 to 321.30 nm. The polydispersity index (PdI) was determined to be 0.549 for *R. officinalis*, 0.809 for *S. officinalis* and 0.209 for *T. satureioides*. In terms of antioxidant activity, the *R. officinalis* nanoemulsion exhibited the highest effect. A moderate effect was observed for the *S. officinalis* nanoemulsion while the *T. satureioides* nanoemulsion demonstrated the least antioxidant inhibition. These results suggest potential applications of these nanoemulsions as natural preservatives in food and cosmetic products.

Key Words: Essential oils, Aromatic and medicinal plant, Nanoemulsion, Antioxidant effect.

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POSTER PRESENTATION ABSTRACT

EXPLORING THE THERAPEUTIC POTENTIAL OF *CRATAEGUS:* TRADITIONAL USES, PHARMACOLOGICAL PROPERTIES, AND CLINICAL PERSPECTIVES IN CARDIOVASCULAR HEALTH

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In the last 20 years, a significant proportion of new drugs for various diseases have been derived from natural health products. *Crataegus*, commonly known as hawthorn, and its various extracts are among such natural health products. In Turkey, various species of *Crataegus* have been used for many years in traditional medicine, particularly in the treatment of cardiovascular diseases and numerous other ailments. According to studies conducted in Turkey, vinegar made from *Crataegus azarolus* L., vinegar made from the fruits of *Crataegus monogyna* J.Jacq. subsp. *monogyna*, infusion obtained by boiling the flowers of *Crataegus orientalis* Pall. ex M.Bieb. var. *orientalis*, decoction of the flowers of *Crataegus pentagyna* Waldst. & Kit, powder of the fruits of *Crataegus aronia* L., infusion of the flowers of *Crataegus aronia* L., and infusion of the fruits of *Crataegus astrosanguinea* Pojark. are used against cardiovascular diseases. Currently, *Crataegus* products are marketed as alternative treatments for hypertension, angina, arrhythmia, and early-stage congestive heart failure, with mechanisms targeting multiple levels. The flavonoids, triterpenic acids, and phenolic carboxylic acids present in *Crataegus* are among the primary compounds responsible for these effects. Studies suggest that hawthorn extracts possess a wide range of cardiovascular pharmacological properties, including antioxidant activity, positive inotropic effects, anti-inflammatory properties, inhibition of cardiac remodeling, prevention of platelet aggregation, vasodilation, protection of endothelial cells, inhibition of smooth muscle cell migration and proliferation, shielding against ischemia/reperfusion injury, antiarrhythmic actions, lipid-lowering properties, and reduction of arterial blood pressure. Clinical studies have shown efficacy in the early stages of congestive heart failure. Additional investigation is necessary to delve into and confirm these effects, especially concerning the prevention of cardiac remodeling and the reduction of smooth muscle cell migration and proliferation. Conducting large-scale, randomized controlled trials is imperative to assess the effectiveness and safety of *Crataegus* products in treating cardiovascular diseases.

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POSTER PRESENTATION ABSTRACT

INHIBITION OF α-GLUCOSIDASE AND ANTIOXIDANT ACTIVITY OF *THYMUS LONGICAULIS* SUBSP. *CHAUBARDII*

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The *Thymus* genus a member of the Lamiaceae family renowel for its aromatic and medicinal properties. Most taxa of this genus are endemic to the Mediterranean region. This genus, which has a high polymorphic feature, is included in the Flora of Turkey with 39 species and 64 taxa, and its endemism rate is 47% compared to the flora of Türkiye. *Thymus* species are known as "thyme" in Turkish and are widely used in making spices, herbal teas and medicinal plants in Türkiye. The dried aerial parts of the plant have been traditionally used to treat problems such as cold, flu, fever, sore throat, cough, asthma, acute pharyngitis, bronchitis, chest infections, respiratory disorders, mouth sores. The main components of the *Thymus* species include flavonoids and terpenic compounds in its essential oil. Among the major compounds found in the oil, thymol and carvacrol have been reported to have the highest antioxidant activity. Additionally, it is known that extracts and chemical components of *Thymus* species show antimicrobial, antioxidant, antitumor, anti-inflammatory, analgesic, antispasmodic, antitussive, carminative, antihypertensive, antidiabetic, anthelmintic activities in vitro and in vivo. Accordingly the our study aims to obtain a methanol extract from the aerial part of the plant using the maceration method. The antioxidant activity of this extract was analyzed with 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) methods while its antidiabetic activity was determined with α- glucosidase enzyme inhibition assay. Our findings indicate that methanol extract of the *Thymus longicaulis* subsp. c*haubardii (*Syn: *Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus)* had significant antioxidant and anti-diabetic activity.

Key Words; *Thymus longicaulis* subsp. *chaubardii* (Syn*: Thymus longicaulis* subsp. *longicaulis* var. *subisophyllus)*, antioxidant activity, alpha-glucosidase, enzyme inhibition

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POSTER PRESENTATION ABSTRACT

ANTIOXIDANT AND ANTI-UREASE ACTIVITIES OF *ORIGANUM MAJORANA* L.

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The genus *Origanum* in the Lamiaceae family is very important among aromatic and medicinal plants. Most of the species in this genus are scattered around the Mediterranean region, Eurasia and north of African. The species in this genus gains economic and medical importance in terms of the essential oil they contain. These species have traditionally been used for gastrointestinal diseases (such as diarrhea, stomach pain, colic and peptic ulcers), respiratory diseases (asthma, cough and chest pain), abnormal menstrual cycles, kidney and liver diseases, metabolic, hormonal and neuronal disorders, skin and urogenital disorders. Additionally, *Origanum* species are used to treat nausea, rheumatism, arthritis, hemorrhoids, sexual diseases, animal bites and poisoning, and to control diabetes and obesity. These species are also known to be used as a source of carminative, diaphoretic, tonic and antimicrobial compounds. Our study plant, *Origanum majorana* L. (Syn: *Origanum dubium*) is known as one of the economically important thyme species grown wild in Turkey. The most important feature of this type of thyme is its high essential oil yield content (6%-8%). It is also known to contain high levels of carvacrol and linalool compounds. Since the species in this genus have antimicrobial effects and are traditionally used against peptic ulcers, the aim of our study is to examine the in vitro anti-urease activity of the methanol extract obtained from the *Origanum majorana* by the maceration method, according to the indophenol method. According to the findings, it was observed that the methanol extract of the *Origanum majorana* could be used as a potential therapeutic agent, as it showed urease enzyme inhibition, with scientific studies that will support this study in the future.

Keywords; *Origanum majorana* (Syn: *Origanum dubium*), antioxidant activity, anti-urease activity, enzyme inhibition

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POSTER PRESENTATION ABSTRACT

ADDING VALUE ON POMEGRANATE FRUIT PROCESSING

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The consumption of pomegranate fruit and its derivatives, especially juice, is growing due to their perceived health benefits leading to an increase in production and trade. However, the processing of pomegranate fruit, results to large amounts of byproducts which are considered wastes with limited alternative uses and value. Similar to other fruit byproducts, those materials are rich in valuable substances presenting an opportunity for valorization and the manufacturing of added value food products. Here we propose a valorization workflow of the main pomegranate fruit components, including peels and seeds, towards a minimal waste solution. The seeds were extracted using $scCO_2$ and hexane-assisted ultrasound, yielding oil with 8.6% and 10.2% yields, respectively. Additional oil was recovered through sequential $scCO_2$ extraction using isopropanol as a co-solvent, while fatty acid analysis with GC-MS was conducted. The defatted residue of extracted seeds can be potentially used as a flour substitute. The peels were freeze dried and grounded yielding a fine powder at 32%, which was assessed for phenolics and antiradical activity by Folin–Ciocalteu assay and DPPH, respectively. Incorporating peel flour in wheat flour for bread production was assessed. We also conducted a comparative analysis for the extraction of peels with water and isopropanol to obtain bioactives and extracts were analyzed by LC-ELSD and LC-MS. We also established the use of pomegranate juice as a source of phenolics and sugars. The lyophilized juice mixed with an equal amount of maltodextrin produces a power, and separation of phenolics from sugars through XAD-7 resin resulted in a 1.6% phenolics recovery yield. The remaining sugar-rich residue shown potential as a cane sugar substitute in food products such as bars. The conversion of a pomegranate wastes into valuable components could create new economic opportunities for food and agricultural industries, while reducing the environmental impact associated with this waste.

Key Words: Pomegranate fruit, valorization, extractions, chemical analysis

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POSTER PRESENTATION ABSTRACT

A NOVEL BIO-FUNCTIONAL FOOD PRODUCT, ENRICHED WITH HT EXTRACTS FROM OLIVE LEAVES

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Nowadays, olive leaf polyphenols have been at the center of scientific interest due to their beneficial effects on human health. Oleuropein (OLE) is the most abundant polyphenol in olive leaves, comprised of three different components hydroxytyrosol (HT), Elenolic acid and a glucose moiety. The biological properties of OLE are mainly due to HT, a drastic catechol group whose biological activity has been established after the approval of European Food Safety Authority (EFSA)'s health claim in 2012 (EU Regulation 432/2012), recognizing the beneficial effect of HT on human health. In recent years many nutritional supplements, food products and cosmetics enriched in HT, have been developed and marketed by pharmaceutical, food and cosmetic industries, with unexpected positive results. However, the concentration of HT in olive leaves depends on several factors, such as olive tree variety, cultivation practices, and is also corelated with the activity of endogenous enzymes (polyphenol oxidase, peroxidase, β-glucosidase, and esterase). Since HT derived from OLE, it is obvious that olive leaves could be an ideal raw material for the production of HT rich extracts. The aim of this study is to develop a rapid and easy methodology for the production of HT-enriched extracts from olive leaves, based on the direct acidic hydrolysis of olive leaves, where the extraction procedure and the hydrolysis of OLE is carried out in one step. To our knowledge, this is the first time that a one-step procedure is applied in olive leaves to produce HT-enriched extracts. The ultimate goal of this study was the enrichment of food products with HT-extracts from olive leaves and the determination of olive bioactives in the final products, utilizating HPLC DAD and QTOF-HRMS analytical methodologies.

Key Words: OLs, HT, direct acidic hydrolysis, functional food products

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POSTER PRESENTATION ABSTRACT

INVESTIGATION ANTIOXIDANT ACTIVITIES OF DIFFERENT EXTRACTS FROM ENDEMIC SPECIES; *GALIUM DUMOSUM* BOISS.

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The *Galium* L. genus, which is a member of the Rubiaceae family. This genus is the largest genus in the tribe Rubieae, with about 667 species distributed worldwide. *Galium dumosum* Boiss. is an endemic species with low perennial with strongly woody rootstock - sometimes subshrub, 10-25 cm; corolla brownish-purple, *flowering time* 5-9. months, it's habitat generally *dry rocks and rocky steppe.* Various classes of bioactive compounds, including triterpenes, anthraquinones, phenolic compounds, and iridoid glycosides, along with trace amounts of tannins, saponins, and essential oils, have been identified from *Galium* taxa thus far. It is known that many species of this genus have antioxidant, antibacterial, spasmolytic, diuretic and antifungal effects. Therefore, the aim of this study is to comparatively examine the antioxidant activities of different extracts of (n-heptane, chloroform, methanol) obtained from the plant using DPPH, FRAP and CUPRAC methods. The findings showed that methanol extract exhibited higher antioxidant activity than other extracts.

Key Words: Rubiaceae, *Galium*, *Gallium dumosum*, Antioxidant activity

POSTER PRESENTATION ABSTRACT

PHYTOCHEMICAL PROFILE OF METHANOLIC EXTRACTS of MARRUBIUM VULGARE FROM DIFFERENT BIOTOPES OF THE REGION OF TIARET- ALGERIA

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Marrubium vulgare L. (Lamiaceae), commonly known as horehound, is widely used in traditional medicine in Algeria. It has anti-hypertensive, anti-inflammatory, vasorelaxant, hypoglycemic, hypolipidemic, antioxidant and antimicrobial effects. The present study aims to determine the phytochemical profile (quantitatively and qualitatively) of methanolic extracts of the aerial parts of *M. vulgare* from different biotopes of the region of Tiaret (Frenda, Sougueur,Tiaret and Mellakou). A phytochemical screening was carried out by specific coloring and precipitation reactions. The colorimetric method Folin- Ciocalteu was used for the quantification of total phenolic content. The method of aluminum chloride was employed for the quantification of total flavonoid content and the method of vanillin for the determination of tannins. The results of the various phytochemical tests, revealed the presence of polyphenols, flavonoids, tannins, terpenoids and saponosides.The total concentrations of total polyphenols, flavonoids and tannins in different methanolic extracts of the aerial part of *M. vulgare* varied respectively between 17.26±0.40 and 31.50 ±0.50 mg GAE/g, 114.12±0.50 and 186.85±0.80 mg of QE/g of extract and 423.85 ± 3.60 and 933.25±3.84 mg of CE/g of extract. In general, the methanolic extracts of *M.vulgare* L. that were harvested from Sougueur were richer in secondary metabolites, and showed higher concentrations of polyphenols, flavonoids and tannins. This study suggests *M.vulgare* L. harvested from Sougueur as a potential source of natural antioxidants.

Key Words: *Marrubium vulgare*, methanolic extract, phytochemistry, biotope, Tiaret

POSTER PRESENTATION ABSTRACT

VALORIZATION OF GRAPE POMACE: A MINIMUM WASTE APPROACH

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Grape pomace, a byproduct of the grape processing and winery industry constitutes a significant waste stream, reaching almost 20 million tons annually. It is currently either disposed or used as a cheap feed component. This loss represents a considerable cost to both growers and consumers, as this waste accounts for around 30% of the mass of a grape. Grape stalks, another grape byproduct, are also produced in considerable amounts. Both wastes contain a high concentration of several potentially bioactive compounds, with potential health benefits making their efficient valorization crucial for economic opportunities and the production of healthier, nutritionally advanced foods. In our proposed valorization scheme, both grape pomace and stalks are identified as promising sources of valuable substances due to their richness in beneficial compounds. Simple drying of the pomace generated a fine powder, rich in phenolics as found by Folin–Ciocalteu analysis. Substituting wheat flour with grape pomace powder at a 10% level produced a processable dough with satisfactory properties and higher phenolic content compared to the control. Pomace was extracted with $scCO_2$ alone and with 5%, 10% isopropanol as a co-solvent. Accelerated solvent extraction and ultrasound assisted extraction with H_2O, isopropanol and H_2O:isopropanol were also conducted resulting in promising yields. A sequential extraction of the same starting material was also conducted because it has a higher potential for industrial applications. Typical analysis of the extracts includes total phenolics, total anthocyanins, antiradical activity, as well as LC-MS and GC-MS. The stalks were also treated using a similar approach and certain fractions were found to be rich in phenolics, while displaying high antiradical activity as found by DPPH. Our comprehensive approach aims to maximize the valorization potential of grape waste components, offering economic benefits to producers and delivering nutritionally enriched products to consumers.

Key Words: grape byproducts, pomace, stalks, valorization, extractions, chemical analysis

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POSTER PRESENTATION ABSTRACT

TARGETED INVESTIGATION ON PYRROLIZIDINE ALKALOIDS OF GREEK BORAGINACEAE SPECIES

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Greece has one of the highest levels of biodiversity in Europe, due to its geophysical background and variety of microclimatic conditions, although, based on resent bibliography, most of it remains untapped. Such a case is the plants of the Boraginaceae family, that are mostly considered as weeds. Many of the plants of the family are edible, while they are characterized by the strong presence of pyrrolizidine alkaloids (PA) and their N-oxides (PANOS), which are transferred to humans through food chains and can also be found in traces in many products, like honey and beverages, and are among the most hepatotoxic naturally occurring molecules[1]. Thus, the aim of this study was the phytochemical investigation of the Greek Boraginaceae species and specifically their methanol extracts where the PAs dwell. 60 different Greek Boraginaceae species were collected all over Greece, with many of them being endemic, and were extracted using a Soxhlet apparatus. The extracts were evaluated on hepatoma cell line (H4IIE) using the MTT assay to assess their proliferation rate and cytotoxicity. As expected, most extracts showed significant hepatotoxicity. Then an extensive investigation of their methanol extracts was performed using UHPLC-ESI-HRMS/MS, in positive and negative ionization modes, which led to more than 1600 compounds belonging to groups like fatty acids and phospholipids, cinnamic derivatives, terpenes, sugars, flavonoids, amino acids, phenolic compounds, some unknowns and PAs/PANOs. Approximately 250 PAs and PANOs were tentatively identified, and their identification procedures were described based on their retention indexes, fragmentation patterns and multiple data gathered by different extracts accompanied by 25 standard solutions. Parallel to that, the methanolic extracts of the roots of *Alkanna methanaea* Hausskn. were studied resulting in the isolation of 18 compounds. Of the five isolated PAs two of them were novel and one was first described in the Boraginaceae family.

Key Words: Boraginaceae, Alkanna methanaea, UHPLC-ESI-HRMS/MS, Dereplication, pyrrolizidine alkaloids

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* The authors declare that there is no conflict of interest.

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POSTER PRESENTATION ABSTRACT

AN ADVANCED METHODOLOGY FOR OBTAINING MASLINIC ACID AND OLEANOLIC ACID FROM OLIVE POMACE

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During the process of pomace oil production, significant quantities of by-products are generated in olive mills, which subsequently end up as untreated biomass, exhibiting considerable environmental hazards and high pollutant potential. The main by-products are wet olive pomace, dried olive pomace, exhausted olive cake, olive pomace skin, and olive stones. Despite their classification as by-products, these materials are sources of valuable bioactive compounds, such as simple phenols, phenolic acids, secoiridoids, flavonoids, lignans, and triterpenic acids. Among the triterpenic acids, Maslinic acid (MA) and Oleanolic acid (OA) have attracted attention within the global market due to their recognized health-promoting activities. Plethora of scientific data prove the antioxidant, anti-inflammatory, antidiabetic, anti-HIV, and antitumor properties attributed to MA and OA, underlining the necessity for the isolation of these compounds in a high purity. The aim of this study is to develop a rapid and effective methodology for the isolation of Maslinic acid and Oleanolic acid from olive pomace by-products, applying modern advanced techniques, such as Centrifugal Partition Chromatography (CPC) and Supercritical Fluid Chromatography (SFC). CPC is a chromatographic technique with many advantages, since it is characterized by total sample recovery, high repeatability, low solvent consumption and high capacity, while SFC fulfills high demands with respect to selectivity, versatility, sensibility and low cost. Structural elucidation of Maslinic acid and Oleanolic acid was performed using tandem mass spectrometry (HRMS-QTOF). Overall, MA and OA isolated from olive pomace by-products in high purity can be used as high added value products in numerous applications.

Key Words: Maslinic acid, Oleanolic acid, SFC, CPC, Olive pomace

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POSTER PRESENTATION ABSTRACT

ANTIOXIDANT ACTIVITIES OF *BITUMINARIA BITUMINOSA* CALLUS CULTURES PRODUCED UNDER DIFFERENT CONDITIONS

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Bituminaria bituminosa (L.) C.H. Stirton (syn. *Psoralea bituminosa* L., Fabaceae), also known as "Arabian pea" or "pitch trefoil", is part of the *Bituminaria* genus and commonly found in the Mediterranean. This species has been known to produce important secondary metabolites that are useful in the fields of food, drugs, and cosmetics. *In vitro* callus culture is an important tool for producing a larger quantity of secondary metabolites in a shorter amount of time under optimal conditions. There is a limited amount of research about the callus culture of *Bituminaria* species. Plants are rich sources of antioxidants and they protect organisms against oxidative damage caused by reactive oxygen species (ROS). *Bituminaria* species are known to have considerable amounts of important antioxidant agents. Our study aims to investigate the antioxidant activities of *B. bituminosa* cultures produced in different media and also compare them by examining their total phenolic contents. For callus cultures obtained from roots and leaves, we used three different media conditions containing varying quantities of 2, 4-D, BAP, and kinetin. The antioxidant activity and total phenolic content of callus cultures were determined by the total antioxidant status (TAS) assay and Folin Ciocalteu's method.

Key Words: *Bituminaria bituminosa L.*, Callus culture, Antioxidant activity

POSTER PRESENTATION ABSTRACT

ETHNOBOTANICAL SURVEY ON CONDIMENT PLANTS USED DURING RAMADAN IN BISKRA (SOUTHERN EAST OF ALGERIA)

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When the month of Ramadan approaches, housewives begin their preparations to receive it, renewing their stocks of “Ras el hanout” a spcial spice and the “Frik” by-product of cereal, for the preparation of soup, the main dish of the all table of fasting families. It has been reported that "Ras el hanout" encompasses the virtues of each of the spices that constitute it: which calm down the pain of diarrhea, facilitate digestion and flatulence after one fasting day and last one month. The aim of this ethnobotanical survey is to collete informations about the condiment species involved in the mixture of Ras el hanout, we conduct un ethnobotanical investigation from May –June 2021, a sample of 30 herbalists answer the questionnaire and explain in their stores organoleptics differences between Ras el hanout. The results of our investigations we identify 22 species included in the composition of the mixture of Ras-el-Hanout, most cited are coriander, caraway, curcuma, pyrethrum, anise, ginger, black pepper, cubeb, rose petals and nutmeg. The condiment of Ras el hanout from the Ziban region has specific properties according to the herbalists its the specific taste, strong odor and greenish yellow color. The spice is called in Ziban with more than 5 names the more used Ras el hanout (the best of the store) and Dawa rgig (thin medication), it’s very used among the inhabitants of Ziban during Ramadan month 71% or during the wedding ceremonies 20%. This investigation comes to preserve the knowledge of the local herbalists in the processing of this spice and confirm its benefits for improving the taste of food and maintaining the human health.

Key Words: Ras el hanout, herbalist, facilitate digestion, Ziban

POSTER PRESENTATION ABSTRACT

COMPARATIVE STUDY: IDENTIFYING THE PHARMACOLOGICAL POTENTIAL OF CATECHIN COMPLEXES WITH PLURIVALENT METAL IONS

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Polyphenols, prevalent in plant species, play a crucial role in human health by offering antioxidant benefits, thus mitigating oxidative stress and lowering susceptibility to chronic illnesses such as cancer and cardiovascular diseases. In pharmaceutical applications, their antioxidant and anti-inflammatory properties are valuable for creating treatments for diverse conditions. Conjoining polyphenols with metal ions can optimize their stability and bioavailability, potentially magnifying their health-promoting effects. The method for obtaining the catechin-zinc and catechin-selenium complexes is a standardized one (using 1:1 molar ratio). After the dissolution and mixing with the metallic, the reaction was refluxed for 3 hours. A colored precipitate was obtained which was washed several times with acetone and dried in an oven. Out of all of the tested solvents, the superior yield was obtained when using methanol as the solvent. The synthesized complexes underwent morphological and spectroscopic analysis which confirmed the successful complexation. To further assess the properties and clinical potential of these complexes, various tests were done. These complexes showcased antioxidant potential, particularly in the DPPH assay where the catechin-selenium complex was shown to be a better radical scavenger than the zinc complex. Similar results were registered in the inhibition of lipoxygenase by these two compounds, while in the chelation of ferrous ions and the inhibition of hydroxyl radicals, the catechin-zinc and selenium complexes had similar results. To further assess their pharmacological potential, the two complexes were subjected to test their inhibition of alpha-amylase and alpha-glucosydase. In both of the tests, the catechin-zinc complex presented elevated inhibition of these carbohydrases. Catechins while forming complexes with metal ions like zinc and selenium can enhance their therapeutic potential. Standardized synthesis methods yield promising results, indicating potential in treating diverse conditions and managing chronic illnesses through various pharmacological pathways.

Key Words: polyphenol, catechin complex, selenium compound, antidiabetic, antioxidant potential

POSTER PRESENTATION ABSTRACT

NUTRITIONAL AND ANTI-NUTRITIONAL DYNAMICS OF ARTHROCAULON MACROSTACHYUM CULTIVATED UNDER DIFFERENT SALINITIES

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Arthrocaulon macrostachyum (Moric.) Piirainen & G. Kadereit is an edible perennial halophyte rich in fatty acids and phenolic compounds such as phenolic acids and flavonoids with antioxidant, metal chelating, anticholinesterase and cytotoxic activities [1,2]. Despite the growing interest in the commercial exploitation of halophytes, either as food or sources of bioactive products, scientific efforts for its cultivation are scarce. Thus, the goal of this work was to compare the agronomic and biochemical features of *A. macrostachyum* cultivated in an integrated multi-trophic aquaculture (IMTA) system for 12 weeks. The effect of the irrigation salinity (20.1, 35.3, 40.3 and 49.0 mS/cm) was evaluated on plant growth and the nutritional and anti-nutritional properties. Maximum productivity and growth were achieved at lower salinity levels, whereas the highest survival rates were observed at the highest salinity. At lower irrigation salinities, *A. macrostachyum* showed an increase in the concentrations of chlorophylls a and b, and at higher irrigation salinities exhibited higher total carotenoid content. The protein and crude fat content were higher in *A. macrostachyum* cultivated at low irrigation salinities. However, the ash content as well as the neutral and acid detergent fiber were found in greater quantities at 40.3 mS/cm. In terms of anti-nutritional profile, *A. macrostachyum* cultivated at 49.0 mS/cm of salinity showed lower levels of phytic acid (11.50 mg/g) and condensed tannins (10.79 mg/g) and a high capacity to inhibit α-amylase was observed. In this study, the potential of *A. macrostachyum* as a valuable resource in saline agriculture with the ability to thrive in extreme salinity conditions while maintaining productivity and good nutritional properties has been demonstrated. Thus, *A. macrostachyum* is a promising food product for agricultural saline production in an economically, socially and environmentally viable IMTA system.

Key Words: Glaucous glasswort, integrated multi-trophic aquaculture (IMTA) system, nutritional composition, anti-nutritional profile

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POSTER PRESENTATION ABSTRACT

VOLATILE COMPOUNDS OF SOME *HYPERICUM* L. SPECIES GROWING IN TURKIYE

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Anatolia has hosted many cultures throughout history has led to the formation of a rich ethnobotanical cultural heritage and folk medicine knowledge. *Hypericum* L. (Hypericaceae, Guttiferae, Clusiaceae) species, known as sarı kantaron, binbir delik otu, mayasıl otu etc. in Anatolia, are important medicinal plants used in the treatment of various diseases in our country and in the world. The genus *Hypericum* is distributed around the world with about 500 species. In Turkey, it is represented by 20 sections and 111 taxa, of which 48% are endemic to the country. Phytochemical studies have shown that *Hypericum* species contain numerous compounds such as naphthodiantrons, phloroglucinol derivatives, flavonoids, phenolic acids, proanthocyanidins, xanthones and essential oils. As a part of our ongoing studies on *Hypericum* species growing in Turkey, in this study, volatile compounds in the aerial parts of *H. adenotrichum* Spach, *H. alacamdaglariense* H.Duman & Çakır, *H. empetrifolium* Willd., *H. microcalycinum* Boiss. & Heldr., *H. montbretii* Spach, *H. olympicum* subsp. *olympicum* L., *H. origanifolium* var. *origanifolium* Willd., *H. origanifolium* var. *depilatum* (Freyn & Bornm.) N.Robson, *H. perforatum* L. and *H. scabroides* N.Robson & Poulter, were obtained using the microdistillation technique and determined by GC and GC/MS analyses.

Key Words: *Hypericum*, microdistillation, GC and GC/MS

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POSTER PRESENTATION ABSTRACT

ANATOMICAL AND ULTRASTRUCTURAL INVESTIGATION ON *HYPERICUM ALACAMDAGLARIENSE* H.DUMAN & ÇAKIR, A RECENTLY DESCRIBED ENDEMIC IN TURKIYE

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Hypericum L., an important genus of the Hypericaceae family, is distributed mostly in temperate regions, with 36 sections and nearly 500 trees, shrubs and herbs. Members of the genus *Hypericum*, which have been used in folk medicine since ancient times, are still used today in the treatment of various diseases such as depression, menstrual disorders, arthritis, neuralgia, sciatica, gastro-intestinal diseases and for wounds worldwide.

The Mediterranean Basin, including Türkiye, is an important center for the genus *Hypericum*. The genus consists of 111 taxa and 20 sections, 48% of which are endemic in our country. Black glands, translucent glands and secretory canals, which are called secretory structures, are important in distinguishing this genus. The type of secretory structures of *Hypericum* varies from species to species, as well as the status and frequency of presence of these structures in different organs of the plant.

In this study, the anatomical features and secretory structures of the *H. alacamdaglariense* H.Duman & Çakır, which was recently described as a new *Hypericum* species in the literature, were investigated for the first time using light microscopy and scanning electron microscopy (SEM).

Key Words: *Hypericum*, endemic, anatomy, secretory structures, light microscopy, SEM

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This study was supported by Anadolu University Scientific Research Project Commission under the grant numbers 2205S057.
Merve Has would like to thank the Higher Education Institution (YÖK) for providing the opportunity for a PhD scholarship in the field of YÖK 100/2000-Pharmaceutical Studies.

POSTER PRESENTATION ABSTRACT

BIOACTIVITY STUDIES ON *LIMONIUM LILACINUM* (BOISS. & BAL.) WAGENITZ VAR. *LAXIFLORUM* DOGAN & AKAYDIN

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Soil salinity and drought, which are the major abiotic stress in plants, cause over-production and accumulation of reactive oxygen species (ROS) which are highly toxic and reactive. Halophytes (salt resistant plants), due to their powerful enzymatic and non-enzymatic antioxidant systems, are known for their ability to quench ROS. Synthesis and accumulation of polyphenols in halophytic plants are usually stimulated in response to salt and drought stress. Nowadays, phenolic compounds are in the center of attention as they play a crucial role in preventing oxidation processes and have highly valued functions in improving health and preventing disturbances ranging from skin disorders to cancer.

Limonium Mill., which belongs to Plumbaginaceae, is represented by 27 taxa, of which 14 are endemic. In our country, taxa of this genus grow wildly in seashores, sand dunes or rocky coasts of Aegean and Mediterranean regions as well as salty inlands. Member of the genus are used for arthritis, fever, cold, neuralgia, hepatitis, bronchitis, diarrhea, menstrual disorders, cramps, genitourinary infection, etc. in folk medicine. Literature survey indicated that researches on *Limonium* species are very limited. In this study, ist was aimed to investigate total phenolic content, antioxidant capacity and cytotoxic effects on cancer cell lines of halophytic *L. lilacinum* (Boiss. et Bal.) Wagenitz var. *laxiflorum* Dogan et Akaydın. For this aim, methanolic extracts from aerial parts of plants and their hexane, dichloromethane, ethyl acetate and water fractions were prepared, total phenolic contents were estimated by Folin-Ciocalteau colorimetric method, antioxidant capacity was evaluated by DPPH and TAC assays and cytotoxic activity studies were performed on MCF7 (human breast adenocarsinoma) ve A549 (human lung carsinoma) cell lines.

Key Words: *Limonium*, phenolics, antioxidant, cytotoxic, MCF-7, A549

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POSTER PRESENTATION ABSTRACT

INVESTIGATION OF THE ANTINOCICEPTIVE ACTIVITY OF CROCIN, THE PRINCIPAL COMPOUND OF SAFFRON FROM TALIOUINE REGION, AND ELUCIDATION OF ITS POTENTIAL MECHANISM OF ACTION IN RODENTS

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Crocin, extracted from *Crocus sativus*, is a carotenoid compound responsible for saffron's distinctive color. It exhibits various therapeutic properties including antipyretic, antioxidant, anti-inflammatory, and anticancer effects. This study examined the pain-relieving properties of Crocin in rodents and aimed to uncover its potential physiological mechanism. The effectiveness of Crocin in reducing pain was tested using two animal models: the hot plate test and writhing test. Additionally, the involvement of different receptors associated with pain relief was investigated by administering various receptor antagonists. Crocin demonstrated a centrally-mediated, dose-dependent anti-nociceptive response to thermal stimuli, as well as a peripheral analgesic effect in the acetic acid-induced contortion test. The anti-nociceptive activity of crocin was totally or partially reversed by the co-administration of receptor antagonists, naloxone, atropine, haloperidol, yohimbine, and glibenclamide. Crocin influenced signal processing, by the modulation of the opioidergic, adrenergic, and muscarinic systems, and by the control of the opening of the ATP-sensitive K+ channels at the peripheral and central levels. In contrast, haloperidol moderately reduced the analgesic effect of crocin in central level. The data obtained indicate a multimodal mechanism of action for crocin as a modulator, via different physiological pathways, of the electrical signal generated by nociceptors.

Key Words: Crocin, antinociceptive, central, peripheral, receptor systems

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POSTER PRESENTATION ABSTRACT

PLECTRANTHUS VENTERI VAN JAARSV. & HANKEY- THE FUTURE OF PLANT BIOTECHNOLOGY?

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Plectranthus is one of the largest genera within the Lamiaceae family, with approximately 350 species distributed mainly in Africa, India, Japan, Malaysia, and Australia. *Plectranthus venteri* is a narrow species endemic to the Sekukuniland region of South Africa and was discovered in 1997. Literature data indicate that this plant is a source of, among other things, bioactive acetophenones with antimicrobial properties [1,2]. In this work, we present a genetic transformation strategy for *Plectranthus venteri* to induce hairy roots by *Rhizobium rhizogenes*. Hairy roots are interesting *in vitro* plant cultures that are of increasing interest in basic and applied research. The main advantages of such a system are rapid growth, genetic stability, ease of manipulation or independence of the culture from external conditions. Such roots often also show a higher productivity of valuable biologically active compounds. Here we present the results of work related to the induction of hairy roots on *in vitro* cultures of *Plectranthus venteri*. The starting material was seed subjected to initial surface sterilisation and germinated *in vitro* on MS medium. Young seedlings were then cocultivated with a prepared bacterial suspension of *Rhizobium rhizogenes* A4. The procedure led to the induction of the first hairy roots just 20 days after the start of cocultivation. These roots, further cultured in a liquid medium, even on a larger scale, may be an interesting material for further phytochemical research and a possible future source of very valuable secondary metabolites with wide applications.

Key Words: *Plectranthus venteri*, in vitro cultures, *Rhizobium rhizogenes*, hairy roots, bioactive acetophenones

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POSTER PRESENTATION ABSTRACT

CHYMOTHRIPSIN INHIBITORY ACTIVITY OF MEDICAGO SATIVA

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Chymotrypsin enzyme has an important role in many physiological events in mammals such as inflammation, digestion of proteins and fibrin clots, and removal of proteins around cancer cells. Therefore, it is extremely important to identify inexpensive compounds that can inhibit chymotrypsin and have few/no side effects. In this regard, plants are an extremely important starting point in new drug development studies with their rich phytochemical diversity.

In this research, which was planned to be carried out as a graduation project, the in vitro chymotrypsin inhibitory activity of the methanol extract prepared from *Medicago sativa* L. was investigated. As a result of the study, it was determined that methanol extract inhibited chymotrypsin by 44%. Then, the prepared methanol extract was fractionated using the liquid-liquid fractionation technique and the chymotrypsin inhibitory activity of the resulting fractions was investigated. Among the fractions studied, *n*-hexane and dichloromethane fractions stood out by inhibiting chymotrypsin by 73 and 71%, respectively.

Key Words: *Medicago sativa*, chymotrypsin, bioactivity guided fractionation

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POSTER PRESENTATION ABSTRACT

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF JUGLANS REGIA L. BARK

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Juglans regia L. is a medicinal plant from the Juglandaceae family which is cultivated in Algeria. It contains a variety of phenolic compounds to which various biological activities are attributed. The present study aimed to study the phytochemical profile of ethanolic extract from the bark of Juglans regia L. from four different regions (Rahouia, Mellakou, Sidi Ouadah and Melaab) and to evaluate its antioxidant and antimicrobial properties. The ethanolic extract of this plant contains considerable levels of phenolic compounds (total polyphenols, flavonoids and condensed and hydrolysable tannins). The results of phytochemical screening showed the presence of starch, anthocyanins, coumarins, flavonoids, lipids, polyphenols, proteins, quinones and saponins. The antioxidant activity of our extract measured by DPPH method was potent with an inhibition percentage of 44.4, 45.8, 40.6, 63.19 % in Rahouia, Mellakou, Sidi Ouadah and Melaab respectively, and an IC_{50} of 183.1 mg/ml (Mellakou), 156.48 mg/ml (Melaab), 106 mg/ml (Rahouia) and 30.38 mg/ml (Sidi Ouadah). Its antimicrobial activity evaluated by the microtiter broth method gave a good effect on two bacterial strains Staphylococcus aureus and Escherichia coli, and a fungal strain Candida albicans, with a different MIC and MBC from one extract to another. The results suggest that examined extract is promising agent worthy of further studies to develop a phytodrug.

Key Words: *Juglans regia* L., phytochemical, antioxidant activity, antimicrobial activity

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POPULATION STRUCTURE ANALYSIS BASED ON ISSR MOLECULAR DATA REVEALED TWO SUBPOPULATIONS AMONG IRANIAN PURSLANE (*PORTULACA OLERACEA* L.) ACCESSIONS

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Abstract
Purslane (*Portulaca oleracea* L.), which belongs to the Portulacaceae family containing valuable vitamins and nutrients, has high tolerance for salt and drought stress growing under different environmental conditions. In this study, the population structure of Iranian purslane accessions was investigated using 25 molecular Inter Simple Sequence Repeat markers. The 25 primers yielded a total of 92 bands, of which 62 were polymorphic bands. Population structure analysis was performed using bioinformatics tools based on the molecular data. In this regard, the number of possible K was calculated and a two-dimensional plot was generated. The number of subpopulations for maximum likelihood was considered as the optimal number of subpopulations. The initial values of K ranged from one to ten, and in order to increase the accuracy, three repetitions were used for each of the subpopulations. Burn-in duration (10000) and MCMC (100000) were selected to obtain the maximum likelihood plot. Finally, the obtained results divided the Iranian purslane populations into two subpopulations. The results of this study can be useful and effective in identifying genetic diversity and preserving Iranian purslane germplasm.

Key Words: Inter Simple Sequence Repeat, likelihood, Population structure, Purslane, Subpopulation

1. Introduction

Purslane (*Portulaca oleracea* L.) is an annual plant from the Portulacaceae family. The leaves of this plant are opposite and placed in clusters at the end of the stems. The stems are bright dark green, prostrate, and succulent. The flowers are yellow with five heart-shaped petals. The fruit is a capsule and has many tiny seeds. The distribution of purslane is very wide, but it is probably native to North Africa, the Middle East, and the Indian subcontinent (Nyffeler and Eggli, 2010; Ocampo and Columbus, 2012; Kumar et al., 2022; Chandrabhan and Pratiksha, 2023). On the other hand, it can grow in fields, waste areas, gardens, and coastal areas. Purslane can also tolerate poor soil and drought stress conditions. Valuable compounds such as terpenoids, alkaloids, flavonoids, fatty acids, vitamins, proteins, polysaccharides, minerals, and sterols have been identified in purslane, many of which are used as an anticancer, antidiabetic, anti-inflammatory, antioxidant, neuroprotective, hepatoprotective, antimicrobial, antiulcerogenic, skeletal muscle relaxant, anti-insomnia, analgesic, gastroprotective, and antiseptic (Zhou et al., 2015; Kumar et al., 2022).

Considering all the metabolites present in purslane and their applications in various industries, studying the population structure and genetic diversity can be the basis for purslane breeding to create new potential cultivars. By breeding purslane, (i) the diversity and availability of various types of cultivars with different morphological characteristics and nutrient values will be modified depending on the breeding purpose. (ii) Its agronomic performance and yield can be increased under different cultivation systems. (iii) New cultivars can be resistant to biotic and abiotic stresses such as diseases, pests, drought, salinity, and temperature stresses. (iv) In terms of secondary metabolites, some modified varieties may

have more or less specific metabolites that can be used in different industries, and (v) etc. In this regard, population structure analysis is an efficient method to study the diversity and genetic differences and relationships among individuals of the same group or different groups of plant species (Ogden, 1970; Hayward and Breese, 1993; Allen et al., 2017). Population structure analysis can be useful and effective in identifying evolution, origin, adaptation of species, etc. In this study, the purslane accessions were collected from different regions of Iran and their population structure was analyzed based on the ISSR molecular markers data.

2. Material and Methods

2.1. Collecting and planting the seeds

In this study, the seeds of 20 native genotypes of purslane medicinal plant were collected from different regions of Iran and stored at 4°C in the laboratory. The details of the collection place of purslane plant seeds are given in Table 1. After checking the seed viability, the seeds were planted in pots with clay medium (2), sand (1) and manure (1) in a completely randomized design (CRD) with 4 replications in the greenhouse condition.

2.2. Leaf sampling and DNA extraction

Young, healthy and fresh leaves were used for DNA extraction. The leaves from the plants of all 4 replicates of the same genotype were combined, wrapped in a foil and immediately transferred into the liquid nitrogen tank. To prevent the possible destruction of genomic DNA by intracellular nucleases, after freezing in liquid nitrogen, the samples were kept in -80°C freezer until DNA extraction. A modified Cetyltrimethylammonium bromide (CTAB) method was used to extract genomic DNA. To check the quantity and quality of extracted DNA, 0.8% agarose gel was used in electrophoresis. Spectrophotometer (model 6705 UV/Vis) was used to determine the purity and concentration of DNA samples. Finally, the extracted DNAs were diluted with double-distilled water (DDW) and stored in a refrigerator at -20 degrees until PCR reaction (Farmanpour Kalalagh et al., 2017; Mehri et al., 2022).

2.3. PCR amplification

A total of 25 ISSR molecular primers were used to perform the PCR reaction (Table 2). The PCR reaction was run in a thermocycler in a total volume of 8 µl containing 1.2 µl of primer, 0.8 µl of template DNA, 3.6 µl 2 × Master Mix buffer (Dream Taq green PCR master mix-2X containing green buffer, dNTPs and 4 mM of MgCl2, supplied by Fermentas-Fisher Scientific, UK), and 2.4 µl DDW. The PCR program was conducted on an initial denaturation for 5 min at 95˚C, then 40 cycles of a denaturation step at 94˚C for 45 s, primer(s) annealing at 40-60˚C for 45 s (touchdown starting at 2˚C up and down Tm), and 72˚C for 8 min, with a final extension at 72˚C for 6 min. Amplified bands were separated on 1% agarose gel prestained with 1 mL of Midori green (dye) using 1x TBE buffer. The gels were run for 90 minutes at 85 voltage and separated bands were visualized by Gel Doc SUV/SN: G089301.

2.4. Population structure analysis

Population structure analysis was performed using STRUCTURE 2.3.4 (Pritchard et al., 2000). In this regard, the number of possible K was calculated and a two-dimensional plot was generated. The number of subpopulations for maximum likelihood was considered as the optimal number of subpopulations. The initial values of K ranged from one to ten, and in order to increase the accuracy, three repetitions were used for each of the subpopulations. Burn-in duration (10000) and MCMC (100000) were selected to obtain the maximum likelihood plot.

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3. Results and Discussion

The 25 primers yielded a total of 92 bands, of which 62 were polymorphic bands. In this study, effective analysis of population structure and accurate classification of genotypes into appropriate subpopulations were performed. For this purpose, the number of K or possible sub-populations was calculated and its two-dimensional graph was drawn. The number of subpopulations in which the maximum likelihood was observed was chosen as the optimal number of subpopulations. To calculate the number of subpopulations, the method of Evanno et al. (2005) was used (Figure 1). In this way, from the two summarized columns K and L(K) and the standard deviation (Stdev) of repetitions were calculated. After that, the difference of the average repetitions for adjacent groups is determined as the difference between the average of the higher group and the average of the lower group under the L'(K), and then the difference of L'(K) for the adjacent groups is obtained under the L"(K). Using these calculations, ΔK was calculated from the $|L''(K)|$/Stedv formula. Finally, by generating the two-way graph of K and ΔK, the peak of the curve was obtained, which was the optimal number of K. Based on Figure 2, a two-way diagram is given to determine the optimal K for the studied purslane accessions. The peak of the curve is at the point equal to 2, which indicates that the optimal number of K is equal to 2. Therefore, accessions are divided into 2 groups in terms of population structure, which can be separated. Figure 3 also shows the inferred structure for the accessions in this study, which are divided into 2 groups, and mixed accessions are also observed among them.

Table 1. Collecting regions of studied purslane (*Portulaca oleracea* L.) accessions

Region	Altitude	Longitude	Latitude	Region	Altitude	Longitude	Latitude
Ardabil	1377	48	38	Shahrekord	2063.4	50	32
Esfahan	1579.8	51	32	Shiraz	1513.9	52	29
Eqlid	2244.4	52	30	Qazvin	1316.2	52	29
Bandar Abbas	10.77	56	27	Karaj	1274.7	50	35
Bushehr	11.84	50	28	Maragheh	1510.2	46	37
Behbahan	326.6	50	30	Mashhad	955.9	59	36
Parsabad	75	47	39	Nurabad	988.1	51	30
Tehran	1148.8	51	35	Nehbandan	1187.8	60	31
Zanjan	1810.3	48	36	Hamedan	1829.7	48	34
Sirjan	1752.9	55	29	Yasuj	1837.6	51	30

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	3	-1360.933333	0.493288	—	—	—
2	3	-1007.600000	0.888819	353.333333	208.400000	234.468319
3	3	-862.666667	4.277071	144.933333	89.833333	21.003472
4	3	-807.566667	6.992377	55.100000	13.700000	1.959277
5	3	-738.766667	4.554485	68.800000	305.900000	67.164565
6	3	-975.866667	36.913457	-237.100000	335.166667	9.079796
7	3	-877.800000	181.200745	98.066667	179.400000	0.990062
8	3	-600.333333	35.350295	277.466667	395.733333	11.194626
9	3	-718.600000	145.028135	-118.266667	263.666667	1.818038
10	3	-573.200000	70.772099	145.400000	—	—

Figure 1. Calculated statistics for the optimal value of K using STRUCTURE 2.3.4 software in this study.

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Table 2. ISSR molecular primers for population structure analysis of Iranian (*Portulaca oleracea L.*) accessions.

#	Primer name	Primer Sequence (5'-3')	Tm (°C)
1	ISSR1	CACACACACACACACARG	56
2	ISSR2	GAGAGAGAGAGAGAGAYT	53.5
3	ISSR3	GAGAGAGAGAGAGAGAYC	55.5
4	ISSR4	GAGAGAGAGAGAGAGAYG	55.5
5	ISSR5	AGAGAGAGAGAGAGAGYT	53.5
6	ISSR7	GACACGACACGACAC	51.5
7	ISSR8	CACACACACACARY	43.5
8	ISSR9	TGTGTGTGTGTGTGTGA	51.5
9	ISSR10	GACAGACAGACAGACA	50.5
10	ISSR11	BDBACAACAACAACAACA	49.5
11	ISSR20	GACGACGACGACGAC	55
12	ISSR21	ACTCACTCACTCACTC	50.5
13	ISSR23	GAGAGAGAGAGAGAGAC	53.5
14	ISSR24	CACCACCACGC	40
15	ISSR25	GTGTGTGTGTGTCG	46
16	ISSR26	GAGAGAGAGAGACC	46
17	ISSR27	CTCTCTCTCTCTCTCTTC	55
18	ISSR28	CTCTCTCTCTCTCTCTAC	54.5
19	ISSR29	GACACACACACACACACAC	57.5
20	ISSR30	CCACTCTCTCTCTCTCTCT	57.5
21	ISSR31	ATGATGATGATGATGATG	48
22	ISSR32	GGAAGAGAGAGGAGA	49
23	ISSR33	GGGTGGGGTGGGGTG	60
24	ISSR34	AGAGAGAGAGAGAGAGGC	57
25	ISSR35	GGATGGATGGATGGAT	54.5

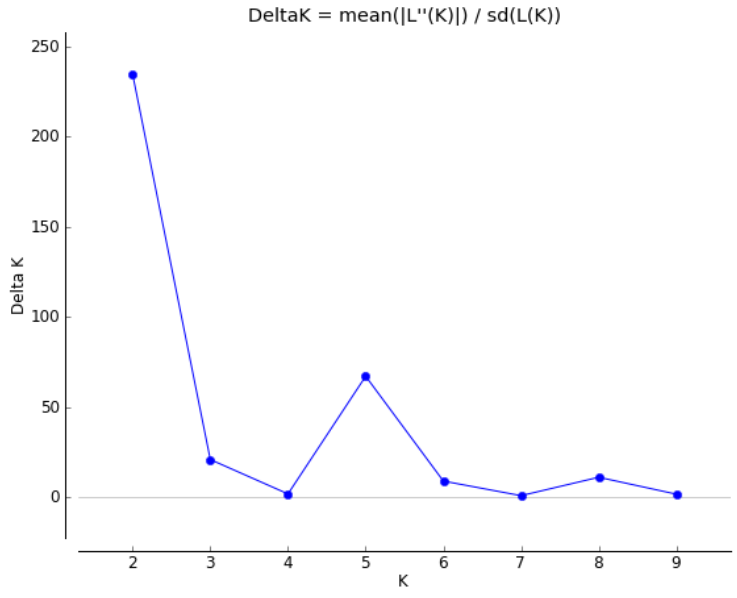

Figure 2. Estimation of DeltaK from calculated K and LnP(K) using the web-based STRUCTURE HARVESTER program in this study.

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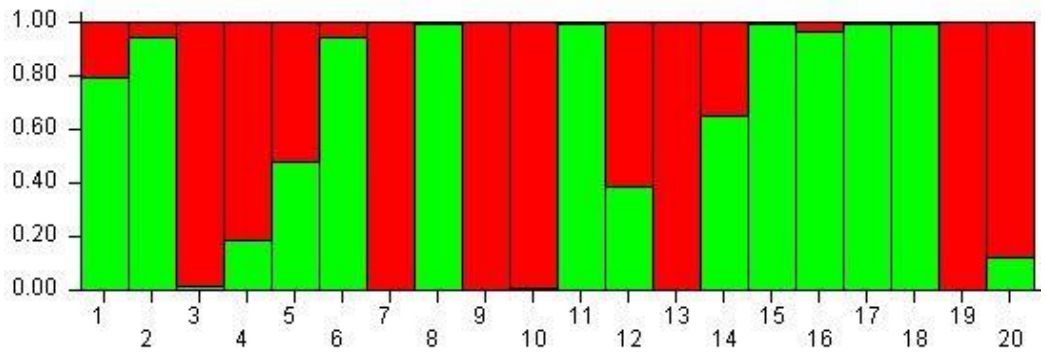

Figure 3. Inferred population structure of 20 Iranian purslane (*Portulaca oleracea* L.) accessions based on ISSR molecular markers in this study.

4. Conclusion

A population can be divided into different subgroups in terms of genetic structure, which is caused by the difference in allelic frequency. A point that should be considered is that if the analysis of the relationship between molecular markers and traits is done without identifying the genetic structure of the studied population, it will lead to the identification of false relationships. Therefore, it is very important to determine the genetic structure in populations and germplasm collections. Therefore, the obtained results can be used as the required information to identify the gene locations controlling the quantitative traits in studied accessions that may be due to the difference in the population structure.

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Conflict of Interest
The authors of this study do not have any conflicting interests.

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MORPHOLOGICAL FEATURES OF THE SECTION *MESOCENTRON* (ASTERACEAE) OF GENUS *CENTAUREA* IN TÜRKİYE

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Abstract
The genus *Centaurea* L. is a member of the Asteraceae family which includes medicinal and aromatic plants. Section *Mesocentron* (Cass.) DC. is represented by *Centaurea solstitialis* L. species in the Flora of Turkey. With the addition of the *C. verutum* L. species, which was later published as a new record, the number of species of the section in Turkey increased to two and the number of taxa to four. *C. solstitialis* spp. subsp. *solstitialis*, subsp. *carneola* (Boiss.) Wagenitz and subsp. *pyracantha* subsp. (Boiss.) Wagenitz, including three subspecies. *C. solstitialis* subsp. *carneola* and *C. solstitialis* subsp. *pyracantha* are endemic taxa for Türkiye and the endemism rate of the *Mesocentron* section is 50%. Among these, there are reports of the use of *C. solstitialis* subsp. *solstitialis* against different diseases. Detailed studies have been conducted on the morphology of the *Mesocentron* section within the genus *Centaurea*. Within this framework, measurements were taken of the stem, leaves, involucre, appendages, achenes, and pappus. Floral and involucral characteristics were sampled from the terminal capitula, and the measurements were conducted using dried herbarium materials. The results indicate that *C. verutum* distinguishes itself from other taxa based on stem indumentum, basal leaf shape, involucre size, and spine length. *C. solstitialis* subsp. *carneola* stands out from other taxa due to differences in spine color, flower measurements, and coloration. *C. solstitialis* subsp. *solstitialis* differs from other taxa in possessing dimorphic achenes and *C. solstitialis* subsp. *pyracantha* differs from other taxa based on pappus measurements.

Key Words: Compositae, endemism, knapweed, taxonomy

1. Introduction

Members of the Asteraceae family have been used in traditional medicine for many years, with some members being cultivated as food and medicinal plants for over 3000 years. It is a large family of flowering plants consisting of 1600-1700 genera and 24,000 species. In Turkey, there are 138 genera and 1336 species. Many species are used worldwide in medicine, food, and various industries, such as sunflower, lettuce, dandelion, and chamomile. Various parts of family members are used for the treatment of hemorrhoids, cardiac, liver diseases, urinary tract infections and stones, stomach problems, abdominal pain, herpes infection, colds, headaches, migraines, nausea, vomiting, rheumatism and body inflammation, dysentery, osteoporosis, diarrhea, gout, and other diseases in many countries (Mulabagal et al., 2009; Amorim et al., 2013; Achika et al., 2014; Bessada et al., 2015; Nikolic et al., 2015; Koc et al., 2015; Jafarinia et al., 2019; Rolnik and Olas, 2021; Özel and Maesaroh, 2023). In Turkey, *Achillea aleppica* and *Achillea biebersteinii* species are used as tea for the treatment of abdominal pain, *Chrysophthalmum montanum* for wound treatment, their roots for regulating blood pressure, *Matricaria aurea* for bronchitis and cough treatment, and *Notobasis syriaca* for liver diseases (Rolnik and Olas, 2021). *Centaurea*, one of the largest genera of the Asteraceae family, is a group of plants rich in secondary metabolites commonly used in alternative medicine.

The genus *Centaurea*, which contains approximately 650 species worldwide, is the largest genus of the Centaureinae subtribe (Mabberley, 2008). One of the main diversity centers for the genus *Centaurea* is Turkey (Wagenitz, 1986), and with recent publications, the number of taxa has reached 247 (Uysal et al., 2024). Of these taxa, 145 are endemic to our country, with an endemism rate of 58.7%. The genus

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Centaurea is known by different local names such as "peygamber çiçeği" (prophet's flower), "zerdali dikeni" (thistle), "çoban kaldıran" (shepherd's lifter), "timur dikeni" (thistle), and "gelin düğmesi" (bride's button). *Centaurea* flowers come in yellow, pink, purple, blue, and whitish colors. They are used in carpet dyeing and garden landscaping. Additionally, members of the genus are important for beekeeping and pollen production. Due to its geographical location, climatic zones, and topographical features, Turkey exhibits regional differences in climate and vegetation. Plants adapt to their geographical regions through their metabolites, and metabolites show differences in content and quantity among individuals of the same species living in different regions (Güvensen et al., 2019; Özel and Maesaroh, 2023). Since genus *Centaurea* members have been used for many years as antidiabetic, anti-inflammatory, anti-rheumatic, diarrhea preventive, diuretic, blood pressure regulator, cytotoxic, and antibacterial agents, elucidating the biological active components and effects of taxa is important (Özel and Maesaroh, 2023).

C. solstitialis subsp. *solstitialis* has been used as a remedy due to its wide distribution, including for common colds, herpes infections around the lips, malaria, peptic ulcers, stomach upset, and abdominal pain. (Yesilada et al., 1995; Fujita et al., 1995; Honda et al., 1996). The section *Mesocentron* is represented in Turkey by two species and four taxa. Two of these taxa (*C. solstitialis* subsp. *carneola* and *C. solstitialis* subsp. *pyracantha*) are endemic to Turkey, with an endemism rate of 50%. With this study, the section *Mesocentron*, which includes *C. solstitialis* subsp. *solstitialis*, has been thoroughly studied morphologically, interspecific relationships have been revealed, and new keys have been proposed for diagnosis.

2. Material and Methods

The morphology of the section *Mesocentron* of the genus *Centaurea* has been studied in detail. In this scope, the stem, leaves, involucre, appendages, achenes and pappus were measured. Floral and involucral features were sampled from the terminal capitula. The measurements were carried out using dried herbarium materials. The statements of size relate to herbarium material with capitula not deformed by pressing. The appendage border was included in the measurements, but never in the involucral bracts. The morphology of the specimens was examined under a stereo miscoscope. The localities of the specimens studied are shown in Table 1.

Table 1. Localities of the studied taxa

Taxa	Locality
C. solstitialis subsp. *carneola*	Kahramanmaraş: Pazarcık, 590 m, 08 vi 2023, step, *E. Şirin* 801
C. solstitialis subsp. *solstitialis*	Konya: Şelçuklu, 1212 m, 31 vi 2023, roadside, *E. Şirin* 802
C. solstitialis subsp. *carneola*	İçel: Mut, 187 m, 01 vii 2023, roadside, *E. Şirin* 803
C. solstitialis subsp. *pyracantha*	İçel: Anamur, 356 m, 01 vii 2023, roadside, *E. Şirin* 804
C. solstitialis subsp. *carneola*	İçel: Mut, 187 m, 19 vii 2023, roadside, *E. Şirin* 805
C. solstitialis subsp. *pyracantha*	İçel: Anamur, 356 m, 19 vii 2023, roadside, *E. Şirin* 806
C. solstitialis subsp. *solstitialis*	Konya: Şelçuklu, 1212 m, 21 vii 2023, roadside, *E. Şirin* 807

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3. Results and Discussion

As a result of detailed examination of taxa, the proposed species and subspecies diagnostic keys are as follows:

Diagnostic key for section *Mesocentron*
1. Stem adpressed–tomentose, spine yellow or suffused with red but not blackish at base, involucre shorter than 15 mm, achene shorter than 3.5 mm *C. solstitialis*
1. Stem scabridulous and somewhat cobwebbed, spine yellow and blackish at base, involucre longer than 15 mm, achene longer than 3.5 mm *C. verutum*

Diagnostic key for subspecies of *C. solstitialis* (Adapted from Wagenitz et al. 1975)
1. Flowers pink, achene monomorphic and without pappus subsp. *carneola*
1. Flowers yellow, achene dimorphic or monomorphic (if so with pappus)
2. Anther tube in flowers yellow, spines straw coloured, usually longer than 15 mm, achene dimorphic subsp. *solstitialis*
2. Anther tube in flowers purple, spines suffused with red, usually shorter than 15 mm, achene monomorphic subsp. *pyracantha*

The detailed morphological characteristics of the taxa in the section *Mesocentron* are as follows:
C. solstitialis L. subsp. *solstitialis*: Annual, adpressed–tomentose, 15–45 cm. Stem rigid, erect, simple or many branched from median, 2–2.5 mm diameter at base. Leaves green, adpressed–tomentose, lower and basal leaves lyrate–pinnatipartite with 3–4 lateral segments, upper and median linear–lanceolate to lanceolate, toothed to entire or lobed, decurrent into entire wings, basal leaves 28–50 × 9–18 mm and lower leaves 15–45 × 2–18 mm. Capitula solitary at the end of branches. Involucre 14–15 × 13–14 mm, ovoid, phyllaries 3–4 seriate, arachnoid–tomentose, outer ones ovate, 4–6 × 3.5–5 mm, median ones linear–lanceolate 6.5–7 × 3 mm, inner ones linear 9–9.5 × 2–2.5 mm. Appendage with a patent straw–coloured and 15–20 mm spine, and 2 spinules (1.5–3.5 mm) on each side at base, outer phyllaries with short spinules. Flowers yellow, marginal not radiant, 14–15 mm, lobes 9–10 mm, tubes 5 mm, anther tube yellow. Achene dimorphic: Marginal obovoid, dull, blackish, without pappus, 2,1 × 1 mm; central obovoid, glossy, greyish to brown, 2.4–2.7 × 1.1–1.2 mm, pappus 3.8–4.5 mm.

C. solstitialis subsp. *carneola*: Annual, adpressed–tomentose, 16–26 cm. Stem rigid, erect, simple or many branched from median, 2–2.5 mm diameter at base. Leaves green, adpressed–tomentose, lower and basal leaves lyrate–pinnatipartite with 3–4 lateral segments, upper and median linear–lanceolate to lanceolate, toothed to entire or lobed, decurrent into entire wings, basal leaves 28–50 × 9–18 mm and lower leaves 15–45 × 2–18 mm. Capitula solitary at the end of branches. Involucre 12–15 × 9–10 mm, ovoid, phyllaries 3–4 seriate, glabrous, outer ones ovate, 5–5.5 × 4.5–5 mm, median ones linear–lanceolate 7–8 × 5–5.5 mm, inner ones linear 10–12 × 2.5–3 mm. Appendage with 13–19 mm spine, and 2–3 spinules (2.5–3.5 mm) on each side at base, outer phyllaries with short spinules. Flowers pink, marginal not radiant, 17–21 mm, lobes 11–13 mm, tubes 6–8 mm, anther tube purple. Achene monomorphic; obovoid, glossy, dark brownish, 3–3.3 × 1.4–1.6 mm, without pappus.

C. solstitialis subsp. *pyracantha*: Annual, adpressed–tomentose, 15–35 cm. Stem rigid, erect, simple or many branched from median, 2–2.5 mm diameter at base. Leaves green, adpressed–tomentose, lower and basal leaves lyrate–pinnatipartite with 3–4 lateral segments, upper and median linear–lanceolate to lanceolate, toothed to entire or lobed, decurrent into entire wings, basal leaves 25–60 × 12–20 mm and lower leaves 20–75 × 2–30 mm. Capitula solitary at the end of branches. Involucre 11–12 × 8–10 mm, ovoid, phyllaries 3–4 seriate, glabrous, outer ones ovate, 3.5–4 × 2.5–3 mm, median ones linear–

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lanceolate 5–5.5 × 2–2.5 mm, inner ones linear 7–7.5 × 2–2.5 mm. Appendage with 9–14 mm spine, and 2–3 spinules (3–3.8 mm) on each side at base, outer phyllaries with short spinules, spines and spinules suffused with red. Flowers yellow, marginal not radiant, 13–15 mm, lobes 7–9 mm, tubes 6 mm, anther tube purple. Achene monomorphic; obovoid, dull, light brown, 1.8–2.2 × 0.8–1 mm, pappus 2.2–2.6 mm.

C. verutum: Annual, scabridulous and somewhat cobwebbed, 30–45 cm. Stem rigid, erect, simple or many branched from median, 2–2.5 mm diameter at base. Lower leaves pinnatifid–lyrate, 50–85 × 3.5–8 mm, basal leaves oblong, long decurrent, forming narrow entire wings 2–3.5 × 0.5–0.6 mm. Capitula solitary at the end of branches. Involucre 15–20 × 13–20 mm, broadly ovoid, truncate at base, tomentose later glabrous, leathery, phyllaries 3–4 seriate, outer ones ovate, 5–5.5 × 5–6 mm, median ones linear–lanceolate 7–7.5 × 4–6 mm, inner ones linear 13–14 × 2.5–3.5 mm. Bracts ending a rigid spine, spine yellow and blackish at base, straight, spreading, 25–35 mm, and 2–3 spinules (3–3.5 mm) on each side at base, outer phyllaries with short spinules. Flowers yellow, marginal not radiant, 15–16 mm, lobes 8–10 mm, tubes 6–7 mm, anther tube yellow. Achene monomorphic; obovoid, dull, light brown, 3.6–4.1 × 1.3–1.4 mm, pappus 2.5–3.5 mm.

According to results *C. verutum* differs from other taxa by stem indumentum, basal leaves shape, involucre and spine length. *C. solstitialis* subsp. *carneola* becomes different other taxa by spine colour, flowers measurements and colour. *C. solstitialis* subsp. *solstitialis* differs from other taxa by having dimorphic achene. *C. solstitialis* subsp. *pyracantha* becomes different other taxa by pappus measurements (Table 2).

Table 2. Morphological comparison of section *Mesocentron*

Characters	*C. solstitialis* subsp. *solstitialis*	*C. solstitialis* subsp. *carneola*	*C. solstitialis* L. subsp. *pyracantha*	*C. verutum*
Stem indumentum	Adpressed–tomentose	Adpressed–tomentose	Adpressed–tomentose	Scabridulous and somewhat cobwebbed
Basal leaves	Lyrate to pinnatipartite with 3–4 lateral segments	Lyrate to pinnatipartite with 3–4 lateral segments	Lyrate to pinnatipartite with 3–4 lateral segments	Oblong, long decurrent, forming narrow entire wings
Involucre	14–15 × 13–14 mm, ovoid	12–15 × 9–10 mm, ovoid	11–12 × 8–10 mm, ovoid	15–20 × 13–20 mm, broadly ovoid
Median phyllaries	6.5–7 × 3 mm	7–8 × 5–5.5 mm	5–5.5 × 2–2.5 mm	7–7.5 × 4–6 mm
Spine	15–20 mm, straw–coloured	13–19 mm, straw–coloured	9–14 mm, suffused with red	25–35 mm, yellow and blackish at base
Flowers	Yellow, 14–15 mm	Pink, 17–21 mm	Yellow, 13–15 mm	Yellow, 15–16 mm
Achene	Dimorphic	Monomorphic	Monomorphic	Monomorphic
Pappus	3.8–4.5 mm	Absent	2.2–2.6 mm	2.5–3.5 mm

The samples collected from Gaziantep province in 2010 and 2012 were published as a new record for Turkey belonging to *C. verutum* (Duran et al., 2014). In this study; leaves measurements, involucre width, shapes and sizes of phyllaries, flowers measurements, and achene width were investigated for the first time. *C. solstitialis* is represented by three subspecies in Turkey (Wagenitz, 1975). Leaves measurements, shapes and sizes of phyllaries, flowers measurements, and achene width were investigated for the first time with this study of all subspecies. Additionally, the distribution map of the taxa within the section *Mesocentron* is shown in Figure 1.

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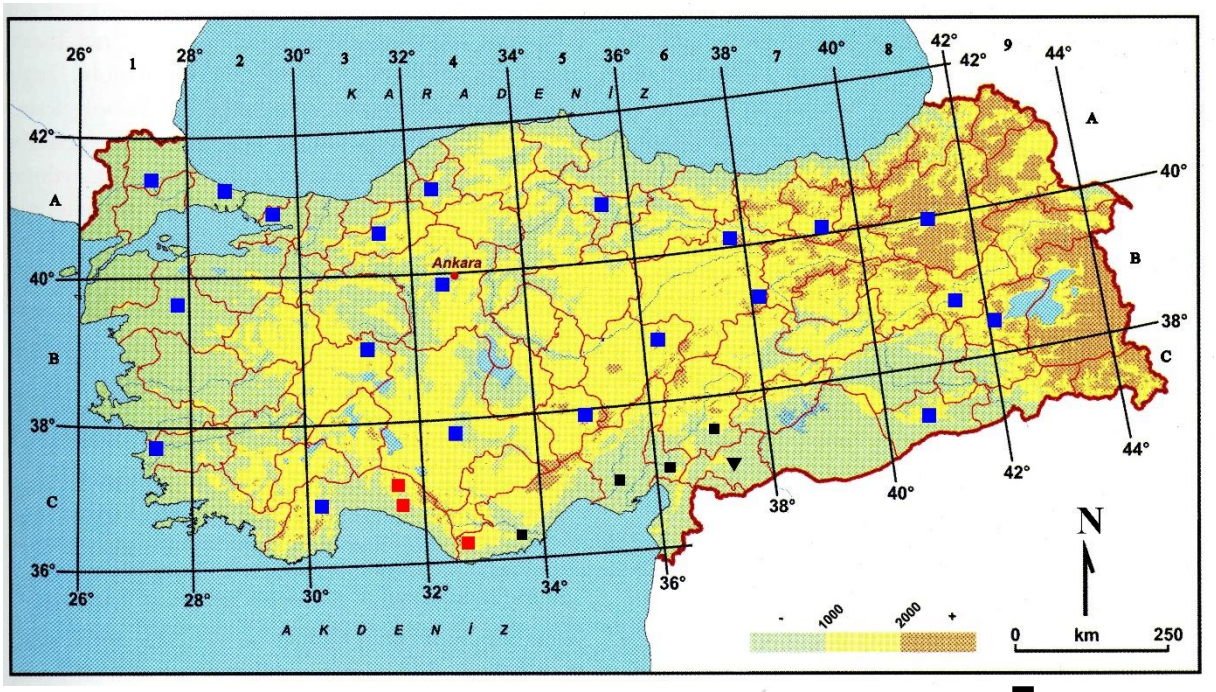

Figure 1. Distribution map of section *Mesocentron*: *C. solstitialis* subsp. *solstitialis* (■), *C. solstitialis* subsp. *pyracantha* (■), *C. solstitialis* subsp. *carneola* (■), *C. verutum* (▼)

4. Conclusion

In conclusion, contributions have been made to the discrimination of taxa within the section *Mesocentron* of the genus *Centaurea*. Species and subspecies diagnostic keys have been created, and the distribution of taxa in Turkey has been shown on a map. Karyological, micromorphological, anatomical, and palynological studies will provide additional contributions to determining taxonomic relationships.

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Conflict of Interest
There is no conflict of interest.

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BEHAVIORAL PREFERENCE OF SILVERLEAF WHITEFLY (*BEMISIA TABACI*) ON BASIL (*OCIMUM BASILICUM*) GENOTYPES WITH DIFFERENT MAIN COMPONENTS OF ESSENTIAL OILS

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Abstract
Silverleaf whitefly (*Bemisia tabaci* (Gennadius), (Hemiptera: Aleyrodidae)) is an important pest of crops worldwide. It is among the most harmful species, especially in subtropical and tropical regions. Chemical control methods are commonly used in the control against this very significant pest. However, essential oils, composed of various components, can act against insect pests through complex mechanisms. This study investigated the behavioral preferences of silverleaf whiteflies on basil genotypes with different main components, including eucalyptol, estragole, geraniol, methyleugenol, methyl cinnamate, citral, and linalool based on the completely randomized design. In the genotypes, during the initial observation on July 2, 2022, the density of silverleaf whiteflies recorded was 12.58 individuals per leaf, whereas in the subsequent observation on July 19, 2022, the density decreased to 4.66 individuals per leaf. Across both observations, the highest mean number of silverleaf whiteflies per leaf was found in genotype PI 172998 (Estragole chemotype) with a mean observation of 20.08 individuals, while the lowest mean was in genotype PI 652070 (Linalol-Estragole chemotype) with a mean observation of 1.43 individuals. Since the linalool-methyleugenol and linalool-estragole chemotypes exhibited the lowest frequency of occurrence in both sets of observations, it is advisable to investigate the potential of these components as insect repellents. Furthermore, since the estragole, linalool–eucalyptol, and citral–neral chemotypes appeared most frequently in both observation groups, it is recommended to investigate the potential of these compounds as insect attractants. This study highlights the varying behavioral responses of silverleaf whiteflies to distinct basil chemotypes, offering valuable insights for pest management approaches.

Key Words: Basil, silverleaf whitefly, chemotype, essential oil components, hemiptera

1. Introduction

Basil (*Ocimum basilicum*), a plant belonging to the Lamiaceae family, known for its valuable bioactive phytochemicals (Zhakipbekov et al., 2024). It has various metabolites, including terpenoids, flavonoids, alkaloids, and more. Major studies on basil have focused on determining its phytochemical composition, primarily through essential oils extracted from various plant parts. These essential oils, containing volatile organic components, serve a protective role for the plant (Kačániová et al., 2022). Basil exhibits significant variations within its genotypes, demonstrating diverse features like differing leaf dimensions, a continuum of hues spanning from green to deep purple, and an array of flower shades encompassing white, red, lavender, and purple. The plant also displays distinct growth features, encompassing differences in shape, height, and flowering time, as well as a diverse array of aromas. The basil essential oil comprises a diverse array of fragrant and volatile elements, encompassing oxygenated and aromatic monoterpenes such as linalool, p-allyl-anisole (estragole), neral, geranial, and eugenol (Branca et al., 2024). The silverleaf whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a critical pest that phloem-feeding mostly on herbaceous plants (De Barro et al., 2011). It is among the most harmful species, especially in subtropical and tropical regions (Ashfaq et al., 2014; Carvalho et al., 2017). It is highly polyphagous and has over 600 host plants (Oliveira et al., 2001). In addition to the damage it

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causes directly by feeding, it also causes damage indirectly by transmitting more than 100 plant pathogenic viruses (Czosnek et al., 2017; Jones, 2003; Wu et al., 2023). *Bemisia tabaci* genetic diversity is quite high. This situation is considered as a biotype complex (Xu et al., 2010) or a complex consisting of different cryptic types (Boykin & De Barro, 2014; De Barro et al., 2011). The most widespread biotype worldwide is Middle East Asia Minor 1—MEAM1 (De Barro et al., 2011). Chemical control is widely used in the control against this very significant pest (Sani et al., 2020). As a result of long-term use of insecticides, resistance development is observed in *B. tabaci* populations (Luo et al., 2010; Roditakis et al., 2005; Satar et al., 2018). It is known that aromatic plants have a repellent effect on B. tabaci adults (Carvalho et al., 2017).

Traditional farming methods, which rely heavily on growing one type of crop and using strong chemical insecticides, have been heavily criticized because they harm the environment and people's health, and cause loss of biodiversity. However, having a variety of plants in fields can naturally help control pests in several ways, like using smells and visual signals, affecting their life cycles, and improving soil health (Matu et al., 2021). This approach, known as companion planting, includes tactics like trap cropping, which can help reduce pest damage. Trap crops are described as plant populations strategically positioned or manipulated to lure, redirect, intercept, or detain specific insects or the diseases they transmit, thereby minimizing harm to the primary crop (Bengtsson et al., 2005). Understanding how different types of basil affect the behavior of pests like the silverleaf whitefly could lead to new ways of managing them without relying on harmful chemicals. Since these pests can cause a lot of damage and spread diseases to plants, finding out if certain basil plants can repel them could be a sustainable solution, especially as these pests become more resistant to insecticides (Birgücü et al., 2015). Within this context, behavioral preference of silverleaf whitefly investigated on basil genotypes with different main components including eucalyptol, estragole, geraniol, methyleugenol, methyl cinnamate, citral, and linalol.

2. Material and Methods

2.1. Plant material and experiment

The greenhouse experiment was conducted according to the completely randomized design at the experimental area of the Department of Plant Protection at Çukurova University in Adana, Türkiye (37°01′45.60″ N, 35°21′39.70″ E) during the 2021–2022 season (Figure 1.). The seeds were sourced from United States Department of Agriculture (USDA) (Table 1). The seeds were sown in plastic pots (170 mm diameter–150 mm depth–2400 ml) filled with soil:perlite:peat mixture (1:1:1). Basil required between 13 and 15 days to complete the germination process. Ten seeds were sown in individual pots, and after a germination period of 15 days the plants were reduced to a single plant per pot through thinning. Each pot received a weekly irrigation of 200 milliliters of distilled water. The plants were manually harvested upon reaching the onset of the flowering stage. Following the harvest, the number of insect individuals was quantified by examining 20 leaves in each treatment (Figure 2.).

2.2. Essential oil extraction

A 20-gram portion of dried basil was weighed and introduced into a glass vessel containing 200 milliliters of distilled water. This assembly was subsequently placed into a Clevenger apparatus for a duration of 3 hours to facilitate the extraction of essential oil. The process involved extraction of essential oil from the samples, which then gathered on the water surface in the equipment. Following this, the volume (in mL) was measured from the marked section of the equipment. The essential oil concentration was expressed as a percentage relative to the weight of dry tissue, calculated as mL/100 g.

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Figure 1. Experimental site overview and different basil accessions.

Figure 2. The view of silverleaf whitefly nymphs, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae).

2.3. Gas chromatography ± mass spectrometry (GC-MS) analysis

GC-MS examinations were conducted at the Department of Biology within Kahramanmaras Sutcu Imam University. 10 µl essential oil was mixed with 250 µl dichloromethane, and subsequently, 1µl mixture was injected into the column. Analysis of essential oil component protocol were performed as specified in our previous study (Barut et al., 2023).

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Table 1. List of studied basil genetic resources

No	Accession name	Plant name	Origin	Collection site	Source type	Chemotype
1	PI 170579	226-3	İzmir, Turkey	Garden of Ali Kucuk Kirca, Bergama, Izmir.	Collected	Methyl cinnamate
2	PI 172998	Reyhan	Van, Turkey	Seed dealer, Van.	Collected	Estragole
3	PI 173746	Reyhan	Malatya, Turkey	Seedsman, Malatya	Collected	Methyleugenol
4	PI 368699	Edar	North Macedonia	Topolovic, North Macedonia	Collected	Eucalyptol
5	PI 379413	Krupnolsen	North Macedonia	Nerezi, North Macedonia	Collected	Geraniol
6	PI 584458	Sweet Dani	United States	-	Developed	Citral
7	PI 652070	Sweet Basil	Pennsylvania, United States	-	Donated	Linalol

2.4. Statistical analysis

Principal component analysis was conducted using the JMP 14.0 statistical software. The heat map was generated utilizing Flourish studio. The network correlation plot was utilized to analyze the relationships between observations and main components of essential oils. This analysis was conducted using the corrr Package within the R Studio software environment.

3. Results and Discussion

A look at the basil accessions examined in the preference for silverleaf revealed a wide range of variation. The essential oils extracted from dried basil leaves were subjected to analysis using gas chromatography-mass spectrometry (GC-MS), with the findings presented in Table 2. There were different chemotypes such as methyl cinnamate (49.84%) – linalol (20.05%), estragole (89.7%), linalol (47.93%) – methyleugenol (19.54%), linalol (48.7%) – eucalyptol (23.59%), linalol (41.15%) – geraniol (33.67%), citral (37.29%) – neral (28.18%), and linalol (69.51%) – estragole (13.91%).

In the genotypes, during the initial observation on July 2, 2022, the density of silverleaf whiteflies recorded was 12.58 individuals per leaf, whereas in the subsequent observation on July 19, 2022, the density decreased to 4.66 individuals per leaf (Table 3). In both observations, the density of silverleaf whiteflies showed similar trends in the genotypes. Across both observations, the highest mean number of silverleaf whiteflies per leaf was found in genotype PI 172998 (estragole chemotype) with a mean observation of 20.08 individuals, while the lowest mean was in genotype PI 652070 (linalol-estragole chemotype) with a mean observation of 1.43 individuals.

Since the linalool-methyleugenol and linalool-estragole chemotypes exhibited the lowest frequency of occurrence in both sets of observations, it is advisable to investigate the potential of these components as insect repellents. Some accessions have relatively low insect activity throughout both observations (e.g., PI 652070), while others exhibit more substantial fluctuations between the first and second observations (e.g., PI 172998). This low insect activity is thought to be due to the chemotypic structure of genotypes.

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Table 2. Essential oil content and its composition in used basil accessions

Compounds	RT (min)	PI 170579	PI 172998	PI 173746	PI 368699	PI 379413	PI 584458	PI 652070
		Relative Peak Area (%)						
Essential oil content (%)		1.15	1.31	1.37	0.90	0.79	0.95	0.85
Myrcene	11.132	ns	ns	0.86	ns	0.8	ns	0.76
Limonene	11.610	0.2	0.2	0.22	0.5	0.22	0.12	ns
δ-3-carene	12.161	ns	ns	1.14	0.84	1.34	ns	0.87
Eucalyptol	12.848	7.37	ns	3.32	23.59	0.59	ns	0.61
p-Cymene	13.063	0.66	ns	ns	ns	ns	ns	ns
cis-3-Hexenyl acetate	13.811	ns	0.03	0.05	ns	0.14	0.19	0.02
Furan	14.274	ns	ns	ns	ns	ns	0.45	ns
Sulcatone	15.010	ns	ns	ns	ns	ns	1.55	ns
trans-α-Bergamotene	15.981	1.26	0.69	3.88	7.01	1.02	1.26	ns
Linalol	16.316	20.05	ns	47.93	48.70	41.15	ns	69.51
trans-β-Farnesene	17.103	ns	0.43	ns	0.61	ns	0.5	ns
β-Ocimene	17.194	0.14	ns	ns	0.58	ns	ns	ns
β-Caryophyllene	17.236	0.09	2.04	1.03	ns	0.4	1.65	ns
cis-Caryophyllene	17.402	ns	ns	ns	ns	ns	0.83	ns
β-Bergamotene	17.562	ns	0.72	0.5	ns	ns	ns	ns
Isoneral	17.645	ns	ns	ns	ns	ns	2.33	ns
Guaiyl acetate	17.761	1.81	2.18	1.19	1.71	1.37	2.93	1.65
Humulene	18.245	ns	0.68	ns	0.5	ns	ns	ns
γ-Muurolene	18.340	1.4	ns	2.23	ns	3.66	2.56	1.59
β-Selinene	18.459	ns	ns	ns	ns	ns	4.2	ns
β-copaene	18.613	ns	1.25	1.39	3.38	2.28	ns	1.62
Estragole	18.840	ns	89.7	0.89	ns	ns	0.86	13.91
α-Terpineol	18.987	1.48	ns	1.11	4.29	1.63	1.33	ns
Isoborneol	19.426	ns	ns	ns	ns	1.57	ns	0.34
Nerol	19.690	ns	ns	ns	ns	0.64	0.42	ns
Geraniol	20.201	ns	ns	0.44	ns	33.67	ns	ns
Neral	20.387	ns	ns	ns	ns	ns	28.18	ns
Citral	20.932	ns	ns	0.04	ns	1.37	37.29	ns
Nerolidol	21.676	0.08	0.07	0.17	0.25	0.37	0.23	0.16
Epicubenol	22.895	0.38	ns	0.65	ns	0.71	ns	0.58
Methyleugenol	23.308	9.16	0.96	19.54	0.16	0.03	0.17	0.04
Caryophyllene oxide	23.346	ns	ns	ns	ns	ns	4.71	ns
τ-Cadinol	23.937	3.11	0.19	3.26	0.13	5.33	ns	4.53
Methyl cinnamate	24.497	49.84	ns	ns	ns	ns	ns	ns
Eugenol	25.377	ns	ns	5.23	5.85	ns	0.8	2.08
Total		97.03	99.14	95.07	98.1	98.29	92.56	98.27

Using chemical pesticides to control pests harm the environment and natural predators of pests. In a way that confirms our study, Matu et al. (2021) reported that the main volatile compenents of basil were

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linalool, 1,8-cineole, eugenol and β-elemene. Out of these, 1,8-cineole strongly attracted greenhouse whitefly, while linalool strongly repelled them. Similiar to our study, silverleaf whiteflies were also previously found to be attracted to 1,8-cineole and eugenol Cao et al. (2008). Al-Harbi et al. (2021) investigated the insecticidal effects of basil, black seed, and lavender essential oils on *Sitophilus oryzae*, a significant pest of stored products, due to concerns over synthetic insecticide risks. The essential oils from all three plants showed toxicity against *S. oryzae*, with basil and lavender oils inducing 100% mortality at 6 mg/cm2 after 48 and 24 hours respectively, while basil oil also demonstrated significant repellent activity at 0.75 mg/cm^2.

Table 3. Density of silverleaf whiteflies nymphs (*Bemisia tabaci*) per leaf

Accessions	1st observation	2nd observation	Mean observation
PI 170579	3.07	2.97	3.02
PI 172998	34.33	5.83	20.08
PI 173746	2.37	1.40	1.88
PI 368699	25.87	6.77	16.32
PI 379413	7.97	5.67	6.82
PI 584458	12.63	8.97	10.80
PI 652070	1.83	1.03	1.43
Mean	12.58	4.66	

Ling Chang et al. (2009) reported that basil oil and its primary components (trans-anethole, estragole, and linalool) demonstrated rapid insecticidal activity with a strong dose-dependent effect against three fruit fly species. Furthermore, linalool, a natural compound found in perfumes and flavorings, has demonstrated antimicrobial and insect-repellent properties, suggesting its potential for controlling pathogens or pests (Beier et al., 2014). On the other hand, Rodríguez-González et al. (2019) investigated the potential of basil essential oil for controlling the bean weevil (*Acanthoscelides obtectus*) a major pest of common bean. They showed that essential oil effectively reduced bean weight losses and the number of damaged beans, with higher doses exhibiting greater efficacy, suggesting their potential as environmentally friendly alternatives for pest control in bean storage. All of these investigations underscore the significance of the basil plant in various contexts. Through comprehensive analysis and experimentation, researchers have elucidated the multifaceted roles and potential applications of basil, highlighting its importance in areas such as agriculture, pest management, and health sciences.

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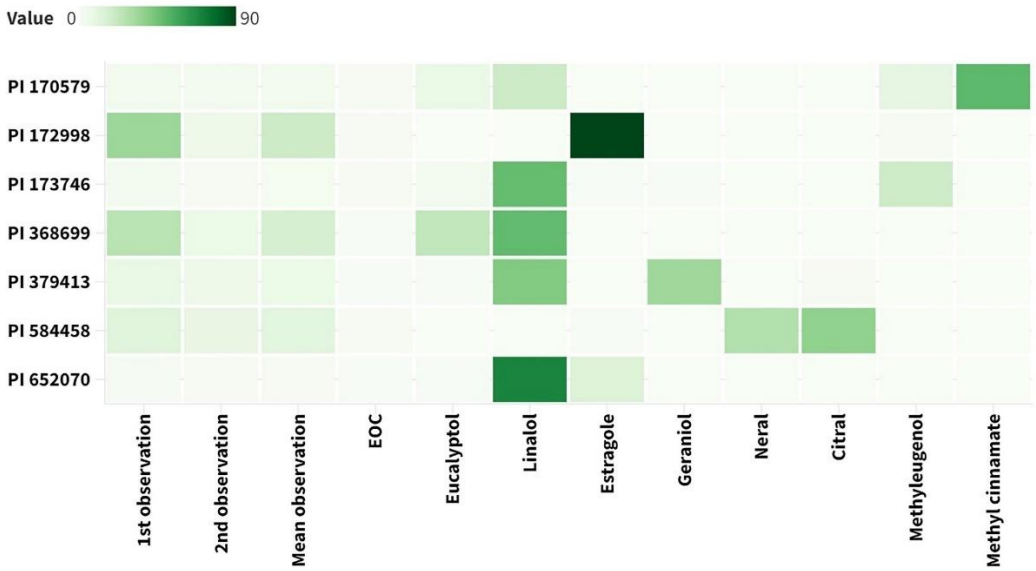

Figure 3. Heatmap based on observations and the main essential oil components of basil (*Ocimum basilicum* L.) (Value=%)

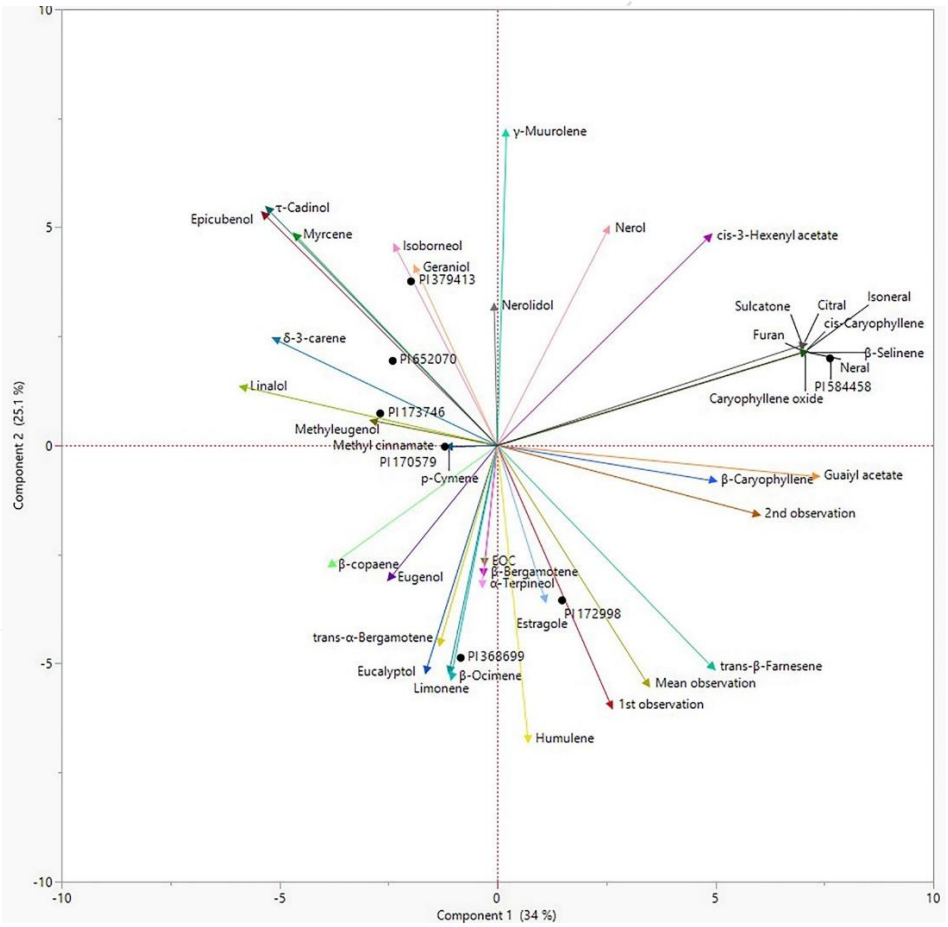

Figure 4. Principal component analysis on correlations of observations and essential oil components

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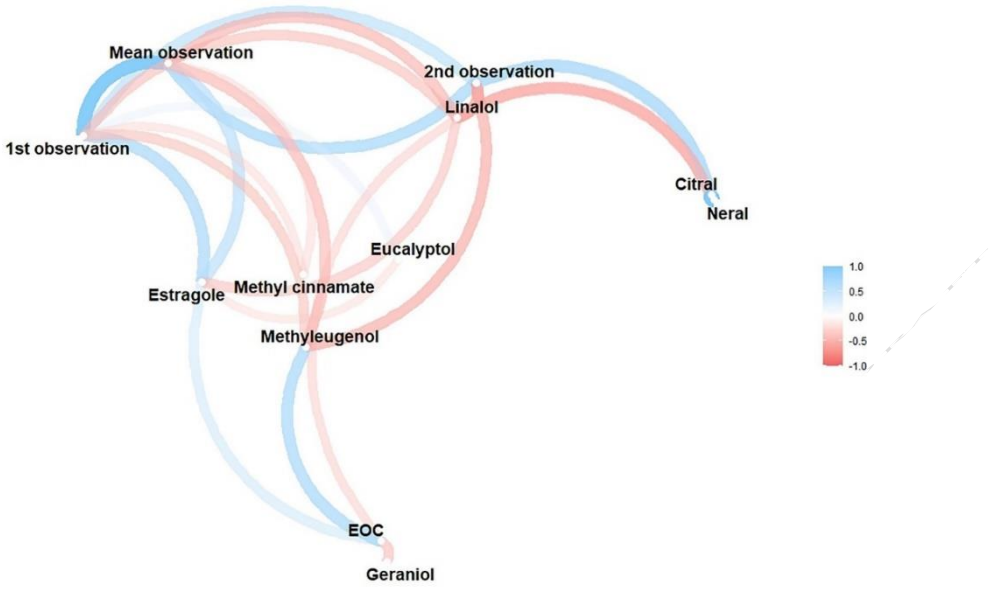

Figure 5. Network correlation plot (NCP) among observations and main essential oil components

The values among the genotypes for the examined traits can be seen in the heatmap in Figure. 3. Additionally, to assess the constituents identified in the essential oil extracted from desiccated leaves, the data were subjected PCAbiplot analysis. A significant portion of the variability, accounting for 59.10% of the overall variation, was found to be correlated along the TBA1 and TBA2 axes, as shown in Figure 4. Figure 5 presents the correlation analysis between the constituents of essential oils and the population density of silverleaf whiteflies, with color codes denoting either positive or negative correlations. The figure revealed an inverse relationship between the presence of linalool, methyl cinnamate, methyleugenol, and the average density of silverleaf whiteflies. Conversely, a direct relationship was noted between the presence of neral, estragole, and the average density of silverleaf whiteflies.

4. Conclusion

This study demonstrated that basil produce volatile signals which influence silverleaf whitefly behaviour. These volatile compounds show promise as bio-repellents and could be implemented in a "push-pull" semiochemical strategy for controlling greenhouse whiteflies. Overall, the study suggests exploring the insect repellent potential of linalool-methyleugenol and linalool-estragole chemotypes, while also considering the insect attractant properties of estragole, linalool-eucalyptol, and citral-neral chemotypes. In future studies, exploring the long-term effects of implementing a novel strategy on pest population dynamics and crop health would be beneficial for sustainable pest management practices.

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Conflict of Interest
All authors declare that there is no conflict of interest in writing upon submission of the manuscript.

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INFLUENCE OF SOLVENT POLARITY ON PHYTOCHEMICAL PROFILE, ENZYME INHIBITORY ACTIVITY, TOTAL PHENOLIC COMPOUNDS, AND ANTIOXIDANT ACTIVITY OF BLUEBERRY (*VACCINIUM CORYMBOSUM* L.): A COMPREHENSIVE CORRELATION ANALYSIS BETWEEN CHEMICAL PROFILES AND BIOACTIVITY

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Abstract
Plant products are a virtually unlimited reservoir of bioactive compounds that can be used to develop new candidates for plant-based medicines or functional foods. With their known health-beneficial effects and rich phytochemical content, fruits are an excellent source for the discovery of plant phytochemicals, and blueberries, which are rich in nutrients and polyphenols, have a promising place among them. Herein, phytochemical profiles, enzyme inhibitory activities, antioxidant capacity, and total polyphenol contents (TPC) of polar and non-polar extracts of dried *Vaccinium corymbosum* L. fruits were examined, along with the statistical analysis to determine the main components that are accountable for bioactivity. The phytochemical composition of the extracts was analyzed using LC-MS/MS and GC-MS techniques using 53 phenolic and seven triterpenoid standards. A total of 23 phenolics were detected in both extracts, while no triterpenoids were detected. The inhibitory potential of the extracts was investigated against certain enzymes targeted in the treatment of neurodegenerative disorders (tyrosinase, acetylcholinesterase, and butyrylcholinesterase), ulcers (urease), hyperpigmentation (elastase, and collagenase), and hypertension (angiotensin-converting enzyme). The maximum enzyme inhibitory activity against ACE (99.75%) and urease (91.66%) was obtained from the non-polar extract. On the other hand, the polar extract showed low ACE inhibitory activity (7.04%), and no activity was observed against urease. The non-polar extract also showed the highest β-carotene bleaching capacity (91.15%), DPPH radical scavenging activity (IC_{50} of 92.92 µg/mL), and TPC (42.13 mg GAE/g). The relationship between each specific phenolic and bioactivity was investigated using principal component analysis and Pearson correlation analysis.

Key Words: *Vaccinium corymbosum* L., Enzyme inhibitory activities, antioxidant capacity, phytochemical profile, Pearson correlation, principal component analysis

1. Introduction

Phytochemicals are a group of bioactive chemicals naturally produced by plants for their defense systems. Although plants have been part of traditional medicine in many parts of the world since ancient times, the consumption of plant-derived bioactive compounds and their use as pharmaceutical raw materials and in the production of functional foods has increased in the last two decades as human beings seek natural products to improve their health (Pawase et al. 2024). More than a thousand phytochemicals have been discovered, and positive evidence of phytochemical activities has been reported. While their presence and varieties vary from plant to plant, they are abundant in fruits, whole grains, vegetables, nuts, legumes, seeds and flowers. In addition, different parts of a plant, such as fruits, leaves, roots, and stems, may present different phytochemical profiles (Gonfa et al. 2023).

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Fruits are a great source of phytochemicals with their rich content of health-promoting phytochemicals, and blueberries, which are rich in nutrients and polyphenols, have a promising place among them. Blueberries, belong to the Ericaceae family, are grown primarily in the United States, Canada, Sweden, Poland, Germany and Türkiye. Blueberries (*Vaccinium* spp.) and are found in two forms: wild-growing low blueberries (*V. angustifolium*) and cultivated high-bush blueberries (*V. corymbosum* and *V. virgatum*). Blueberries are a rich reservoir of vitamin C, folate, flavonoids, phenolic compounds and especially anthocyanins, essential for nutritional physiology. Several studies have shown that blueberries have antioxidant, antimicrobial, anticarcinogenic, anti-angiogenesis, anti-allergic, anti-ulcer, and anti-inflammatory activities. In addition, blueberries added to tea as part of the food-to-food fortification approach have been reported to inhibit the formation of iron-polyphenol complexes. Although many studies have been conducted on blueberries, the information on phytochemical content, bioactivities, and the main components responsible for bioactivity have not been comprehensively elucidated (Dasdemir et al. 2023) .

The present study aims to reveal the relationship between individual phenolics and bioactivity by addressing the effect of solvent polarity on the phytochemical profile and bioactivity of blueberries. For this purpose, the phytochemical profile of the polar and non-polar extracts of dried blueberries was comprehensively examined by LC-MS/MS and GC-MS techniques. The enzyme inhibitory activities of the extracts against seven enzymes were assessed. DPPH radical scavenging activity and linoleic acid/β-carotene bleaching assay were used to investigate the antioxidant activity of the extracts. The Folin-Ciocalteu method. Pearson correlation and principal component analysis were performed to determine the relationship between specific phenolic compounds and enzyme inhibitory activities.

2. Material and Methods

2.1. Material

The fresh blueberries (*V. corymbosum* L.) were purchased from a local market in Rize, Türkiye. The fruits were sorted and washed with sterile distilled water to remove surface contaminants. The fruits were freeze-dried until the final moisture content of the fruits reached 12.68±1.24%. The dried fruits were stored at -80°C without light until use. The moisture content of the fruits was measured by the gravimetric method (Ng, Soh, and Yong 2022). The results were calculated as percentages (%) using the following equation (1):

$$\text{Moisture (\%)} = (\text{fresh weight} - \text{dry weight}) / \text{fresh weight} \times 100 \quad (1)$$

2.2. Preparation of dried blueberry polar and non-polar extracts

Freeze-dried blueberries (25 g) were ground to powder in a laboratory blender (Waring Commercial Blender, USA). The resulting powder (3 g) was extracted with 20 mL of either deionized water or a mixture of ethanol and water (50:50, v:v) by sonication in an ultrasonic water bath (SK06GT Kudos ultrasonic water bath, Korea) for 30 min. The temperature of the ultrasonic bath was maintained between 30 and 40 °C by adding ice. The extract was centrifuged at 8,000xg (Hanil Science Industrial Combi 514R, Korea) for 15 min. The supernatant was collected in a different tube. 20 mL of a mixture of ethanol and water (50:50, v:v) or deionized water was added to the pellet and sonicated again. This process was repeated three times, and the three supernatants were combined. The extracts were stored at -80°C until analysis (Meng et al. 2011).

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2.3. Determination of the phytochemical composition of polar and non-polar blueberry extracts

The presence of the 53 phenolic compounds in the extracts was determined using a Shimadzu-Neexera model UHPLC (ultra-high performance liquid chromatograph) combined with a Shimadzu-LCMS 8040 model triple quadrupole mass spectrometer according to the method developed and validated by Yilmaz et al. (2018) and Yilmaz et al. (2020). An analytical column (Inertsil ODS-4 model C18, 100 mm×2,1 mm, 2µm), an autosampler (SIL-30AC model), binary pumps (LC-30 AD model), a degasser (DGU-20A3R model), and a column oven (CTO-10ASvp model) were parts of the liquid chromatography system. Samples were filtered prior to injection, and the final concentrations of the extracts were adjusted to 250 mg/L. The solvent flow rate was set to 0.5 mL/min. The injection volume was 5 mL. The eluent A consisted of water, 5 mM ammonium formate, and 0.1% formic acid; the eluent B consisted of methanol, 5 mM ammonium formate, and 0.1% formic acid. The elution profile was 20–100% B (0–25 min), 100% B (25–35 min), and 20% B (35–45 min)(Yilmaz 2020; Yilmaz et al. 2018). The triterpenoid composition of the extracts was analyzed using an Agilent 7890A gas chromatography and an Agilent 5977B model mass spectrometry system according to the method suggested by Bakir et al. (2020) (Bakir et al. 2020). The concentration of the samples was 1000 mg/L.

2.4. Determination of enzyme inhibitory activities of polar and non-polar blueberry extracts

2.4.1. ACE inhibition assay: The inhibitory activity of the extracts was evaluated according to the method proposed by Kwon et al. (2006), with some modifications. A mixture of 200 µL of NaCl-borate buffer (0.3 M, pH 8.3) containing 2.0 mU of ACE-I solution was added to 50 µL of the extracts (50 mg DW/mL). The mixture was preincubated for 10 minutes at 25 ºC. 100 µL of hippuryl-histidyl-leucine (5.0 mM) solution was added to the reaction mixture and then incubated for an additional hour at 37 ºC. To terminate the reaction, 150 µL of 0.5 N HCl was used. The formation of hippuric acid was monitored by HPLC with a UV detector at 228 nm (Kwon, Vattem, and Shetty 2006). Lisinopril was used as a standard. The following formula was used to calculate the inhibition percentage:

$$\text{Inhibition\%} = [\text{Area}_{\text{control}} - (\text{Area}_{\text{sample}} - \text{Area}_{\text{blank}})]/(\text{Area}_{\text{control}} - \text{Area}_{\text{blank}}) \times 100 \quad (3)$$

2.4.2. Urease inhibition assay: The inhibitory activity of urease was evaluated according to the method described by Hina et al. (2015) (Hina et al. 2015). Urease solution (25 µL) was mixed with extracts (20 µL, 50 mg DW/mL) in a 96-well plate and preincubated at 30 °C for 15 min. Afterward, 40 µL of 100 mM urea was added and further incubated at 30 °C for 15 min. 45 µL of phenol reagent (1%, w/v, phenol and 0.005%, w/v, sodium nitroprusside) and 70 µL of alkali reagent (0.5%, w/v, NaOH and 0.1% NaOCl) were added to the wells, and incubated again at 30 °C for 15 min. The absorbance of the samples was measured at a wavelength of 630 nm. Thiourea was used as a standard.

2.4.3. Elastase inhibition assay: The elastase inhibitory activity of extracts was determined as described in Kraunsoe et al. (1996) with slight modifications (Kraunsoe, Claridge, and Lowe 1996). Shortly, 10 µL of dried fruit extract (50 mg DW/mL) and 20 µL enzyme solution were added to 40 µL tris-Cl buffer (0.1 M, pH:8). The mixture was preincubated for 10 min at 37 °C. As a substrate, 30 µL of N-succinyl-(Ala)3-nitroanilide (1.015 mM) in Tris-Cl (0.1 M, pH:8) was added to the mixture. The solution was incubated at 37 °C for 20 min. The release of p-nitroaniline was monitored by reading the absorbance at 410 nm. Oleanolic acid was used as the standard.

2.4.4. Tyrosinase inhibition assay: The tyrosinase inhibitory activity of the extracts was determined by the method of Hearing and Jiménez (1987) (Hearing and Jiménez 1987).

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2.4.5. Collagenase inhibition assay: The method recommended by Thring et al. (2009) was used to measure the inhibitory activity of the extracts against collagenase (Thring, Hili, and Naughton 2009).

2.4.6. Acetylcholinesterase and butyrylcholinestase inhibition assay: The AChE and BChE inhibition assays were performed according to the method of Ellman (Ellman et al. 1961).

2.5. Determination of the antioxidant capacity and total polyphenols content (TPC)

The antioxidant properties of the extracts were determined using the DPPH free radical scavenger and linoleic acid/beta-carotene bleach tests. Three duplicates of each experiment were conducted. Standard references were butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). The Folin-Ciocalteu method was used to determine the TPC of the extracts. Gallic acid was used as the standard, and the results were expressed as mg GAE/g (Ciniviz and Yildiz 2020).

2.6. Statistical analysis

The statistical differences among the extracts were assessed through ANOVA, a one-way analysis of variance. Mean values were compared using Duncan's multiple comparison test, and significance was determined at $P < 0.05$. The statistical analysis was performed using SPSS statistics software, version 22.00 (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp). Pearson correlation coefficient was calculated for correlation analysis, with significance set at $P < 0.05$. A confidence level of 95% ($P < 0.05$) was used to determine significance. Principal component analysis (PCA) was employed to condense the interactions between numerous variables into a smaller set of fundamental dimensions, aiding in data visualization and interpretation. PCA was applied to correlate data from phenolic profiles and ACE inhibition. The software R package version 4.2.0 was utilized to create PCA plots and conduct analyses using the libraries "factoextra," "FactoMineR," "ggcorrplot," available at https://CRAN.R-project.org/package=here.

3. Results and Discussion

3.1. Phytochemical composition of polar and non-polar blueberry extracts

The polyphenols profile of the blueberry extracts was determined by LC-MS/MS with the use of 53 standard phenolic compounds. A total of 23 phenolic compounds were identified in both extracts. Of these, 17 phenolic compounds were found in the polar extract, and 21 compounds were detected in the non-polar extract. The results are presented in Table 1.

Table 1. Quantitative summary of the phenolic compounds identified in blueberry extracts

Reference Phenolic Compound	M.I. (m/z)[1]	F.I. (m/z)[2]	U[3]	Quantification (mg/100 g DW)	
Simple Phenols					
Phenolic acids				**Polar**	**Non-polar**
Hydroxycinnamic acids					
Caffeic acid	179.0	134.0	0.0354	0.066	2.474
Chlorogenic acid	353.0	85.0	0.0213	10.752	7.492
p-Coumaric acid	163.0	93.0	0.0516	ND	0.076
Ferulic acid	192.8	149.0	0.0181	ND	3.152
Quinic acid	190.8	93.0	0.0082	330.61	57.792
Hydroxybenzoic acids					
Gallic acid	168.8	79.0	0.0282	0.178	2.21
Protocatechuic acid	152.8	108.0	0.0411	0.42	3.586
Syringic acid	196.9	182.2-167.3	0.0238	ND	1.412
Polyphenols					
Flavonoids					
Flavonols					

Astragalin	447.0	255.0	0.0114	0.380	0.032
Kaempferol	285.0	239.0	0.0209	ND	0.558
Nicotiflorin	592.9	255.0/284.0	0.0276	1.116	0.578
Rutin	608.9	301.0	0.0159	1.208	0.386
Quercetin	301.0	272.9	0.0543	0.100	5.808
Quercitrin	447.0	301.0	0.0268	0.986	ND
Isoquercitrin	463.0	271.0	0.0220	3.122	0.048
Flavanones					
Hesperidin	611.2	449.0	0.0262	1.208	0.468
Hesperetin	301.0	136.0/286.0	0.0562	ND	0.452
Naringenin	270.9	119.0	0.0521	0.004	0.030
Non-Flavonoids					
Tannins					
Tannic acid	182.8	78.0	0.019	ND	5.882
Hydroxybenzaldehydes					
Protocatechuic aldehyde	137.2	92.0	0.0396	0.060	0.368
Syringic aldehyde	181.0	151.1	0.0215	0.042	0.052
Organic acids					
Aconitic acid	172.8	129.0	0.0247	0.024	ND
Fumaric acid	115.2	40.9	0.0124	1.682	1.000
Rutin-D3-IS[d]	612.2	304.1	ND	IS	IS
Ferulic acid-D3-IS[d]	196.2	152.1	ND	IS	IS
Quercetin-D3-IS[d]	304.0	275.9	ND	IS	IS

[1]MI (m/z): Molecular ions of the standard analytes (m/z ratio). [2] FI (m/z): Fragment ions. [3] U (%): percent relative uncertainty at 95 % confidence level (k = 2). ND: Not determined. IS: Internal standard.
Epigallocatechin, epigallocatechin gallate, epicatechin, epicatechin gallate, catechin, gentisic acid, 1,5-dicaffeoylquinic acid, 4-hydroxybenzoic acid, vanilic acid, vanillin, daidzein, daidzin, piceid, sinapic acid, coumarin, salicilic acid, cynaroside, miquelianin, *o*-coumaric acid, genistein, genistin, rosmarinic acid, ellagic acid, fisetin, luteolin, apigenin, amentoflavone, chrysin, and acacetin were not detected either of the extracts.

When the phenolic acid content of the fruit extracts was compared (Fig. 1), the diversity of phenolic compounds in the non-polar extract was found to be more than that of the polar extract. However, the polar extract was found to have higher concentrations of quinic acid (330.61 mg/100 g DW) and chlorogenic acid (10.752 mg/100 g DW) than the non-polar extract. In the present study, ten flavonoids were detected in the fruit extracts. Seven of these flavonoids were found in both extracts. Whilst quercitrin was detected only in the polar extract, kaemferol, and hesperetin were detected only in the non-polar extract. The dominant species in the polar extract was isoquercitrin (3.122 mg/100 g DW), whereas in the non-polar extract it was quercetin (5.808 mg/100 g DW). Among tannins, tannic acid was determined only in the non-polar extract at a concentration of 5.882 mg/100 g DW. Among the organic acids, fumaric acid was the most dominant species detected in the polar and non-polar extracts of the fruit, and its concentration was 1.682 mg/100 g DW and 1.00 mg/100 g DW, respectively. The results showed that blueberries are a rich source of phenolics, especially chlorogenic acid and its derivative quinic acid. They have multiple bioactivities such as anti-inflammatory, antioxidant, anti-diabetic, antimicrobial, and anticancerogenic activities (Aree 2019).

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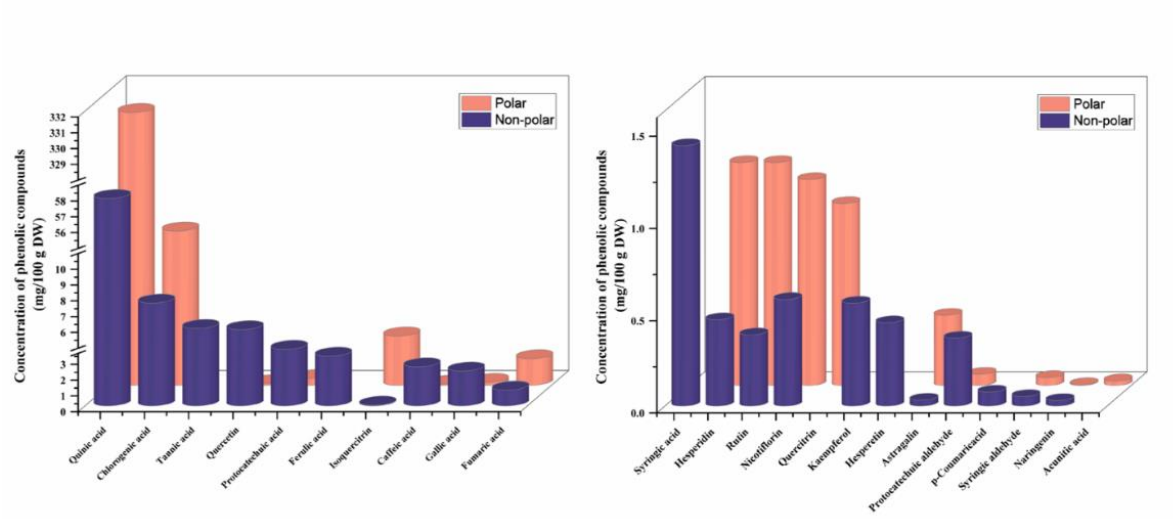

Figure 1. Order of phenolic compounds detected in blueberry extracts from most to least abundant

None of the 7 triterpenoids screened (α-amyrin, betulinic acid, moronic acid, oleanolic acid, oleanonic acid, ursolic acid, and ursonic acid) were detected in either polar or non-polar extracts.

3.2. Enzyme inhibitory activities of polar and non-polar blueberry extracts

In the present study, the inhibitory activities of polar and non-polar blueberry extracts was tested against seven different enzymes. While promising inhibitory activity was observed against ACE, elastase, and urease, no activity was obtained against AChE, BChE, tyrosinase, and collagenase (Fig 2).

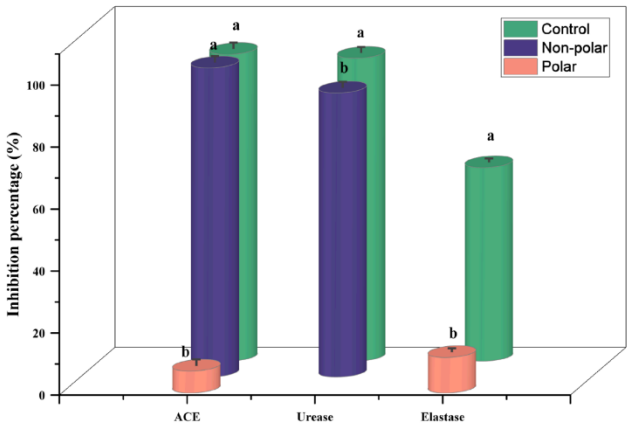

Fig 2. Enzyme inhibitory activities of blueberry extracts. Data are presented as mean ± standard error. The following standards were used as controls: lisinopril, ACE; thiourea, urease; oleanolic acid, elastase. Different lowercase letters denote a significant difference between each enzyme group ($P < .001$)

The highest enzyme inhibition activity was obtained by the non-polar extract against the ACE enzyme. The inhibition activity of the non-polar extract (99.75%) was higher than that of the standard (98.99%), lisinopril, a commercial ACE inhibitor. On the other hand, the polar extract exhibited very low ACE

inhibitory activity (7.04%). ACE is a Zn-dependent peptidase that converts inactive angiotensin I into angiotensin II, constricting blood vessels and increasing blood pressure. Therefore, inhibition of ACE is a key therapeutic target in the treatment of hypertension, the leading risk factor for more than 10 million deaths worldwide. Although commercially available ACE inhibitors such as captopril, enalapril, lisinopril, and lisinopril are used to treat hypertension, long-term use of these drugs is associated with side effects such as extremely low blood pressure, cough, bad taste, and allergic reactions. Therefore, there is a need to find safe and easily accessible natural antihypertensive agents with reduced adverse effects (Memarpoor-Yazdi et al. 2020; Paiva et al. 2023). Blueberries could be an alternative source of natural ACE inhibitors due to their high inhibitory activity, low cost, and easy availability.

The non-polar blueberry extract (99.75%) also showed significant urease inhibitory activity (91.66%), almost as high as the standard (97.74%), thiourea, a commercial urease inhibitor. No activity was observed for the polar blueberry extract. Urease, an Ni^{2+}-dependent enzyme, is a type of hydrolase that accelerates the rate of hydrolysis of urea to ammonia and carbamic acid. Urea adversely affects on agriculture and human health as it is a virulence factor in many pathogenic processes. It has also been reported to cause kidney stones, pyelonephritis, and peptic ulcers. Due to all these effects of urease, urease inhibitors with high stability and low toxicity might provide a practical therapeutic approach against urease-induced diseases (Valentová et al. 2023).

The polar extract of blueberry exhibited elastase inhibitory activity with a value of 11.45%. The inhibitory activity of oleanolic acid, a commercially available elastase inhibitor, was found to be 62.45%. The breakdown of elastin protein is catalyzed by elastase, which cleaves peptide bonds at the carboxyl-terminal end of amino acids containing an alkyl side chain. Loss of skin elasticity due to elastase hyperactivity can lead to the formation of wrinkles (Senol Deniz, Orhan, and Duman 2021).

3.3. Antioxidant activities and total polyphenol content (TPC) of the dried fruit extracts

The results of the antioxidant activities and TPC of the extracts are shown in Fig. 3. The antioxidant activity of the non-polar extracts measured by the DPPH radical scavenging method was much higher than that of the polar extracts. This difference observed between the extracts could be due to the nature of the polyphenols released into the non-polar solvent during extraction. In addition, the activity of the non-polar extract may also be due to other phenolic compounds that cannot be screened. The TPC of the non-polar extract (42.13 ± 0.74 mg GAE/g) was higher than that of the polar extract (26.13 ± 1.04 mg GAE/g) ($P < 0.05$). The effects of solvent polarity on TPC showed similar results to its effect on the antioxidant activity of the extracts.

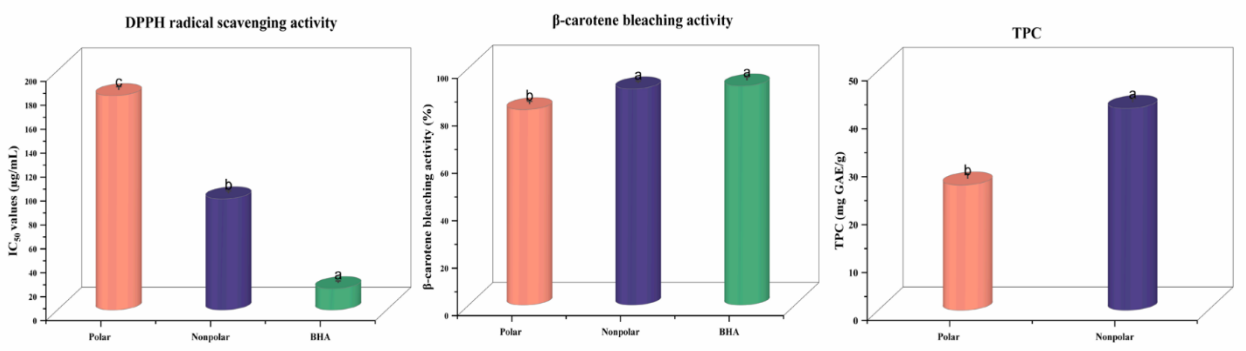

Fig 3. The antioxidant capacities and TPC of blueberry extracts. Data are presented as mean ± standard error. Different lowercase letters denote a significant difference between each group ($P < .001$)

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3.4. Correlation analysis

To highlight the possible correlations between individual phenolic compounds and the enzyme inhibitory activities of the extracts, Pearson's correlation analysis was used (Fig. 4A). The direction of the correlation is either positive (a positive relation) or negative (a negative relation) (Fig. 5). Of the twenty-three phenolic compounds, nineteen showed either a positive or negative correlation with the inhibition of ACE, urease, and elastase. Aconitic acid, syringic aldehyde, *p*-coumaric acid and naringenin showed no correlation with target enzymes. While ten of the individual phenolic compounds showed a positive linear relationship with ACE and urease inhibitory activities, nine of them showed a positive linear relationship with elastase inhibitory activity.

PCA was used to plot the contribution of the variables to the PCs in order to assess the relationship between the individual phenolics and the enzyme inhibitory activities (Fig. 4B). The first principal component (Dim 1) explained 89.1% and the second principal component (Dim 2) explained 8.8% of the total variance. Thus, the PCs accounted for 100% of the variation. The angle between the vectors representing the original variables is used to estimate the degree to which these variables are correlated. Small angles indicate positive correlations, and angles approaching 180 degrees indicate negative correlations. For example, as shown in the PCA plot, ten of the phenolics (protocatechuic aldehyde, hesperetin, caffeic acid, kaempferol, protocatechuic acid, gallic acid, quercetin, syringic acid, ferulic acid, and tannic acid) are grouped on the right side of the PCA plot, indicating positive correlations with ACE and urease inhibition. On the other hand, nine of the phenolics (nicotiflorin, astragalin, hesperidin, rutin, quercitrin, fumaric acid, isoquercitrin, quinic acid, and chlorogenic acid) grouped on the left side of the PCA plot represent the phenolics responsible for elastase inhibition. These data overlapped with Pearson correlation results, indicating that these phenolics could be important parameters for functional food or pharmaceutical production according to their ACE and urease inhibition activities.

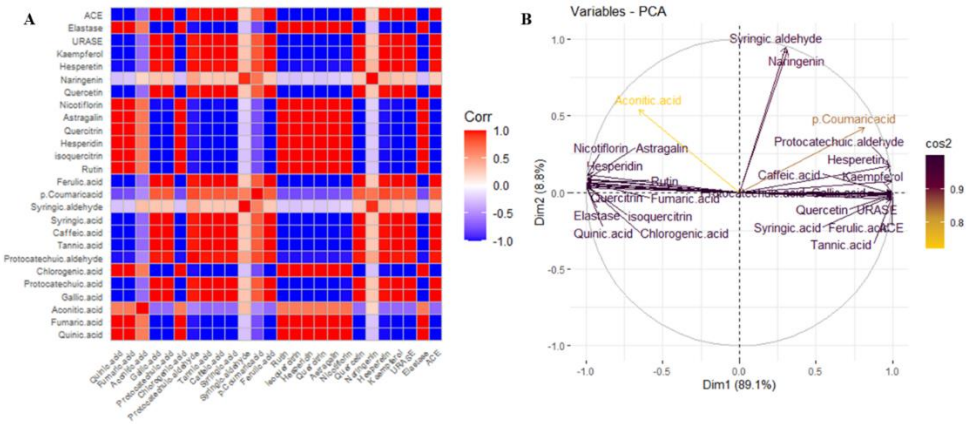

Figure 4. Correlation analysis of the phenolic compounds and enzyme inhibitory activities of blueberry extracts. (A) Pearson correlation: Higher to lower correlation levels are indicated by the intensity of the red and blue colors. A perfect positive correlation is indicated by a correlation coefficient of +1. (B) Principal component analysis: A biplot generated from data used to represent the phenolic composition of different extracts in conjunction with enzyme inhibitory activities.

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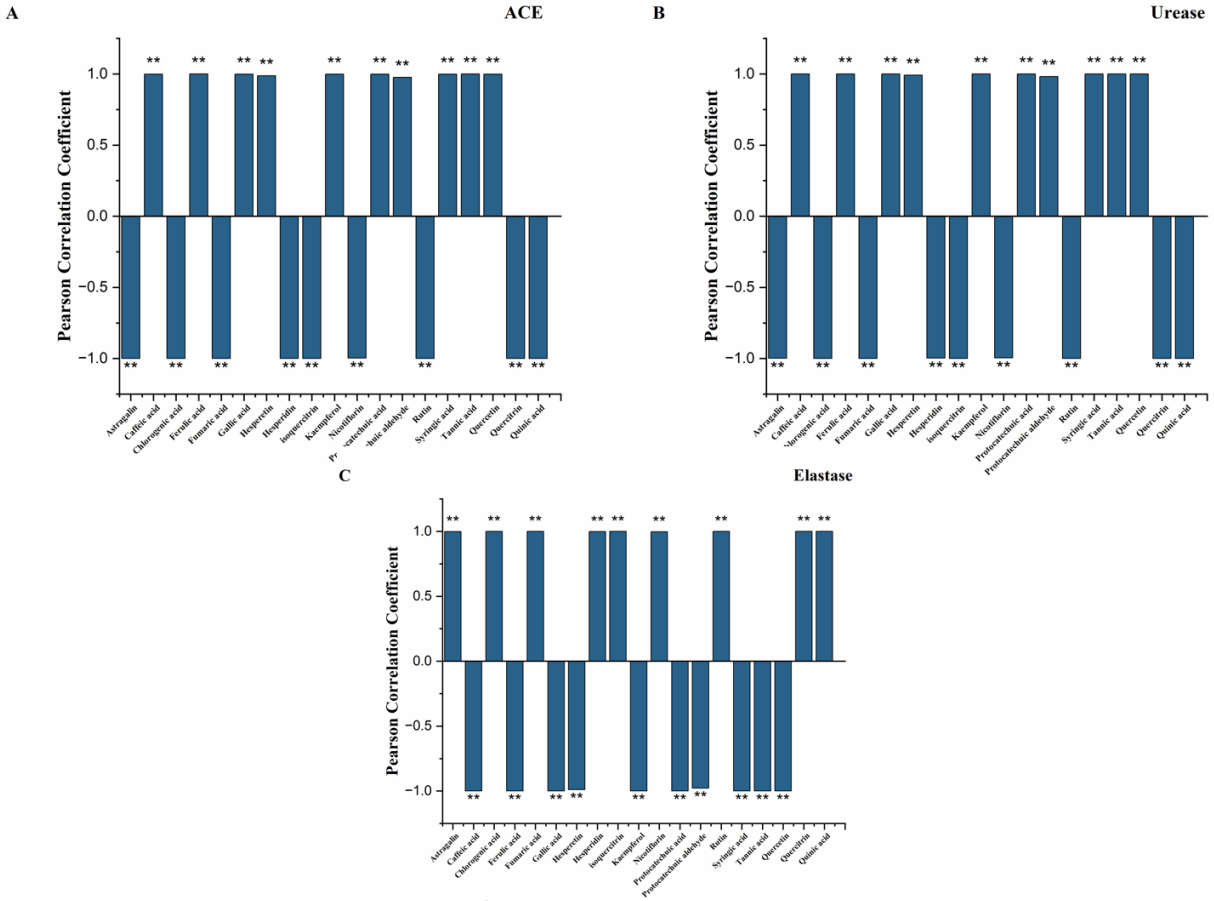

Figure 5. Bar graph showing the direction of the Pearson correlation between each phenolic compound and enzyme. (**) indicates *p* values less than 0.001.

4. Conclusion

The present study evaluated the phenolic profile, antioxidant capacity, and enzyme inhibitory activity of polar and non-polar extracts from blueberries and identified the primary component responsible for the enzyme inhibitory activity by correlation analysis. The findings showed that the polarity of the solvent used in the extraction process was an effective factor in determining the phytochemical content of the extracts and, therefore, their bioactivity. The present study might be useful in the design of emerging studies to be planned for functional food and pharmaceutical applications.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to have influence the work reported in this paper.

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ANTIFUNGAL, ANTIOXIDANT ACTIVITIES AND CHEMICAL CHARACTERIZATION OF BROWN ALGAE FROM MOROCCO

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Abstract
The study aimed to investigate, for the first time, the antifungal and antioxidant properties of methanolic extracts and volatile compounds derived from four brown algae: *Bifurcaria bifurcata*, *Cystoseira humilis*, *Ericaria selaginoides*, and *Ericaria mediterranea*.
The volatile compounds and methanolic extracts of the studied species showed strong antifungal activity, with a more pronounced inhibition of *B. cinerea*, where inhibition zones of 18.7 ± 0.10 and 18.4 ± 0.08 mm were recorded respectively by the volatile compounds of *C. humilis* and *E. mediterranea*. Meanwhile, inhibition zones of 14.8 ± 0.01 and 15.20 ± 0.08 mm were recorded for the methanolic extracts, respectively, for *E. selaginoides* and *E. mediterranea*. The antioxidant activity was observed across all species, particularly in the volatile compounds of *E. selaginoides* that exhibit the highest ability to scavenge the DPPH free radical with an IC50 of 0.471 ± 0.29 mg/ml. The analysis of methanolic extracts using HPLC-MS revealed 9 phenolic compounds. In C. humilis and E. selaginoides, we identified 5 compounds dominated by Deoxyschisandrin (40.67%) and Fucophlorethol (26.44%), respectively. Additionally, GC-MS analysis of methanolic extracts detected 22 volatile compounds, with *E. selaginoides* exhibiting 13 compounds primarily composed of eucalyptol (40.49%) and Bornéol (11.93%). We identified 65 volatile compounds in the seven algae species by CPG-MS analysis, dominated by aromatic hydrocarbons such as Hydroxyeremophilone, carboxylic acids (palmitoleic, palmitic acid, lauric, oleic acids, Bis(2-ethylhexyl) isophthalate, …), terpenes (Linalol, p-Cymene, Alpha-Cadinol, Estafiatin…), and phenols (Durohydroquinone, Alpha-cyclocostunolide Phenol, 2,4-bis-(1,1-dimethyl ethyl), TMS…). Since volatile algae compounds are an inexpensive resource with antifungal solid and moderate antioxidant capacity, their use during postharvest opens a new way of biological control.

Key Words: Volatile compounds; Antifungal activity; Antioxidant activity, Brown algae, Biochemical analysis

1. Introduction

The absence of plant health monitoring can significantly impact field and warehouse product production and quality. As a result, disease control has relied heavily on the extensive use of pesticides for many years. However, these chemicals have exhibited toxicity towards pathogens, plants, and consumers [1,2]. This imperative has spurred researchers to investigate innovative alternative methods, such as harnessing bioactive natural compounds derived from algae. These compounds have the potential to either inhibit phytopathogen growth or induce plant defense mechanisms within plant tissues [3,4].
The Atlantic coast boasts an undeniable wealth of diversity and abundance of macroalgae. Many of these algae are being studied to extract new bioactive compounds with promising applications in various fields, including biological activities such as antibacterial, antifungal, and antioxidant properties [5,6]. Numerous research efforts have been conducted to assess the antimicrobial properties of plant extracts containing different classes of phenolic compounds, which have been widely used as alternative post-harvest control measures due to their strong fungicidal properties [7,8]. Additionally, several other

studies have demonstrated the antimicrobial effectiveness of flavonoids, esters, alkaloids, aldehydes, alkenes, alkanes, and amides [1,9].

Many polyphenolic compounds, such as catechins, flavanols, and flavonoid glycosides, have been discovered in methanolic extracts of red and brown algae [10,11,12]. The volatile compounds found in plants and algae are renowned for their antimicrobial properties [1,13,14]. Despite their lipid content constituting a relatively small percentage (0.3 to 6% of dry weight) [15], they have garnered significant interest among known functional compounds in algae. Interestingly, their levels of polyunsaturated fatty acids may surpass those found in terrestrial plants [16,17].
The extraction of volatile compounds from macroalgae serves to shield them from herbivore assaults and counteract neighboring plant threats. Additionally, these compounds demonstrate antimicrobial properties that safeguard algae against pathogens or alleviate oxidative stress [9,18,19]. Halogenated compounds, aldehydes, alcohols, ketones, hydrocarbons, esters, and terpenes are frequently found in algae [20]. Among various algae groups, brown algae stand out for their highest antioxidant potential, harboring compounds absent in terrestrial plant sources. Several compounds isolated from algae and possessing antioxidant effects belong to the phenolic fraction [16].

In Morocco, scientific investigations have revealed the antimicrobial properties exhibited by algae. However, studies are needed to delve into their potential antifungal and antioxidant capacities, particularly concerning crop diseases encountered post-harvest [10,21]. The present study scrutinized antioxidant and antifungal efficacy against two post-harvest phytopathogens, *B. cinerea* and *P. digitatum*, using organic extracts and volatile compounds sourced from four distinct species of brown algae for the first time. Moreover, the study aims to identify the specific molecules possibly responsible for these observed activities.

2. Materials and Methods

2.1. Sample Preparation and Chromatography Analysis

The algae samples were obtained from the subtidal region of El Jadida city on the Moroccan Atlantic coast (33°-33°16’09’’N, 8°30’-8°45’W) in the Spring of 2019. We collected four species of brown algae: *Bifurcaria bifurcata*, *Cystoseira humilis*, *Ericaria selaginoides*, and *Ericaria mediterranea*. We stored the samples under 4°C and then transported them to the laboratory after collection. Upon arrival, we identified the species based on their morphological and histological characteristics by the Biodiversity and Conservation Research Group at IU-ECOAQUA, University of Las Palmas de Gran Canaria, and the Laboratory of Hydrobiology, Ecotoxicology, Sanitation, and Global Changes (LHEAC-URAC33) at Cadi Ayyad University. The algae were thoroughly rinsed with seawater, followed by tap water to remove extraneous particles and epiphytic organisms. Subsequently, the fresh biomass was freeze-dried and ground into a powder using a mechanical grinder.

Preparation of methanolic extract: We dissolved 20 g of the dried and ground algae samples in 500 mL of methanol and extracted them by maceration for four days at room temperature with continuous stirring. We filtered the material over Millipore filters at 0.45 µm and evaporated under reduced pressure to obtain the crude extracts. We weighed the seaweed extracts and stored them at -4°C in the dark until further use.
Analysis of methanolic extracts using high-pressure liquid chromatography coupled with mass spectrometry (HPLC-MS): The methanolic extracts underwent HPLC-MS analysis following the protocol described by Puigventós et al. [22]. We conducted this analysis using an Ultimate 3000 system (Dionex, CA, USA) equipped with a quaternary pump (HPG 3400 RS), an autosampler (WPS 3000

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TSL), and a column oven (TCC 3000). We used a Kinetex C18 reverse-phase column (250 × 4.6 mm, 2.6 µm particles) supplied by Thermo Fisher Scientific (CA, USA). We established the gradient separation using solvent A (0.1% aqueous formic acid solution) and solvent B (methanol) as follows: 0 to 3 min, linear gradient from 5 to 25% B; 3–6 min, 25% B; 6–9 min, 25 to 37% B; 9–13 min, 37% B; 13–18 min, 37 to 54% B; 18–22 min, 54% B; 22–26 min, 54 to 95% B; 26–29 min, 95% B; from 29 to 29.15 min, then returned to initial conditions with 5% B; and from 29.15 to 36 min, 5% B. We adjusted the flow rate of the mobile phase to 1 ml/min. UV-Vis spectral data for all peaks were collected in the 200 to 400 nm range, while chromatographic profiles were recorded at 280 nm. The mass spectrometer was a triple quadrupole TSQ Endura (Thermo Fisher Scientific, CA, USA) equipped with a heated electrospray ionization (H-ESI) source in negative ion mode; nitrogen (> 99.98% purity) was used as the sheath gas, with collision gas and auxiliary gas flows set at 65, 0, and 40 arbitrary units (u.a.), respectively. The H-ESI vaporizer and ion transfer tube temperatures were set at 350 °C. The electrospray voltage was set to -2.5 kV. Full scan MS acquisition mode (m/z 50-1000) in Q1 (mass resolution of 0.7 m/z full width at half maximum with a scan time of 0.5 s) was primarily employed for characterization and assessment. We identified the volatile compounds by comparing the obtained spectra with those of commercial standard compounds and with published data.

Analysis of methanolic extracts using Gas Chromatography- Mass Spectrometry (GC-MS): The organic compounds in the methanolic extracts were also determined using a gas chromatography-mass spectrometry (GC-MS) system (Fisons Instruments, model 8000) with a scanning range of m/z 40–300 under modified conditions described by Saravanakumar et al. [23]. We equipped the system with a non-polar capillary column (Hewlett-Packard OV-17, 30 m × 0.25 mm, film thickness: 0.25 µm) and a configured FID detector. We programmed the column temperature from 45–175 °C at a 3 °C/min rate, then at 15 °C/min to 300 °C. Subsequently, it was held isothermal for 10 min. The injector and transfer line temperatures were set at 280°C. We diluted a one µl injection volume of each sample in n-hexane. The ionization source temperature was 280°C. The carrier gas (helium) flow rate was adjusted to 30 cm/s, and the ionization energy to 70 eV. We identified the compound by comparison of the spectra using the NIST17 library (National Institute of Standards and Technology mass spectral library).

2.2. Preparation of Volatile Compounds and Chromatography Analysis

Seaweed powder (100 g) was subjected to steam distillation using a steam distillation apparatus for about 3 h until total oil recovery. We performed the extraction three times (3 × 100 g), and the oils were separated and dried with anhydrous sodium sulfate, then stored at 4ºC until use (Ozel and Kaymaz, 2004).

Analysis of volatile compounds using Gas Chromatography- Mass Spectrometry (GC-MS):

We analyzed volatile compounds of seaweed using GC-MS in triplicate (Fisons Instruments, model 8000) following Falcão et al. [24] protocol. The system had a non-polar capillary column Hewlett-Packard OV-17 (30 m × 0.25 mm and film thickness of 0.25 µm) and a set FID detector. We programmed the oven temperature, 45–175 °C, at 3 °C min−1, and at 15 °C min−1 up to 300 °C, then held isothermal for 10 min; injector temperature, 280 °C and the injection volume of 1 µL of each sample diluted in n-hexane. The transfer line temperature was 280 °C; ion source temperature, 220 °C; carrier gas, helium, was adjusted to a linear velocity of 30 cm s−1; split ratio, 1:40; ionization energy, 70 eV; scan range, 40–300 u; scan time, 1s. We carried out the identification of the volatile compounds by comparison of the spectra using the NIST17 library (National Institute of Standard and Technology) and by determining the linear retention index (LRI) based on the retention times of an n-alkanes (C8–C40) mixture analyzed under identical conditions. We performed comparisons with commercial standard compounds and with

published data as much as possible. We quantified compounds as a relative percentage of total volatiles using relative peak area values obtained from total ion current.

2.3. Fungal pathogens and Antifungal Activity Analysis

We isolated Botrytis cinerea, the causal agent of grey mold, from the infected tomato and Penicillium digitatum agent of green mold from Citrus sinensis. We transferred the strains aseptically to Potato Dextrose Agar (PDA) under axenic conditions in Petri plates; the fungal cultures were incubated at 25 ± 1°C for one week. Conidia were collected from two-week-old fungal cultures, adding a few millilitres of distilled sterile water to each petri dish for the antifungal activity test.

Solid medium diffusion method: We spread fungus suspensions (3.8 x 105 spores ml-1) on PDA solid media in Petri plates. Subsequently, we placed Whatman paper disks containing 10 µl of methanolic or essential oil extracted from the algal samples on the media. We tested three concentrations (1, 2, and 3 mg ml-1). Methanol was the negative control, and all tests were conducted in triplicate. Fungal isolate growth was assessed after 3 days of incubation at 25°C. We measured the inhibition zone diameter around the disk (in mm).

Micro-dilution method: determination of the minimum inhibitory concentration (MIC): We prepared a spore suspension of 105 to 106 CFU/ml of B. cinerea and P. digitatum in Sabouraud broth. We prepared the microtiter plates with 96 wells by dispensing 100 µl per well of fungal suspension and 100 µl of each methanolic extract or antibiotic dilution to generate a 2-fold dilution range. We incubated the plates at 25°C for 48 hours. Control wells containing only the microbial suspension or methanol were also tested. We defined the MIC as the lowest concentration that inhibits all visible microbial growth to the naked eye.

2.4. Antioxidant Activity Analysis

DPPH free radical scavenging activity: The free radical scavenging activity of the volatile compounds was measured using a stable free radical DPPH [25]. DPPH solution was prepared in Methanol at the concentration of 4x10-4 M. 100 µL of algal volatile compounds at different concentrations, and 900 µl of freshly prepared DPPH solution were mixed. We incubated the mixture for 30 min in the dark at room temperature. Then, we recorded the absorbance at 517 nm. The inhibition percentage was calculated as below:

$$[1 - (Ai - Ac)/Ac] \times 100$$

Ai: absorbance of extract mixed with DPPH solution; Ac: control absorbance.

FRAP (Ferric reducing ability plasma) assay: We performed the FRAP test according to Khadhri et al. [25]. It depends on the capability of the sample to reduce the ferric iron (Fe^{3+}) present in the potassium ferricyanide complex ($K_3Fe(CN)_6$) to ferrous iron (Fe^{2+}); ferrous iron has an intensive blue-green color. 200 µl of algal volatile compounds at different concentrations was added to 500 µl of phosphate buffer solution (0.2 M, pH 6.6) and 500 µl of potassium ferricyanide solution $K_3Fe(CN)_6$ (1%). We cooled the mixture at room temperature after incubation at 50 °C in a water bath for 20 min. We added 500 µl of trichloroacetic acid (10%), and we did the centrifugation at 3000 rpm for 10 min, we mixed 500 µl of the supernatant with 500 µl of distilled water and 100 µl of iron trichloride $FeCl_3$ (0.1%). We recorded the absorbance at 700 nm after 10 min at room temperature.

2.5. Statistical analysis

We conducted the statistical analysis using one-way ANOVA followed by the Tukey test ($p<0.05$). We carried out the statistical analyses using IBM SPSS Statistics (Version 25).

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3. Results and Discussion

3.1. Analysis of methanolic extracts by HPLC-MS and GC-MS

In this study, HPLC-MS revealed the presence of 9 phenolic compounds, representing 30 to 80% of the identified compounds in the methanolic extracts of algae studied (Table 1). C. humilis and E. selaginoides contain 5 compounds, dominated by deoxyschisandrin (40.67%) and fucophlorethol (26.44%). Exifone is present in all algal species and abundant in E. selaginoides (22.2%). GC-MS analysis highlighted the presence of 22 compounds, most of which are terpenes and fatty acids (Table 2). E. selaginoides contains 13 compounds dominated by eucalyptol (40.49%), borneol (11.93%), α-pinene (10.84%), and camphor (10.34%). E. mediterranea contains 7 compounds, with eucalyptol and α-pinene representing the highest contents at 37.25% and 21.90%, respectively. p-Cresol is presented in 4 algae; B. bifurcata (11.29%) and C. humilis (9.91%). According to the literature, marine algae contain an arsenal of molecules that are the subject of numerous studies, such as polyphenols, terpenoid derivatives, flavonoids, and alkaloids [18,11,12]. Polyphenols, defined as compounds containing one or more aromatic cycles carrying hydroxyl groups, have received considerable attention [27]. It has been demonstrated that polyphenols exhibit antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticancer properties [28] and are classified into phenolic acids, flavonoids, stilbenes, and lignans [29].

Studies have shown the presence of exifone in extracts from several species of brown algae, such as Carpodesmia amentacea, Cystoseira compressa, and Treptacantha ballesterosii. Exifone is an interesting phenolic compound from an ecological standpoint as it reduces environmental pollution levels [30,12]. Caffeoyl tartaric acid was also found in red algae H. musciformi, Grateloupia sp., and Centroceras sp. [31]. Caffeic acid 3-O-glucuronide was also found in the green algae Caulerpa sp. [31]. Phlorotannins, highly complex compounds formed by the polymerization of phloroglucinol [32], including fucophlorethol, are among the phlorotannins recorded in S. polyschides, B. bifurcata, E. selaginoides, and E. mediterranea with high contents.

GC-MS analyses of methanolic extracts revealed the presence of several terpenoid molecules such as camphene, α-myrcene, o-cymene, eucalyptol, camphor, and borneol. These terpenes are brown algae's most predominant class of compounds [29,33]. Thymol is among marine algae's most commonly identified volatile phenolic compounds [34].

Table 1. HPLC-MS analysis of methanolic extracts from the studied algae

N°	Compounds identified	(M-H)- m/z	Uv max	TR	Area %				References
					Bb.	Ch.	Es.	Em.	
	Exifone	277	210, 323	3,37	-	4,41	22,2	20,11	[12]
	20 -Hydroxyenterolactone	314	210, 270	4,33	-	12,01	-	-	[31]
	Deoxyschisandrin	413,2	210, 260	5,5	-	40,67	-	-	[31]
	Fucophlorethol	373	274	6,06	49,57	-	26,44	10,20	[31]
	Caffeic acid 3-O-glucuronide	357,4	210, 270	6,46	4,8	-	3,6	-	[31]
	Rosmanol	345,1	-	11,44	-	12,76	-	-	[31]
	Octaphlorethol A	993,4	-	13,2	21,79	-	-	-	[31]
	Isopeonidin 3-O-arabinoside	432,9	280	26,6	4,50	4,97	6,31	2,02	[31]
	Isopeonidin 3-O-arabinoside isomer 1	432,9	280	27,5	-	-	7,97	5,40	[31]

Bb. : *B. bifurcata* ; Ch. : *C. humilis* ; Es. : *E. selaginoides* ; Em. : *E. Mediterranea*

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Table 2. GC-MS analysis of methanolic extracts from the studied algae. The components were identified by comparing the observed MS spectra with those in the NIST database

N°	Compounds identified	TR	Area %			
			Bb.	Ch.	Es.	Em.
1	α-pinène[Tr]	4,64	-	-	10,84	21,90
2	Camphene[Tr]	4,81	-	-	6,39	-
3	α-Myrcene[Tr]	5,16	-	-	1,42	-
4	o- Cymene[Tr]	5,52	-	-	1,70	-
5	Eucalyptol[Tr]	5,60	-	8,47	40,49	37,25
6	Linalool[Tr]	6,47	-	-	0,46	-
7	Camphor[Tr]	6,64	-	2,73	10,34	8,08
8	Bornéol[Tr.]	6,83	-	-	11,93	3,24
9	Isoborneol[Tr.]	7,05	-	-	0,29	-
10	Terpineol[Tr]	7,18	-	-	3,49	-
11	Trans-2-isopropyl-bicyclo-[4,3,0]-non-3-ene-8-one	7,32	-	-	-	3,45
12	Thymol[Ph.]	7,75	-	-	1,05	-
13	Caryophyllene	8,75	-	-	1,31	-
14	Doconexent	8,77	-	-	-	0,59
15	Arachidonic acid[Ag.]	8,79	-	-	-	-
16	tetradecamethyl cycloheptasiloxane	9,02	3,93	-	-	0,85
17	β-Vatirenene[Tr.]	9,52	3,49	3,12	-	-
18	Caryophyllene oxide[tr.]	9,81	-	-	0,45	-
19	Glycine, N-[(3alpha,5beta)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-, methyl ester	11,67	2,19	-	-	-
20	p-Cresol	13,80	11,29	9,91	-	-
21	Androstane-11,17-dione, 3-[(trimethylsilyl) oxy]-,17-[O-(phenylmethyl) oxime], (3α,5α)-	14,02	2,66	2,18	-	-
22	Glafenin	16,02		2,59	-	-

Bb. : *B. bifurcata* ; Ch. : *C. humilis* ; Es. : *E. selaginoides* ; Em. : *E. mediterranea*

3.2. Analysis of volatile compounds by GC-MS

In this study, we identified 65 compounds representing more than 80% of the volatile compounds in the four species of algae studied (Table 3). *B. bifurcata* and *E. mediterranea* contain 24 volatile compounds dominated by aromatic hydrocarbons, terpenes, and carboxylic acids. The main constituents of these volatile compounds were aromatic hydrocarbons (Hydroxyeremophilone, 13R,3aR,4aS,5R,9aS) -3,5,8-Trimethyl-3a,4,4a,5,6,7,9,9a -octahydroazuleno [6,5-b]furan-2(3H)-one), carboxylic acids (palmitoleic acid, palmitic acid, lauric acid, oleic acid, Bis(2-ethylhexyl) isophthalate, Bis(2-ethylhexyl) terephthalate…), terpenes (Linalol, p-Cymene, Alpha-Cadinol, Estafiatin…), and phenols (Durohydroquinone, Alpha-cyclocostunolide Phenol, 2,4-bis-(1,1-dimethylethyl), TMS…). The volatile compounds of algae act as asexual pheromones and confer antimicrobial properties to the algae, protecting them against pathogens and or not attenuating oxidative stress [19]. Sulfur or halogenated compounds, aldehydes, alcohols, ketones, hydrocarbon esters, terpenes, phenols, and carboxylic acids are the predominant compounds most frequently found in algae [9,20].

Since the 1990s to the present day, volatile compounds from Japanese brown algae *Dictyopteris proliferate*, *D. divaricata*, *Laminaria japonica*, and *Undaria pinnatifida* have been extensively studied [18,35]. Güven et al. [36] identified many volatile compounds in various classes of macroalgae, such as terpenes, benzaldehydes, phenols, various acids, alcohols, and halogenated compounds. Nowadays,

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many researchers have demonstrated that volatile compounds from cyanobacteria and algae have a variety of applications in medicine as antimicrobial, antioxidant, anti-ulcer, anti-inflammatory, and anti-leukemic agents [9,18,35]. Similarly, the role of these compounds in agriculture has been demonstrated as they present interesting antimicrobial and larvicidal activities [13]. Lastly, synthetic fungicidal benzaldehyde has been found in *Polysiphonia sphaerocarpa* and *Ulva lactuca* [36].

Table 3. GC-MS analysis of volatile compounds from the studied algae. The components were identified by comparing the observed MS spectra with those in the NIST database

N°	Compounds identified	LRI [a]	LRI [b]	TR	Area %			
					Bb.	Ch.	Ct.	Cm.
1	p-Xylene [Ha.]	855	857	6,13	-	-	-	1,53
2	Nonane [Al.]	900	900	7,03	-	1,61	-	4,48
3	2,6-dimethyloctane [Al.]	932	934	8,24	-	-	-	3,09
4	2-Methyl-1-phenyl-2-propanol [Alc.]	1124,6	1140,9	8,49	-	-	-	2,28
5	1-Ethyl-3-methylbenzene [Ha.]	954	955	9,31	-	-	0,69	-
6	1-Ethyl-4-methylbenzene [Ha.]	963	948	9,98	-	-	-	2,37
7	1,2,4-trimethylbenzene [Ha.]	984	978,4	10,5	-	1,72	-	-
8	Decane [Al.]	1000	1000	10,8	1,7	2,49	-	6,82
9	Mesitylene [Ha.]	995	995	11,7	-	1,78	-	9,09
10	1-Methyl-4-propylbenzene [Ha.]	1042	1046	12,9	-	-	-	4,05
11	2-Ethyl-p-xylene [Ha.]	1060	1062	13,2	-	-	-	2,8-
12	1-Ethyl-2,4-dimethylbenzene [Ha.]	1071	1070	14,1	-	-	-	1,59
13	Undecane [Al.]	1100	1100	15,1	-	-	-	2,64
14	Linalol [Tr.]	1096	1098	15,1	2,45	-	-	-
15	6-Methyl-3,5-heptadien-2-one [Ctn.]	1074	1074,9	15,3	2,3	-	-	-
16	4-caranol [Alc.]	-	-	19,1	1,85	-	-	-
17	Vitispirane [Tr.]	1260	1268	22,5	-	1,21	-	-
18	6-Methoxy-3methylbenzofuran [Ha.]	-	-	26,18	-	-	2,03	-
19	Durohydroquinone [Ph.]	1556,3	1556,3	27,3	-	1,38	9,84	-
20	1-tetradecanol [Alc.]	1664	1647	27,8	1,35	-	-	-
21	Widdrenal [Ha.]	1724,2	1724,2	29,98	-	-	0,55	-
22	Alpha -cyclocostunolide [Tr.]	-	-	30,9	-	9,68	0,91	-
23	2-[acetylmethyl]-3 -carene [Tr.]	1344	1380	31,6	2,67	-	-	-
24	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, semicarbazone [Ha.]	-	-	31,7	7,16	6,98	-	2,97
25	Hydroxyeremophilone [Ha.]	1865	1865	32,44	-	-	34,04	-

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2	2,4-Di-tert-butylphenol Ph.	1494	1539	32,8	-	1,49	-	1,86
2	Phenol, 2,4-bis-(1,1-dimethylethyl), TMS Ph.	1540	1540	33	1,57	-	-	-
2	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl- Ha.	1698	1698	33,5	-	3,38	0,77	1,79
2	Valeric acid, tridec-2-ynyl ester Ca	2010,7	2010,7	34,3	1,18	-	-	-
3	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester Ac.	1752	1752	34,9	1,37	-	-	-
3	Lauric acid Ac.	1561	1560	35,42	-	2,86	0,76	-
3	Pentafluoropropionic acid, hexadecyl ester Ac.	1859	1859	35,8	2,64	-	-	-
3	[5,5-Dimethyl-6-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]hept-1-yl]-methanol Ha.	-	-	36,2	-	2,12	-	-
3	3-Methylbut-2-enoic acid, 4-nitrophenyl ester Ha.	1778	1778	36,7	6,86	-	-	-
3	Neoisolongifolane, hydroxy- Ha.	1621	1621	37	-	-	-	1,45
3	Acetate, (2,5,5,8a-tetramethyl-3,4,4a,5,6,7,8,8a-octahydro-1-naphthalenyl) ester Ha.	1293,4	1293,4	37	-	1,37	-	-
3	Alpha-Cadinol Tr.	1627	1635	38,1	-	1,23	-	-
3	Phthalic acid, 2-ethylbutyl isobutyl ester Ac.	2066	2066	38,26	-	1,29	6,86	-
3	Caryophyllene oxide Tr.	1581	1575	38,6	2,19	-	-	-
4	(3R,3aR,4aS,5R,9aS)-3,5,8-Trimethyl-3a,4,4a,5,6,7,9,9a-octahydroazuleno[6,5-b]furan-2(3H)-one Ha.	1899	1899	39,40	-	-	11,98	-
4	Myristic acid Ac.	1748	1752	42	2,88	10,76	-	5,44
4	Estafiatin Tr.	-	-	44,3	-	1,26	-	-
4	2-pentadecanone Ctn.	1682	1671	44,5	-	1,49	-	1,42
4	Benzeneethanol, 3-(trifluoromethyl)- Ha.	1151	1151	44,5	-	1,29	-	-
4	Reynosin Tr.	2266,1	2266,1	45,04	-	-	2,81	-
4	Cis-lanceol Alc.	1583	1583	46,2	5,64	-	-	-
4	Palmitoleic acid Ac.	1948	1953	46,2	-	8,13	-	2,47
4	Zotepine Am.	-	-	46,49	-	-	10,69	-
4	Palmitic acid Ac.	1968	1959	46,5	2,82	12,9	-	6,64
5	12-Hydroxy-5,8,10-heptadecatrienoic acid Ac.	-	-	47,06	-	-	2,24	-
5	1,5,9-Cyclododecatriene, 1,5,9-trimethyl- Ha.	-	-	47,3	8,02	-	-	-
5	1-Heneicosyl formate Ca	-	-	47,9	-	1,96	-	-
5	Azuleno[4,5-b]furan-2(3h)-one Tr.	-	-	48,18	1,7	-	1,29	-
5	Oleic acid Ac.	2141	2142	48,3	-	4,84	-	5,47

5	1-Monolinolenoyl-rac-glycerol Ac.	2161	2161	48,47	-	-	1,00	-
5	Tributyl citrate Es.	2150	2150	48,70	-	-	1,15	-
5	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, (S-(E,Z,E,E))- Ha.	1916	1916	49	19,1	-	-	-
5	2H-pyran Pyr.	1775	1775	49,7	2,45	-	-	-
5	(E)-atlantone Tr.	1743	1744	50	11,7	-	-	-
6	Hexanedioic acid, bis(2-ethylhexyl) ester Ac.	2382	2381	50,1	-	3,12	0,90	1,54
6	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-etheny e-10,14-dimethylene-pentadec-4-enyl)cyclohexane Ha.	-	-	50,7	1,18	-	-	-
6	Bis(2-ethylhexyl) phthalate Ac.	2562	2550	51	1,12	-	0,74-	-
6	Phthalic acid, 2-pentyl propyl ester Ac.	2527	2527	51	-	1,25	-	1,44
6	Bis(2-ethylhexyl) isophthalate Ac.	2730	2730	52,1	2,27	-	-	-
6	Bis(2-ethylhexyl) terephthalate Ac.	2509	2509	52,10	-	-	7,48	1,9
6	Decanedioic acid, bis(2-ethylhexyl) ester Ac.	2792	2792	52,3	-	-	-	8,75

Ac.: Carboxylic acids; Al.: Alkanes; Ha.: Aromatic hydrocarbons; Tr.: Terpenes; Ph.: Phenols; Alc.: Alcohols; Am.: Amines; Ene.: Alkenes; Es.: Esters; O.: Organosiloxane

3.3. The antifungal activity

Table 4 presents the results of the antifungal activity of volatile compounds and methanolic extracts from 4 species of brown algae at different concentrations (1, 2, and 3 mg/ml) against *B. cinerea* and *P. digitatum* using the solid medium diffusion method. The volatile compounds and methanolic extracts of the studied species showed strong inhibition of the growth of both fungal species compared to Amphotericin, with a more pronounced inhibition of *B. cinerea*, where inhibition zones of 18.7 ± 0.10 mm and 18.4 ± 0.08 mm were recorded respectively by the volatile compounds of *C. humilis* and *E. mediterranea*. Meanwhile, inhibition zones of 14.8 ± 0.01 mm and 15.20 ± 0.08 mm were recorded for the methanolic extracts, respectively, for *E. selaginoides* and *E. mediterranea*. The results also noted a significant difference between the tested concentrations against the two fungal species primarily. For *P. digitatum*, the strongest inhibition was observed at 3 mg/ml of volatile compounds from *C. humilis*, *E. selaginoides*, and *E. mediterranea* with inhibition zones of 16.4 ± 0.09 mm, 15.3 ± 0.18 mm, and 15.8 ± 0.02 mm, respectively. The table also shows a high MIC for *E. mediterranea*, particularly against *P. digitatum*.

Cette activité antifongique peut être due aux différents groupes de produits chimiques détectés par analyse HPLC-MS et CPG-MS tels que, les acides carboxyliques, les alcanes, les hydrocarbures aromatiques, les terpènes, les phénols, les amines, les cétones et alcools. Les acides gras à activité antimicrobienne sont présents en concentration élevée essentiellement chez *C. humilis* et *E. mediterranea* ce qui explique leurs fortes activités antifongiques. Une hypothèse a été avancée selon laquelle les acides gras tueraient les micro-organismes, à savoir champignons, bactéries et levures, par la perturbation des membranes cellulaires [37]. Les acides palmitique et oléique d'origine végétale sont connus pour leurs activités antibactérienne et antifongique potentielles [38,39]. Dans plusieurs études, l'acide palmitique a été rapporté comme étant le principal composé antimicrobien dans un mélange

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d'acides gras isolés de *L. digitata, L. japonica, U. pinnatifida* et *P. umbilicalis* [1,18,35]. D'autre part, le rôle des composés phénoliques pourrait être important dans la réduction de la croissance fongique. Les hydrocarbures aromatiques possèdent un potentiel antifongique. En effet, les p-xylène et o-xylène ont été détectés chez l'algue verte *Capsosiphon fulvescens* [20]. Les terpènes continuent d'être la classe prédominante de composés chimiques des algues brunes [40]. Les résultats de l'analyse CPG-MS ont montré aussi la présence des sesquiterpènes tels que le linalol et le p-cymène. Ces produits ont été détectés chez les plantes et les algues et ils ont montré une forte activité antimicrobienne. Plusieurs études ont mentionné l'activité antifongique des terpènes et des phénols [40].

Table 4. Antifungal activity expressed by the diameter of the inhibition zone in mm of volatile compounds and methanolic extracts of the studied algae against the fungi *B. cinerea* and *P. digitatum.*

Espèce d'algue	Fungal species	Volatile compounds				Methanolic extracts			
		Concentrations testées mg/ml			MIC (µg/ml)	Tested concentrations mg/ml			MIC (µg/ml)
		1	2	3		1	2	3	
B. bifurcata	*B. cinerea*	8,90 ±0,05^{a,A}	11,5 ± 0,13^{a,A,B}	13,9 ± 0,08^{a,B}	6,25 ± 0,00	13,00±0,09^{a,A}	13,80±0,03^{a,A,B}	13,90±0,08^{a,B}	12,5± ,00
	P. digitatum	2,3 ± 0,05^{a,A}	3,9 ± 0,10^{a,A,B}	7,6 ± 0,09 ^{a,B}	50 ± 0,00	7,2 ± 0,05^{a,A}	8,20 ± 0,10^{a,A,B}	8,60 ± 0,12^{a,B}	25 ± 0,00
C. humilis	*B. cinerea*	14,8 ±0,06^{b,A}	17,4 ± 0,05^{b,A,B}	18,7 ± 0,10^{b,B}	1,56 ± 0,00	12,80±0,06^{b,A}	13,00±0,05^{b,A,B}	13,7 ±0,10^{b,B}	12,5± ,00
	P. digitatum	16,1 ± 0,06^{b}	16,2 ± 0,14^{b,A,B}	16,4 ± 0,09^{b,B}	0,4 ± 0,00	5,40 ± 0,06^{b}	7,79 ± 0,04^{b,A,B}	8,20 ±0,10^{b,B}	25± 0,00
E. selaginoides	*B. cinerea*	2,3 ± 0,04^{a,b,A}	6,1 ± 0,09^{a,b,A,B}	23,6 ± 0,11^{a,b,B}	1,56 ± 0,00	11,9± ,04^{a,b,A}	12,7±0,03^{a,b,A,B}	14,8±0,01^{a,b,B}	12,5±0,00
	P. digitatum	12,6±0,10^{a,b,A}	14,9±0,04^{a,b,A,B}	15,3 ± 0,18^{a,b,B}	50 ± 0,00	6,20± 0,6^{a,b,A}	7,70±0,04^{a,b,A,B}	8,70±0,08^{a,b,B}	25 ± 0,00
E. mediterranea	*B. cinerea*	13,9 ±0,08^{b,A}	18,0 ± 0,07^{b,A,B}	18,4 ± 0,08^{b,B}	1,56 ± 0,00	14,00± ,08^{b,A}	14,80±0,07^{b,A,B}	15,20±0,08^{b,B}	12,5±0,00
	P. digitatum	15,2 ±0,12^{b,A}	15,8 ± 0,03^{b,A,B}	15,8 ± 0,02^{b,B}	0,4 ± 0,00	5,80± 0,02^{b,A}	6,90 ± 0,03^{b,A,B}	7,70±0,02 ^{b,B}	25± 0,00
T+ (Nystatin) (1 mg/ml)	*B. cinerea*	24,6 ± 0,05^{b}	-	-	6,25±0,00^{a,B}	-	-	-	-
	P. digitatum	16,4 ± 0,00^{b}	-	-	6,25±0.00^{a,B}	-	-	-	-
T+(Amphotericin B) (1 mg/ml)	*B. cinerea*	2,1 ± 0,00^{a}	-	-	00 ± 00	-	-	-	-
	P. digitatum	2,1 ± 0,00^{a}	-	-	00 ± 00	-	-	-	-

3.4. Antioxidant Activity

The results of our samples' ability to scavenge the DPPH free radical and to reduce ferric iron (Fe3+) to ferrous iron (Fe2+) are shown in Table 5. According to the latter, the volatile compounds of *E. selaginoides* exhibit the highest ability to scavenge the DPPH free radical with an IC50 of 0.471 ± 0.29 mg/ml, followed by the methanolic extract of *B. bifurcata* (1.78 ± 0.05 mg/ml). In comparison, the strong ability to reduce ferric iron to ferrous iron is recorded by the volatile compounds of *C. humilis* with an IC50 of 1.95 ± 0.06 mg/ml respectively.

We have noted a positive correlation between the total content of phenolic compounds and the antioxidant activities. Indeed, previous studies have mentioned that phenolic compounds are the main responsible agents for the excellent antioxidant properties found in algae [41,42]. However, other molecules could be involved in these activities, namely carboxylic acids, terpenes, and phenolic compounds. Numerous authors have highlighted their antioxidant effect [43,44]. Some studies have shown a positive correlation between fatty acids (such as palmitic acid, oleic acid, etc.) and antioxidant activity [45,46]. Several studies have also demonstrated a highly significant correlation between phenolic compounds' content and algae extracts' antioxidant activity. Moreover, some studies have

described the antioxidant activity of certain purified phenolic compounds from *Eisenia bicyclis* and *Sargassum kjellmanianum* [47]. Blasi and Cossignani [48] reported that the antioxidant properties of phenolic compounds are more effective and comparable to synthetic antioxidants.

Table 5. Antioxidant activity expressed in IC50 (mg/ml) of volatile compounds and methanolic extracts of the studied species and ascorbic acid.

	Volatile compounds		**Methanolic extracts**	
Espèce d'algue	**IC50 DPPH (mg/mL)**	**IC50 FRAP (mg/mL)**	**IC50 DPPH (mg/mL)**	**IC50 FRAP (mg/mL)**
B. bifurcata	1,78 ± 0,05	2,02 ± 0,03	44,32 ± 1,53^{d}	31,06±0,48^{c}
C. humilis	4,08 ± 0,09	1,95 ± 0,06	0,556 ± 0,47^{a}	26,14±4,07^{b,c}
E. selaginoides	2,90 ± 0,04	3,26 ± 0,01	0,471 ± 0,29^{a}	21,08±1,67^{a,b}
E. mediterranea	4,58 ± 0,02	4,28 ± 0,02	0,593 ± 0,36^{a}	28,52±1,82^{c}
Acide ascorbique	0,03 ± 0,00	89,80±1,00	-	-

4. Conclusion

The volatile compounds and methanolic extracts obtained from the four macroalgae contain significant amounts of phenols, carboxylic acids, terpenes, aromatic hydrocarbons, and alcohols. These compounds exhibited pronounced inhibitory effects on the growth of two phytopathogenic strains, with strong antioxidant activity, suggesting their potential eco-friendly applications in biological control. It is recognized that the interplay between compounds may lead to synergistic or antagonistic effects, thereby enhancing their antimicrobial and antioxidant properties. Therefore, further investigation into algal compounds' isolation and chemical characterization could enhance their efficacy or lead to the discovery of novel antimicrobial agents. Moreover, in vivo studies are essential to validate the antimicrobial efficacy of macroalgae volatile compounds, potentially offering alternatives to harmful chemical fungicides commonly used in agrochemical practices. In regions like Morocco, where agriculture plays a pivotal role in the economy, and the utilization of seaweed in agriculture remains underexplored, the development of eco-friendly products based on algal volatile compounds could promote organic farming practices and contribute to the establishment of sustainable and environmentally friendly food systems, an international priority in contemporary times

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EFFECT OF HARVEST TIMES ON ESSENTIAL OIL COMPONENTS AND ANTI-MICROBIAL ACTIVITY IN PLANT ORGANS OF FERULA LYCIA BOİSS.

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Abstract
Pharmaceutical active ingredients, which vary in concentration across a range of medicinal plants, serve as potent natural anti-microbial agents in medical applications. These oils were extracted through hydrodistillation from samples that had been air-dried. The extraction yield of the essential oil (Eos), expressed as a percentage of weight to weight (w/w%), varied across different developmental stages, ranking as follows: floral budding stage (1.1%) surpassed immature fruit stage (0.9%), which was followed by both vegetative and flowering stages at an equal yield (0.8%), and finally, the ripening fruit stage (0.5%). The composition of the EOs was determined using Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS). Across the different stages, a total of 27, 38, 41, 28, and 37 components were identified and quantified, respectively. In this study composition, and anti-microbial effects of EOs of Ferula lycia Boiss. root-stem, leaf, flower, flower head samples were evaluated periodically and daily. Major components of the EOs were α-pinene, β-pinene, and limonene. Significant differences in α-pinene, β-pinene, and limonene ratios were determined depending on the phenological periods, plant parts and harvest time. The highest major component rates were detected in camphor obtained from root-stem samples harvested at full flowering period-1 pm, in α-pinene obtained from flower head samples harvested before seed maturation stage, and in borneol obtained from root-stem samples harvested at pre-flowering stage-6 am. A total of 14 new compounds were first detected in F. lycia. When all plant parts are evaluated, it was determined that flower head samples harvested in the evening at seed maturation stage and leaf samples harvested in the evening at post flowering stage showed strong anti-microbial activities against microorganisms. Study results showed that the harvest time of compounds that can be used as anti-microbial agents in the treatment of infectious diseases varies depending on the harvest time.

Keywords: Diurnal variation, microorganisms minimum inhibitory concentration, Umbelliferae

1. Introduction

Phytochemical studies have revealed that coumarins, sesquiterpene lactones, organic acid glycosides, steroidal esters, lignans, and sulfur-containing compounds are the primary constituents of *Ferula* species (Sonigra and Meena, 2020). Among these, sesquiterpene coumarins are the characteristic chemical components. The skeleton of sesquiterpene coumarins (SCs) consists of a sesquiterpene unit and a coumarin framework, commonly connected via an ether C–O–C or C–C bridge (Wang et al., 2023). It is also believed that SCs are responsible for the significant anti-inflammatory effects exhibited by *Ferula* species. Regrettably, in recent years, the endemic Turkish species *F. lycia* has become increasingly endangered due to overharvesting, severe habitat damage, and adverse environmental conditions. The commonly used medicinal part of *F. lycia* is its resin, obtained only by cutting the roots and stems, while the aerial parts are discarded, leading to considerable resource wastage. Therefore, extracting and isolating various natural active components from the aerial parts of *F. lycia* to transform them into valuable resources has great practical importance for environmental conservation and resource utilization of *F. lycia*. As part of the ongoing effort to discover products with biological activity and low

side effects, the aerial parts of *F. lycia* were selected from which 14 previously unidentified new compounds were isolated from the ethanol extract. The genus *Ferula*, belonging to the Apiaceae family, is represented by approximately 180 species, with 9 of them endemic to Turkey. Like other aromatic plants, *Ferula* holds economic significance due to its use in folk medicine, flavor enhancement, organoleptic improvement, and as food preservatives (Mohammadhosseini et al., 2019; Salehi, et al., 2019). Recent studies have indicated that *Ferula* species possess various biological activities, including anti-cancer, anti-diabetic, anti-viral, anti-bacterial, anti-fungal, anti-inflammatory, anti-nociceptive, anti-convulsant, anti-spasmodic, anti-depressant, antioxidant, cytotoxic, phytotoxic, acetylcholinesterase inhibitory, and memory-enhancing properties (Moradzadeh et al., 2017; Upadhyay, 2017; Attarian et al., 2024; Bagheri et al., 2024). Some species of *Ferula* are used by local populations to enhance memory or treat neurological disorders. Recently, a prenylated coumarin discovered in plants of this genus, known as auraptene, has been reported to exhibit neuroprotective and anticholinesterase activities (Dehghan and Habibi, 2024).

EOs are the primary substances used in aromatherapy, obtained from various aromatic parts of plants through methods such as hydrodistillation, steam distillation, or cold pressing. These EOs, along with extracts derived from a range of edible and medicinal plants, herbs, and spices, are potent natural agents with significant biological activity (Açıkgöz, 2019; Açıkgöz, 2020; Ay Batı et al., 2023a; Ay Batı et al., 2023b; Kırgeç et al., 2023). The application of EOs as anti-microbial agents in food systems is increasingly recognized as an intrinsic factor that can enhance food safety and extend shelf life (Khalid et al., 2024; Wang & Su, 2024). However, the chemical composition of EOs and plant extracts, particularly those from medicinal plants, can vary depending on the plant's origin, environmental conditions, and the developmental stage at which the plant materials are collected (Batı Ay et al., 2023c). Research on *F. lycia* is notably scarce. The few studies conducted have analyzed the composition of EO compounds and anti-microbial activity in the aerial parts of the plant (Alkhatib et al., 2010; Kose et al., 2010; Kazemi et al., 2012; Ghafoor et al., 2019). Our literature review did not uncover any studies on the optimal harvest time related to *F. lycia*. Therefore, we have set the objective of this study to determine the most suitable time for harvesting in terms of EO components and to evaluate its anti-microbial activity.

2. 2. Materials and Methods

2.1. Collection of Plant Samples

The aerial parts *of Ferula lycia* Boiss. were gathered from the Hadim region in Konya province, situated at an elevation of 1517 meters. Plant samples, encompassing both root and aerial sections, were collected during various flowering stages—pre-flowering, full-flowering, and post-flowering and at different times of the day: 6 am, 1 pm, and 8 pm. For each collection period, twenty plants were sampled. These samples were then air-dried at ambient temperature in a shaded area with adequate ventilation.

2.2. Extraction of EOs

The EOs were extracted from 100 grams of the dried plant material using a Clevenger apparatus for hydrodistillation over a duration of three hours, following the methodology recommended by Açıkgöz (2019). The distilled EOs were then stored in opaque, airtight containers for subsequent analysis.

2.3. GC-MS Analysis

The EO constituents of *F. lycia* were subjected to analysis using a gas chromatograph-mass spectrometer setup (Thermo Scientific Quantum GC Triple Quadrupole). The fragmentation process was carried out with an electron impact energy of 70 eV, scanning a mass range from 40 to 400 amu. A DB-WAX column (60m x 0.25mm x 0.25µm, Agilent Technologies) was employed for the chromatographic separation of the principal components. Helium was used as the carrier gas. The temperature of the

column was programmed to increase from 50°C to 260°C at a rate of 10°C per minute. An injection volume of 0.5 µL was used with a split ratio of 1:30. The identification of oil components was achieved by comparing their retention indices with those of the (C_8-C_{24}) n-alkane series and cross-referencing with literature. Chemical characterization also involved matching the mass spectra obtained with those in chemical databases, alongside meticulous separation techniques and the use of NIST & Wiley library references (Adams, 2017). Component quantification was performed using peak area normalization without resorting to internal standards.

2.4. Anti-microbial screening

The microorganisms, commercial strain codes, and antibiotics used as positive controls are presented in Table 1. In the disc diffusion assay, the anti-microbial potential of extracts was gauged. Initial cultures of bacteria and fungi were cultivated on Mueller-Hinton and Sabouraud Dextrose Agars, respectively, and incubated at designated temperatures. The main experiment involved saturating sterile discs with the extracts and positioning them on agar plates pre-inoculated with a specific concentration of microorganisms. Following incubation, the extent of microbial growth inhibition was quantified by measuring the zones of inhibition, with established antibiotics serving as reference standards. This entire process was replicated three times for consistency. The microdilution technique was utilized to ascertain the minimum inhibitory concentration (MIC) of plant extracts. The process began by adding the medium to 96-well plates, followed by the extracts, which had been serially diluted. The MIC was determined by incubating bacterial, yeast, and fungal isolates at specific temperatures and identifying the lowest extract concentration that prevented growth. Standard antibiotics were used for comparison, and the McFarland standards guided the preparation of anti-microbial agents. Repetition in triplicate ensured the accuracy of the results.

Table 1. Microorganisms, commercial strain codes and antibiotics used as positive controls

Microorganism species	Antibiotics
Bacillus subtilis ATCC 6059 (gram-positive bacteria)	Ampicillin
Listeria monocytogenes ATCC 7644 (gram-positive bacteria)	Vancomycin
Staphylococcus aureus ATCC 6538 (gram-positive bacteria)	Gentamicin, Penicillin
Streptococcus faecalis ATCC 8043 (gram-positive bacteria)	Ampicillin
Escherichia coli ATCC 25922 (gram-negative bacteria)	Ampicillin
Proteus vulgaris ATCC 13315 (gram-negative bacteria)	Ampicillin
Pseudomonas aeruginosa ATCC 9027 (gram-negative bacteria)	Gentamicin
Aspergillus niger ATCC 16404 (fungi)	Nystatin
Candida albicans ATCC 10231 (fungi)	Nystatin
Saccharomyces cerevisiae ATCC 9763 (yeast)	Nystatin

2.5. Data analysis

The Kolmogorov-Smirnov test was applied to assess the distribution's normality, while the Levene test verified the homogeneity of variances. The one-way ANOVA was conducted to analyze the data, with some datasets undergoing transformation prior to analysis in the SAS-JMP software, version 10. The sample distinctions were articulated using the mean and standard deviation (SD), and the least significant difference (LSD) test was utilized for mean comparisons at a 95% confidence level.

3. Results and Discussion

3.1. The yield and composition of EOs

F. lycia were determined at different harvest times (06:00, 13:00, and 20:00), with results presented in Table 2, categorized by phenological stages (pre-flowering, full flowering, and post-flowering). The EO yields from the plant samples were 0.75%, 0.90%, 0.62%, 0.96%,

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1.24%, 0.76%, 0.80%, 0.93%, and 0.95%. The highest EO yield was observed in samples harvested pre-flowering at 20:00. Previous research has indicated the influence of plant parts and harvest timing on EO yield. Our findings suggest that bioactive compounds are synthesized at varying rates during different physiological growth stages, which may provide protection against micro and macro-organisms, potentially aiding in the genetic transfer to subsequent generations. The composition of EOs from *F. lycia* was analyzed at different stages of flowering (pre-, full-, and post-) and times of day (6 am, 1 pm, and 8 pm), with the chemical profiles detailed in Table 2. Variance analysis revealed notable differences in the EO compositions across various harvesting times and phenological stages. A total of 38 compounds were identified in the EOs, with their overall concentration ranging from 97.30% to 98.10%. The oils were predominantly composed of oxygenated monoterpenes, which varied from 82.30% to 97.20%, while the presence of monoterpene hydrocarbons fluctuated between 0.50% and 9.18%. Comparative studies have indicated a significant presence of oxygenated monoterpenes in the EOs of some *Ferula* species. Oxygenated sesquiterpenes were found in smaller amounts, between 0.33% and 0.79%, and sesquiterpene hydrocarbons ranged from 0.24% to 4.81%. The data showed that the post-flowering stage had the highest levels of monoterpene and sesquiterpene hydrocarbons, whereas the highest levels of oxygenated monoterpene hydrocarbons were observed during the pre-flowering and full bloom stages. Previous studies have reported that sulfur-containing compounds predominate in different *Ferula* species. Moreover, after the results obtained from these studies were subjected to cluster analysis, it is possible to group the compounds characterizing the EOs of *Ferula* species into 4 main groups. 1) monoterpene hydrocarbons consisting of α-pinene (52-69%) as well as β-pinene (36-66%) for the first and second subgroups, respectively, 2) α-terpinyl acetate (73%) and oxygenated monoterpenes including sabinene (20%), verbenone (69%) and ar-curcumene (6%), 3) Organosulfur compounds including 2,3,4-trimethylthiophene (2) (49%) and 2,5-diethylthiophene (6) (28%), 4) monoterpene + sesquiterpene + aliphatic hydrocarbons (fourth cluster) are (Z)-β-ocymene (42%), myrcene (35%), sabinene (75%) and (E)-caryophyllene (16%) (Alkhatib et al., 2010; Kose et al., 2010; Kazemi et al., 2012; Ghafoor et al., 2019).

The EO under examination is distinguished by its exclusive composition of sesquiterpenoids. Notably, the oil extracted from the flowers is predominantly composed of camphor, accounting for 18.3%, and α-pinene, making up 15.3%. These components are also identified as the primary constituents in the essential oils derived from the leaves and flowers of *F. communis* ssp *glauca*, as well as in *F. lycia*, *F. badrakema*, *F. szovitsiana*, *F. ovina*, both the gum and latex as well as the fruits of *F. gummosa*, *F. flabelliloba*, F. stenocarpa, two collections of *F. jaesekheana* (one in May and another in July), and *F. penninervis*. The respective percentages of these key constituents in the essential oils from these species are reported as follows: 11.7%, 24.2%, 59.9%, 10.9%, 8.0%, 5.7%, 18.3%, 10.0%, 48.8%, 9.5%, 30.0%, and 4.7% (Rustaiyan et al., 2001a; Rustaiyan et al., 2001b; Sayyah et al., 2001; Ghannadi and Amree, 2002; Ghannadi et al., 2002; Dehghan et al., 2007; Asili et al., 2009; Maggi et al., 2009; Kose et al., 2010). Research on the volatile compounds of *F. communis* from the Mediterranean region has revealed diverse compositions. For instance, the essential oil from Corsican leaves is predominantly myrcene (53.5%) and aristolene (8.5%), as identified by Ferrari et al. (2005). In contrast, the main constituents of the inflorescence oil from Sardinia are the sesquiterpenes α- and β-gurjunene, which constitute 40.7% and 7.1% respectively, as reported by Marongiu et al. (2005). Furthermore, the aerial parts of Sardinian *F. communis* exhibit significant variability: the toxic chemotype is rich in aristolene (47.1%) and (E,E)-farnesol (21.2%), while the non-toxic chemotype is characterized by a high concentration of allohedycaryol (53.7%), according to Rubiolo et al. (2006). Additionally, the essential oil profile of *F. glauca*, once classified as a subspecies of *F. communis*, from central Italy shows distinct major volatiles: (E)-caryophyllene and caryophyllene oxide in the leaves, α-pinene, myrcene, and germacrene D in the flowers, and α- and β-pinene in the fruits, as detailed by Maggi et al. (2009).

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3.2. Anti-microbial activity

The anti-microbial activity of *F. lycia* EO was evaluated against a variety of microorganisms, including 4 Gram-positive and 3 Gram-negative bacteria, 2 fungi, and 1 yeast, as detailed in Table 3. The EO exhibited modest activity, particularly against four Gram-positive bacteria (*S. aureus* and *S. faecalis*), and one Gram-negative bacterium (*E. coli*), with no significant activity against the others. *P. vulgaris* showed the highest sensitivity with a 14 mm inhibition zone. A comparative analysis of the anti-microbial activities of *F. lycia* EO and antibiotics is presented in Table 3, indicating that the EO was as effective as ampicillin against *B. subtilis*. Antibiotics were generally more effective than the EO against all tested bacteria except *L. monocytogenes*. Previous reports have documented the anti-microbial properties of EOs from various *Ferula* species (Alruways, 2023; Tavakkoli et al., 2023). For instance, a study on *Ferula szovitsiana* found *B. subtilis* to be the most susceptible strain, although the EO displayed weak activity against all tested microorganisms (Dehghan et al., 2007). Similarly, *F. gummosa* EO showed antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, *E. faecalis*, and exhibited weak activity against *P. aeruginosa* (Eftekhar et al., 2004). *Ferula glauca* EO was also found to be effective against *B. subtilis* (Maggi et al., 2009). Another report indicated that the EO of *Ferula latisecta* had significant activity against all tested Gram-positive bacteria and *E. coli* (Habibi et al., 2006).

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Table 2. EO (%) composition in different phenological stages and harvesting times

			Pre- flowering stage			Full flowering stage			Post flowering stage		
			Harvesting times			Harvesting times			Harvesting times		
No	Compounds	RI	6 am	1 pm	8 pm	6 am	1 pm	8 pm	6 am	1pm	8 pm
1	α-pinene	706	53.2 ± 0.00^{c}	49.8 ± 0.00^{e}	54.6 ± 0.00^{b}	55.2 ± 0.00^{b}	52.6 ± 0.00^{c}	56.3 ± 0.00^{a}	49.2 ± 0.00^{b}	49.8 ± 0.00^{e}	51.2 ± 0.00^{d}
2	camphene	1026	nd	nd	0.60 ± 0.00	nd	nd	nd	nd	nd	nd
3	1,8-Cineole	1031	0.29 ± 0.32^{d}	0.45 ± 0.57^{f}	nd	nd	nd	nd	nd	nd	nd
4	α-tolualdehyde	1042	0.21 ± 0.01^{b}	0.75 ± 0.00^{a}	nd	nd	nd	nd	nd	nd	nd
5	sabinene	1044	0.14 ± 0.00^{d}	0.17 ± 0.00^{c}	nd	0.88 ± 0.02^{a}	0.86 ± 0.00^{b}	nd	nd	nd	nd
6	Terpinene <γ->	1059	nd	nd	nd	nd	nd	nd	3.20 ± 0.20^{b}	3.04 ± 0.80^{b}	10.86 ± 0.58^{a}
7	cis-4-thujanol	1098	4.77 ± 0.01^{a}	3.38 ± 0.00^{b}	3.42 ± 0.00^{b}	2.86 ± 0.03^{c}	1.19 ± 0.01^{e}	1.39 ± 0.13^{d}	nd	nd	nd
8	β-pinene	1140	17.2 ± 0.00^{c}	16.8 ± 0.00^{c}	19.6 ± 0.00^{a}	18.2 ± 0.00^{b}	19.8 ± 0.00^{a}	18.6 ± 0.00^{b}	15.2 ± 0.00^{d}	14.8 ± 0.00^{d}	14.6 ± 0.00^{d}
9	Verbenol <trans->	1144	2.10 ± 0.64^{b}	3.03 ± 0.56^{a}	nd	2.05 ± 0.18^{b}	2.02 ± 0.40^{b}	nd	nd	nd	nd
10	myrcene	1146	0.22 ± 0.10^{c}	40.30 ± 0.04^{a}	0.25 ± 0.05^{f}	nd	nd	nd	nd	nd	nd
11	α-phellandrene	1152	0.37 ± 0.02^{d}	nd	20.01 ± 0.80^{b}	nd	nd	22.78 ± 1.74^{a}	5.56 ± 0.42^{c}	nd	nd
12	limonene	1164	0.13 ± 0.02^{b}	nd	nd	0.26 ± 0.00^{a}	nd	nd	nd	nd	nd
13	β-phellandrene	1169	2.22 ± 0.60^{c}	0.50 ± 0.70^{h}	2.10 ± 0.46^{d}	1.98 ± 0.74^{e}	1.28 ± 0.18^{g}	2.49 ± 0.20^{b}	1.91 ± 0.00^{e}	1.71 ± 0.02^{f}	2.62 ± 0.55^{a}
14	ocimene	1169	1.52 ± 0.12^{b}	1.85 ± 0.01^{a}	nd	0.76 ± 0.00^{c}	0.49 ± 0.00^{d}	0.70 ± 0.00^{c}	nd	nd	nd
15	limonene oxide	1171	nd	nd	20.25 ± 1.40^{a}	nd	nd	2.17 ± 0.20^{c}	nd	nd	5.04 ± 0.30^{b}
16	camphenol	1171	0.69 ± 0.03^{b}	nd	nd	nd	nd	18.38 ± 1.08^{a}	nd	nd	nd
17	Terpinen-4-ol	1177	1.98 ± 0.30^{b}	0.57 ± 0.10^{e}	1.00 ± 0.50^{d}	2.49 ± 0.32^{a}	1.89 ± 0.64^{bc}	1.67 ± 0.30^{c}	1.17 ± 0.00^{d}	0.05 ± 0.00^{e}	0.06 ± 0.00^{e}
18	Terpineol <α->	1188	0.18 ± 0.01^{d}	nd	0.42 ± 0.00^{b}	0.35 ± 0.00^{c}	nd	0.64 ± 0.02^{a}	nd	nd	nd
19	Verbenone	1205	1.36 ± 0.30^{d}	2.83 ± 0.24^{a}	nd	1.40 ± 0.02^{c}	1.98 ± 1.02^{b}	nd	nd	nd	nd
20	trans-pinocarveol	1237	nd	nd	1.39 ± 0.12^{b}	nd	nd	1.69 ± 0.18^{a}	nd	nd	nd

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21	**pulegone**	1247	nd	nd	1.03 ± 0.02[b]	nd	nd	1.38 ± 0.10[a]	nd	nd	nd
22	**D-verbenone**	1252	nd	nd	0.37 ± 0.00	nd	nd	nd	nd	nd	nd
23	**isoborneol**	1252	nd	nd	0.42 ± 0.01[b]	nd	nd	0.53 ± 0.01[a]	nd	nd	nd
24	**myrtenol**	1285	nd	nd	nd	nd	nd	nd	1.06 ± 0.04	nd	nd

Table 2 (continued). EO (%) composition in different phenological stages and harvesting times

25	fenchyl acetate	1388	nd	nd	0.86 ± 0.00[a]	nd	nd	0.37 ± 0.02[b]	nd	nd	nd
26	Elemene <β->	1390	0.16 ± 0.00	nd	nd	nd	nd	nd	nd	nd	nd
27	Eugenol	1359	nd	nd	0.08 ± 0.00	nd	nd	nd	nd	nd	nd
28	Caryophyllene <(E)->	1419	nd	nd	1.95 ± 0.45	nd	nd	nd	nd	nd	nd
29	β-caryophyllene	1481	1.05 ± 0.00	nd	nd	nd	nd	nd	nd	nd	nd
30	α-ylangene	1489	0.12 ± 0.00[b]	0.24 ± 0.00[a]	nd	nd	nd	nd	nd	nd	nd
31	α-copaene	1490	nd	nd	nd	nd	nd		4.81 ± 0.62[a]	4.19 ± 0.20[b]	4.22 ± 0.40[b]
32	α-bourbonene	1525	0.30 ± 0.00[c]	0.54 ± 0.00[b]	nd	0.25 ± 0.02[d]	0.84 ± 0.02[a]	nd	nd	nd	nd
33	α-bisabolene	1650	nd	nd	0.44 ± 0.02[c]	0.33 ± 0.00[d]	0.79 ± 0.02[a]	0.65 ± 0.02[b]	nd	nd	nd
34	Germacrene D	1481	1.05 ± 0.00	nd	nd	nd	nd	nd	nd	nd	nd
35	Eudesma-6,11-diene <cis->	1489	0.12 ± 0.00[b]	0.24 ± 0.00[a]	nd	nd	nd	nd	nd	nd	nd
36	Selinene <β->	1490	nd	nd	nd	nd	nd		4.81 ± 0.62[a]	4.19 ± 0.20[b]	4.22 ± 0.40[b]
37	Chavibetol acetate	1525	0.30 ± 0.00[c]	0.54 ± 0.00[b]	nd	0.25 ± 0.02[d]	0.84 ± 0.02[a]	nd	nd	nd	nd
38	Eudesmol <β->	1650	nd	nd	0.44 ± 0.02[c]	0.33 ± 0.00[d]	0.79 ± 0.02[a]	0.65 ± 0.02[b]	nd	nd	nd

Retention indices relative to n-alkans (C5-C22) on HP 5MS column; nd: not detected;
The result are expressed as means ± SD (n=3);
Means with similar letter in % 5 level of LSD test are not significant

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Table 3. Determination of inhibition zone (IZ) and minimum inhibitory concentration (MIC) of *Achillea gypsicola* EO for anti-microbial test

				Bacterial, yeast and fungal strain									
				Gram-negative bacteria			Gram-positive bacteria				Yeast strain	Fungal strain	
Stages				***Escherichia coli***	***Proteus vulgaris***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Bacillus subtilis***	***Streptococcus faecalis***	***Listeria monocytogenes***	***Saccharomyces cerevisiae***	***Candida albicans***	***Aspergillus niger***
Pre-flowering	**H.T.**	**6 am**	**IZ (mm)**	22.2 ± 0.10	15.3 ± 0.30	14 ± 0.50	18.5 ± 0.14	25.4 ± 0.70	15 ± 0.35	13 ± 0.07	8.3 ± 0.09	12.2 ± 0.10	21 ± 0.07
			MIC (µg/mL)	144 ± 0.45	36 ± 0.37	36 ± 0.44	18 ± 0.68	9 ± 0.05	36 ± 0.06	36 ± 0.10	144 ± 0.00	72 ± 0.70	72 ± 0.36
		1 pm	**IZ (mm)**	24.4 ± 0.12	17.5 ± 0.19	12.6 ± 0.00	17.1 ± 0.32	25 ± 0.07	14.8 ± 0.40	13.2 ± 0.00	10.1 ± 0.00	13.5 ± 0.98	19.8 ± 0.75
			MIC (µg/mL)	144 ± 0.20	36 ± 0.12	36 ± 0.34	18 ± 0.07	9 ± 0.0	36 ± 0.52	36 ± 0.05	144 ± 0.40	72 ± 0.11	36 ± 0.15
		8 pm	**IZ (mm)**	20 ± 0.17	18.1 ± 0.04	13.2 ± 0.10	15.6 ± 0.20	26.5 ± 0.15	17.3 ± 0.93	13.1 ± 0.42	10 ± 0.17	11 ± 0.13	18 ± 0.04
			MIC (µg/mL)	72 ± 0.10	36 ± 0.10	36 ± 0.12	36 ± 0.00	9 ± 0.00	36 ± 0.00	36 ± 0.12	144 ± 0.65	72 ± 0.65	72 ± 0.30
Full flowering	**H.T.**	**6 am**	**IZ (mm)**	32 ± 0.51	21.2 ± 0.54	18 ± 0.00	30 ± 0.42	29.5 ± 0.11	17.5 ± 0.13	15 ± 0.44	9 ± 0.70	39.8 ± 0.1	38.5 ± 0.58
			MIC (µg/mL)	18 ± 0.02	18 ± 0.05	36 ± 0.21	18 ± 0.60	2.25 ± 0.12	36 ± 0.80	144 ± 0.55	18 ± 0.92	36 ± 0.1	36 ± 0.36
		1 pm	**IZ (mm)**	35 ± 0.01	24 ± 0.10	17.5 ± 0.43	37.3 ± 0.11	35 ± 0.60	15 ± 0.01	14.2 ± 0.30	12.5 ± 0.35	40 ± 0.44	42 ± 0.01
			MIC (µg/mL)	72 ± 0.05	4.5 ± 0.09	18 ± 0.00	9 ± 0.20	4.5 ± 0.00	9 ± 0.03	144 ± 0.30	36 ± 0.00	36 ± 0.05	36 ± 0.07
		8 pm	**IZ (mm)**	35.7 ± 0.14	23.8 ± 0.30	21.1 ± 0.18	36.9 ± 0.15	35.1 ± 0.71	15.5 ± 0.08	14.5 ± 0.10	12.6 ± 0.00	42.2 ± 0.56	42.3 ± 0.10
			MIC (µg/mL)	72 ± 0.56	4.5 ± 0.00	36 ± 0.13	9 ± 0.00	4.5 ± 0.15	36 ± 0.30	144 ± 0.52	36 ± 0.10	72 ± 0.90	72 ± 0.20
Post flowering	**H.T.**	**6 am**	**IZ (mm)**	16.5 ± 1.10	19.4 ± 0.12	13.8 ± 0.01	20.5 ± 0.98	25 ± 0.02	27.3 ± 0.10	17.2 ± 0.27	12.4 ± 0.30	35 ± 0.20	22.8 ± 0.52
			MIC (µg/mL)	18 ± 0.05	4.5 ± 0.01	18 ± 0.05	18 ± 0.55	4.5 ± 0.20	36 ± 0.00	72 ± 0.20	36 ± 0.32	9 ± 0.30	36 ± 0.45
		1 pm	**IZ (mm)**	15 ± 0.10	20.6 ± 0.10	13.4 ± 0.40	22 ± 0.03	23.8 ± 0.30	26.1 ± 0.25	18 ± 0.16	12 ± 0.54	33.2 ± 0.55	22 ± 0.18
			MIC (µg/mL)	18 ± 0.30	4.5 ± 0.05	18 ± 0.00	18 ± 0.00	4.5 ± 0.00	36 ± 0.84	72 ± 0.05	36 ± 0.06	9 ± 1.08	36 ± 0.10
		8 pm	**IZ (mm)**	14.8 ± 0.41	20 ± 0.30	14.7 ± 0.04	23.1 ± 0.05	24 ± 0.00	26 ± 0.50	17.9 ± 0.00	13 ± 0.05	36.5 ± 0.65	22 ± 0.10
			MIC (µg/mL)	18 ± 0.08	4.5 ± 0.09	18 ± 0.11	18 ± 0.01	4.5 ± 0.05	36 ± 0.10	72 ± 0.51	36 ± 0.10	9 ± 0.98	36 ± 0.44
Penicillin[a]			**IZ (mm)**	nt	nt	nt	27 ± 0.80	nt	nt	nt	nt	nt	nt
			MIC (µg/mL)	nt	nt	nt	0.5 ± 0.03	nt	nt	nt	nt	nt	nt
Ampicillin[a]			**IZ (mm)**	18 ± 0.10	38 ± 0.21	nt	nt	30 ± 0.70	13 ± 0.42	nt	nt	nt	nt
			MIC (µg/mL)	4 ± 0.10	8 ± 0.15	nt	nt	0.5 ± 0.01	2 ± 0.30	nt	nt	nt	nt
Gentamicin[a]			**IZ (mm)**	nt	nt	23 ± 0.05	28 ± 0.06	44 ± 0.15	nt	13 ± 0.30	nt	nt	nt
			MIC (µg/mL)	nt	nt	8 ± 0.45	8 ± 0.10	4 ± 0.18	nt	1 ± 0.05	nt	nt	nt
Vancomycin[b]			**IZ (mm)**	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
			MIC (µg/mL)	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
Nystatin[b]			**IZ (mm)**	nt	nt	nt	nt	nt	nt	nt	25 ± 0.50	42 ± 0.54	40 ± 0.30
			MIC (µg/mL)	nt	nt	nt	nt	nt	nt	nt	1 ± 0.10	2 ± 0.54	8 ± 0.07

H.T: Harvesting times; Diameter of zone of inhibition (mm) including disk diameter of 6 mm; a: Tested at 10 µg/disc, b: Tested at 30µg/disc;
nt: not tested; The result are expressed as means ± SD (n=3).

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4. Conclusion

This research offers a comprehensive report analysis of the phytochemical profile and biological attributes of *F. lycia a*cross various growth stages. It was found that the optimal time for harvesting EOs is during peak bloom at 13:00. Notably, fluctuations in the EO composition were recorded periodically and even daily. Moreover, this study identified several novel compounds within the EO derived from *F. lycia*, marking a first in the research of this species. The most abundant periods for EO components in *F. lycia* were observed during the pre- and full flowering stages. Furthermore, the peak bloom phase also yielded the most effective anti-microbial properties. These findings suggest that *F. lycia* holds potential as a reliable resource for the cosmetic, food, and pharmaceutical sectors.

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Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability
Data will be made available on request.

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THE EFFECT OF TEMPERATURE ON THE VOLATILE OIL COMPOSITION OF *LAURUS NOBILIS* L.

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Abstract
Laurus nobilis L (bay leaf: BL)., a member of the Lauraceae family, occurs naturally in the southern Mediterranean climate. Extracts of this plant can be used in a wide range of applications, from medical applications to foods, cosmetics and sustainable pesticides. It has various beneficial effects such as anti-bacterial, antioxidant, anti-tumoral and acetylcholinesterase inhibition. All these properties can be attributed to the plant volatile oil components. In the realm of medicinal and aromatic plants, the conditions under which they are stored play a pivotal role in determining the quantity and quality of their active substances. However, few studies have delved into the alterations of secondary metabolites post-extraction. This study employed a factorial experiment based on a completely randomized design with three replications. The variables included three storage temperature levels: freezer temperature (–20 °C), refrigerator temperature (4 °C), and room temperature (23 ± 2 °C), along with four durations of storage time (fresh distillation, 3 months, 6 months, 9 months, and 12 months). The volatile oils extracted via hydro-distillation were subjected to analysis using gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). A total of 27 components were detected in the leaves of LVO. The dominant compounds included 1,8-cineole, followed by linalool, α-pinene, α-terpinyl acetate, methyl eugenol, β-pinene and sabinene. Findings indicated that at each storage temperature, certain compounds like 1,8-cineole, nerol, and cis-ocimene experienced a decline. Conversely, the primary components of *L. nobilis* volatile oil, such as linalool, linalyl acetate, and α-terpinyl acetate saw an increase across all temperatures, with the most significant rise at freezer temperature and the least at room temperature. Storing the *L. nobilis* volatile oil in the freezer was found to enhance its quality.

Key Words: Laurel, Oil Quality, Phytochemical Compounds, Shelf Life

1. Introduction

Volatile oils (VOs) are the primary substances utilized in aromatherapy, derived from various aromatic parts of plants through methods like hydrodistillation, steam distillation, or cold pressing. These VOs, along with extracts from a range of edible and medicinal plants, herbs, and spices, are highly potent natural biologically active agents (Açıkgöz, 2019; Açıkgöz, 2020; Ay Batı et al., 2023a; Ay Batı et al., 2023b; Kırgeç et al., 2023). The application of VOs as antimicrobial agents in food systems is increasingly recognized as an intrinsic factor that can enhance food safety and extend shelf life (Khalid et al., 2024; Wang and Su, 2024). However, the chemical composition of VOs and plant extracts, particularly those from medicinal plants, can vary depending on the plant's origin, environmental conditions, and the developmental stage at which the plant materials are collected (Batı Ay et al., 2023c).

In order to sustain agriculture, it has become a necessity to research alternative methods to chemical methods. One of these alternative methods is the use of allelopathic substances to combat weeds, pests and plant diseases (Uludağ et al.,, 2006). The allelopathic effect of plant-derived chemicals on weeds allows them to be used as bioherbicides (Putnam and Duke, 1978; Dudai et al., 1999; Singh et al., 2002; Duke et al., 2000). In recent years, a lot of work has been done on the use of semiochemicals as an

alternative to synthetic drugs in the fight against weeds and harmful insects. Semiochemicals tested included plant extracts, essential oils, and allerocins. These substances can inhibit germination and development in weeds, and have effects such as fumigant, contact insecticide and repellent in insects. The group whose effects on weeds have been most studied are terpene compounds (Aydın and Tursun, 2010).

Despite the extensive literature on the compositional changes of VOs during storage and/or use, research on *L. nobilis* leaves is notably absent. For consumers and stakeholders in aromatherapy, ensuring the safety, efficacy, and quality of VO products is crucial. Studies have shown that storage affects the chemical composition of VOs; for instance, VO from *Leonurus cardiaca* L. wild in Vilnius and from cultivated plants exhibited oxidation of β-caryophyllene and α-humulene when stored in lidded glass containers in a refrigerator (Mockute et al., 2005). Additionally, significant increases in the VO composition, particularly thymol and carvacrol, were noted during the storage of *Thymus daenensis* L. (Rowshan et al., 2013). Thus, this study aims to identify the changes in the VO composition of bay leaves under prolonged storage conditions.

3. Materials and Methods

2.1. Plant material

Laurus nobilis L. (Bay) leaves were collected from local producers in Akhisar district of Manisa province, at an altitude of 117 meters above average sea level. Plants were dried in the shade at room temperature (22-24◦C) for 14 days.

2.2. The isolation of VO

VOs of all dried samples (100 g) were isolated by hydrodistillation for 3 h using a Clevenger-type apparatus according to the method suggested by Açıkgöz (2019). Distilled oils were placed in tightly closed dark bottles for analysis.

2.3. VOs storage conditions

To investigate the effects of different storage conditions on the compositions of distilled oils, oil samples were subjected to various storage temperatures such as a refrigerator (4°C), a deep freezer (-20°C), and room temperature (23 ± 2°C). They were stored for 12 months, with analyses conducted every three months. The oil analyses for all storage processes were performed in three-month periods. Additionally, to determine the precise effects of storage conditions on the VO compositions throughout the experiment, freshly extracted oil was analyzed immediately after extraction. The extracted VOs were pale orange in color and had a distinct, sharp odor.

2.4. Analysis by gas chromatography-mass spectrometry (GC-MS)

The VO components of *L. nobilis* are analyzed using a Thermo Scientific type gas chromatograph coupled with a mass spectrometry (MS) system (Quantum GC Triple Quadrupole). Fragmentation is established with an electron impact energy of 70 eV and a mass scan range of 40-400 amu. The major components are separated on a DB-WAX column (60m x 0.25mm x 0.25µm: Agilent Technologies) used in chromatographic characterization. Helium gas is utilized as the carrier. The column temperature is programmed to rise from 50°C to 260°C at a rate of 10°C per minute. The injection volume is 0.5 µL with a split ratio of 1:30. Oil components are identified by comparing retention indices (RIndex) and literature references with the (C8-C24) n-alkane series. Additionally, chemical characterization involves aligning the obtained mass spectra with entries in the chemical database, a detailed separation process, and the maintenance of NIST & Wiley libraries throughout (Adams, 2017). The quantification of a component is finalized through peak-included normalization without the use of any internal standards.

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3. Results and Discussion

The research analyzed the volatile oils (VOs) of *L. nobilis*, obtained through hydrodistillation, under various temperatures and durations of storage, as detailed in Table 1. Out of the 27 identified constituents, which accounted for over 99% of the total composition, phenolic compounds such as 1,8-cineole and nerol, along with their precursors cis-ocimene and methyl eugenol, were predominant in the *L. nobilis* VO immediately post-distillation and throughout different storage scenarios. The study revealed significant fluctuations in the concentration of these primary compounds when stored at freezer conditions (−20 °C), in stark contrast to other storage settings outlined in Table 1.

Our research demonstrated that VO components of lower molecular weight tend to diminish over time, particularly when stored at ambient temperatures, as shown in Table 1. This reduction is likely due to factors such as evaporation and oxidation, which can lead to unfavorable alterations in the VO's composition during storage, as noted by Baritaux et al. (1992) and Mockute et al. (2005). It was observed that compounds with lower boiling points showed a significant decrease in refrigerated and room temperature conditions over time. Conversely, certain components increased when stored at freezing temperatures (Table 1). For instance, the concentration of 1,8-cineole started at 49.24% post-extraction and then declined to 41.74%, marking a 16% reduction by the end of the storage term. Neurol's progression after 3, 6, 9, and 12 months of storage was 13.4%, 14%, 11.7%, and 15.1%, respectively. Sabinene also followed a similar pattern, initially at 3.14%, then falling to 2.25% at room temperature by the experiment's conclusion, but rising to 5.40% at -20 °C. Methyl eugenol levels after 3, 6, 9, and 12 months of storage at -20 °C were 4.13%, 5.20%, 5.05%, and 6.38%, respectively. This component exhibited a comparable trend at +4 °C, where the storage duration had a positive impact on the proportion of the component (Table 1). A key finding from the investigation is that the proportions of linalool, linalyl acetate, and α-terpinyl acetate in the essential oil tend to rise with prolonged storage at ambient temperatures. Specifically, linalool exhibited an initial concentration of 1.03% at the time of oil distillation, which consistently increased under various storage temperatures, peaking at 2.41% after a six-month period at -20 °C. Previous research, such as that by Usai et al. (2011), has indicated that vital constituents like thymol, terpinene, and carvacrol remain stable in thyme oil samples kept at frozen temperatures. Research on the storage of plant secondary metabolites, particularly VOs, is scarce because these metabolites are volatile and can potentially undergo various changes depending on storage conditions. Kumar et al. (2013) demonstrated that the content and composition of volatile oils are influenced by the time of harvest and storage conditions. The duration of storage at different temperatures also affected the oil content and composition. When flowers were stored at 4°C and at 18 ± 1°C or 25 ± 1°C for 24 hours, there was an approximate 9% and 28% decrease in oil content, respectively. With an increase in storage duration, the content of citronellol + nerol also increased. Another report indicated that different storage conditions could affect the quality of selected volatile oils (Turek and Stintzing, 2012). This study notably observed the degradation of prominent monoterpenes in rosemary oil. Kazaz et al. (2009) reported that the volatile oil of *Rosa damascena* Mill. was not affected by different storage temperatures (0°C and 3°C) but was influenced by the duration of storage (7, 14, 21, and 28 days). In a study conducted by Arabhosseini et al. (2007), the impact of storage conditions on the volatile oil concentration and coloration of French Tarragon (*Artemisia dracunculus* L.) leaves was examined. Findings indicated that both the volatile oil levels and color metrics experienced a decline over the storage duration. Notably, the most significant decrease in volatile oil levels approximately 50% within a 30 days period and alterations in color, as measured by the hue value, were observed in samples that were dehydrated at 90°C. The literature notes that -terpinene undergoes aromatization to become p-cymene, which can then transform into carvacrol or thymol through hydroxylation (Dewick, 2002; Poulose and Croteau, 1978). Research tracing back to the 1960s by Yamazaki et al. (1963) has characterized thymol as an aromatic terpenoid biosynthesis product. Further

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studies in the late 1970s involved feeding radioactively labeled monoterpenes -terpinene and p-cymene to thymol (Poulose and Croteau, 1978). These studies suggest that the biosynthetic pathway for thymol and carvacrol, its isomer, starts with -terpinene and passes through p-cymene. Observations from these studies indicate that during storage, the concentrations of 1,8-cineole and nerol trended inversely compared to sabinene and methyl eugenol. Particularly at room temperature, the levels of the former compounds decreased, while the latter saw increases.

				Storage conditions											
				Freezer (-20 °C)				Refrigerator (4 °C)				Room (23 ± 2 °C)			
				Storage period (month)				Storage period (month)				Storage period (month)			
No	Compound	RI	After distilation	3	6	9	12	3	6	9	12	3	6	9	12
1	*α*-Pinene	935	0.61	0.63	0.63	0.63	1.48	0.73	0.62	0.58	0.43	0.44	0.43	0.48	0.30
2	*β*-Pinene	978	0.13	-	-	-	-	-	-	-	-	0.19	0.18	0.19	0.18
3	*β*-Myrcene	990	0.33	0.33	0.36	0.36	0.36	0.40	0.58	0.40	0.35	0.46	0.33	0.51	0.43
4	1,8-Cineole	1036	49.24	48.35	47.05	43.37	47.40	45.25	41.30	42.52	45.52	42.20	41.00	42.35	41.74
5	*p*-Cymene	1042	-	-	-	-	0.14	-	-	-	-	-	-	-	-
6	*cis*-Rose oxide	1089	0.44	-	-	-	-	0.44	0.43	0.38	0.69	-	-	-	-
7	*trans*-Rose oxide	1092	0.21	0.38	0.63	0.38	0.69	-	-	-	-	0.88	0.87	0.88	0.84
8	*β*-Bourbonene	1098	0.25	-	-	-	-	-	-	-	0.19	-	-	-	0.25
9	Linalool	1105	1.03	1.96	1.74	1.96	2.07	2.00	1.04	1.16	1.01	1.71	1.76	1.74	1.67
10	Terpinen-4-ol	1171	-	0.75	-	0.78	-	0.82	0.82	-	-	-	-	-	-
11	linalyl acetate	1203	1.31	1.98	2.41	2.38	2.27	1.51	1.81	1.54	1.30	1.66	1.74	1.78	1.93
12	2,6-Octadiene	1207	-	0.85	0.94	0.89	1.03	0.94	0.97	0.88	1.00	0.95	0.97	0.95	1.26
13	Citronellol acetate	1330	1.04	0.87	-	-	-	-	-	-	-	-	-	-	-
14	*α*- terpinyl acetate	1337	0.67	0.57	1.12	0.59	1.20	0.64	1.10	0.81	0.51	0.58	1.12	0.68	0.64
15	(*Z*)-Citral	1370	1.22	0.36	1.03	0.37	0.67	0.42	1.07	0.97	0.56	0.40	0.38	0.37	1.18
16	*α*-Terpineol	1374	0.30	0.32	0.43	0.36	0.41	0.36	0.45	0.35	0.35	0.28	0.46	0.26	0.44
17	(*E*)-Citral	1423	-	0.80		0.80		0.40	1.03	0.97	1.02	0.64	0.61	0.61	0.98
18	Nerol	1425	20.30	19.28	19.60	17.33	18.57	16.13	19.00	18.54	17.99	17.57	17.45	17.92	17.24
19	Benzeneethanol	1427	-	2.12	3.22	2.13	3.86	2.18	3.22	-	-	-	2.83	-	0.82
20	Germacrene-D	1430	1.38	-	-	-	-	-	-	1.12	1.16	1.22	1.14	1.23	1.51
21	Geranyl acetate	1441	1.82	-	1.73	-	1.97	-	1.77	1.78	1.89	1.94	1.88	1.75	2.39
22	cis-Ocimene	1445	3.13	1.98	1.41	1.38	1.27	1.31	1.81	1.34	1.30	1.66	1.54	1.68	1.33
23	Sabinene	1447	3.14	5.37	5.40	5.05	5.38	3.59	4.25	4.28	4.15	3.17	2.25	2.57	2.39
24	Methyl eugenol	1451	3.43	4.13	5.20	5.05	6.38	3.59	4.95	4.74	5.01	3.57	3.03	3.57	3.79
25	Eugenol	1501	0.43	0.57	0.28	0.70	0.27	0.78	0.16	0.96	0.74	0.46	0.81	0.67	0.53
26	*β*-Eudesmol	1583	0.12	-	0.16		0.19		0.16	0.11	0.14	0.13	-	-	-
27	*γ*-Eudesmol	1581	-	-	-	-	-	-	-	-	-	-	-	-	0.15

RI: retention indices relative to C8–C25 n-alkanes on the HP-5 column; t: trace < 0.1%

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4. Conclusion

The primary process during the storage of VOs is the evaporation of compounds, especially monohydrocarbons, which have lower boiling points. According to the results of the current study, the VO of *L. nobilis* leaves stored in the freezer maintained its primary quality over nine months compared to other storage conditions and even showed an increase in the quality of certain compounds. Generally, storing bay leaf VO at low temperatures prevents the decrease in concentrations of oil components and helps maintain the primary quality of the VO with minimal changes. However, the results reported here indicate that storage at room temperature reduces the quality of the VO and significantly decreases important index components such as 1,8-cineole, nerol, and cis-ocimene. These findings could be extended to the storage of VOs with similar chemical properties. Moreover, these successes demonstrate that VO producers and consumers, particularly those using these compounds in the pharmaceutical and cosmetic industries, can benefit from this phenomenon. In conclusion, the storage of secondary plant products, especially VOs, is an intriguing area of research that requires further studies with VOs from various aromatic plants composed of different components.

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Conflict of Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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PLANTS USED AS WOUND HEALERS IN FOLK MEDICINE IN TÜRKİYE

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Abstract
Wound healing is a physiological and dynamic process regulated by molecular, cellular, and humoral mechanisms. The process mainly occurs in four stages; proliferation, inflammation, hemostasis, and tissue remodeling. The skin, the body's biggest organ, acts as a protective barrier against harmful stimuli such as bacteria, UV radiation, allergies, and irritants. It serves as the crucial boundary between the body's internal and exterior environments (Midwood et al., 2004; Reinke & Sorg, 2012). The restoration of the skin regenerates itself upon injury. The recovery process involves the interaction of many cellular elements (monocytes/macrophages, fibroblasts, keratinocytes, and endothelial cells) and extracellular matrix components including collagen and fibronectin, which help the wound edges come together. Various variables including mechanical stress, infection, or poisonous chemicals can greatly impact the skin's capacity to repair itself (Myer, 2000). Ethnobotanical investigations conducted using ancient treatment approaches are documented to aid in the advancement of pharmaceuticals. Knowledge about the traditional use of these medicinal or wild plants has been passed down through generations. This study examined theses at the National Higher Education Center and ethnobotanical studies from different regions of Turkey focusing on the usage of regional flora for wound therapy. Extensive ethnobotanical research has identified numerous plants utilized for their healing properties in traditional wound care in Turkey. Due to their numerous favorable physiological advantages, they have been utilized on the skin for cosmetic and medical purposes for a long time (Lin et al., 2018). This study demonstrates that the majority of plants have been utilized as healers for treating wounds.

Key Words: Ethnobotanical research, Medicinal plants, Wound healing, Folk medicine, Turkey

Introduction

The utilization of plants as a form of therapy in traditional medicinal systems can be dated back approximately 60,000 years ago, as evidenced by fossil investigations. In recent times, developed nations have embraced traditional medicinal systems that utilize herbal drugs and remedies. WHO (World Health Organization) reports that approximately 65% of the global population has integrated the use of plants as a primary method of healthcare. A often observed fact is that 25% of the medications currently given are derived from plants. This estimation indicates that pharmaceuticals generated from plants constitute a substantial portion of natural product-based medications. In the last two decades, our comprehension of the wound healing process has significantly advanced. Furthermore, the acquisition of this knowledge has led to the emergence of innovative technologies that enhance the natural healing process of wounds and counteract the underlying pathological mechanisms responsible for the progression of persistent wounds. The future of wound healing shows immense potential, ranging from growth factors to bioengineered skin substitutes (Singh Pawar & Toppo, 2012).

Of the approximately 250,000 species of flowering plants worldwide, only 15% have undergone phytochemical evaluation, and a mere 6% have been tested for biological activity. Although just a small fraction of all plants have been utilized for therapeutic purposes, it is crucial to recognize their

significance, as about 65% of the global population relies on them as their main form of healthcare. Surprisingly, over 33% of traditional herbal medications are specifically designed to cure wounds or skin diseases, while just 1-3% of modern drugs serve this purpose. It is worth mentioning that various experimental investigations have provided support for the positive benefits of plant extracts on the healing of skin wounds. Given that chronic wounds affect a total of 6.5 million persons in the United States alone, and that the global prevalence of chronic wounds is expected to rise due to the growing incidence of age-related conditions and diseases like cardiovascular diseases, diabetes, and obesity, it is crucial not to overlook any therapeutic intervention that aims to enhance the wound healing processes (Budovsky, Yarmolinsky, & Ben-Shabat, 2015). In this study, what is a wound, what are the wound healing mechanisms, and brief information on the most commonly used medicinal plants for wound healing in Turkey will be given.

Wound and its Various Types

A wound is characterized by the destruction of a tissue's anatomical and cellular integrity. The process of wound healing involves a sequence of biochemical and cellular activities that result in the recovery of the damaged tissue's vigor and the restoration of its structural and functional soundness. Wounds are categorized based on their causes, severity, healing duration, depth, and skin condition. Surgically, wounds are typically inspected and grouped into five primary groups: cut wounds, wounds caused by sharp objects, tear wounds, and open wounds. Furthermore, bite, poison, and gunshot wounds might also be included in the tally (Çavuş Alan & Özen, 2021; Singh Pawar & Toppo, 2012).

Process of Wound Healing

Wound healing includes the stages of inflammation (days 0-3), cellular proliferation (days 3-12) and remodeling (3-6 months) with uninterrupted interactions between cells and between cells and the extracellular matrix. Wound repair mechanism is observed as 4 main processes. These are wound contraction, inflammation, epithelialization, and granulation tissue formation. Inflammation initiates promptly following tissue damage, whereby platelets are drawn towards clotting factors and assemble a homeostatic plaque to halt bleeding.

Prostaglandins (PGE and PGE2) are released at the site of inflammation. These factors serve as the ultimate regulators of acute inflammation and have a homeostatic function for fibroblasts and white blood cells. Mobile white blood cells that have been activated move towards the wound and start producing cellular waste. During the final phase, the process of wound contraction begins gradually and then speeds up after 3-4 days. Myofibroblasts located at the edges of the wound function as a mechanism for wound contractionThe wound is covered by the growth and movement of basal cells at the edges of the lesion, resulting in epithelial covering. The hematoma present in the wound is substituted with granulation tissue that consists of newly formed capillaries and fibroblasts. Fibroblasts are the cellular entities that are accountable for the synthesis of mucopolysaccharides in connective tissues. Finally, new nerve fibers and scars are formed (Gökalp & Özay, 2009; Nagori & Solanki, 2011).

Wound healing is an intricate biological process that encompasses numerous types of cells, various cytokines, growth factors and their interactions. In the initial phase of wound healing, fibrin polymerizes rapidly and forms an accumulation. Plasma fibronectin binds to and cross-links with fibrin to form a fibrinous sheath, which promotes the migration and adhesion of leukocytes and fibroblasts. Fibroblasts migrate to the wound site and produce additional fibronectin. Normal wound healing occurs when many cellular activities occur together in an organized manner. These activities are Phagocytosis, Chemotaxis, Collagen synthesis, Mitogenesis, and the synthesis of other matrix components (Berk, Dokumaci, &

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Kaymaz, 2015; Gökalp & Özay, 2009). Wound healing progresses through various stages including coagulation, granulation, epithelization, collagenation, and tissue remodeling. Collagen, the main component that improves and maintains tissue outside of cells, contains substantial amounts of hydroxyproline, which has been used as a biochemical marker for collagen in tissues. Wound contraction is the result of the contraction of myofibroblasts. Platelets secrete growth factors and other cytokines (Singh Pawar & Toppo, 2012).

Medicinal Plants

It is necessary to identify and develop plants or chemical compounds produced from plants that can be utilized for the treatment and management of wounds. Currently, several herbal products are under investigation for this particular situation. Herbal products have been traditionally used to control and treat wounds.

Several plants with wound healing properties and active components such as flavonoids have been discovered. Tannins facilitate wound healing by many biological mechanisms, including the chelation of free radicals and reactive oxygen species, encouraging wound contraction, and enhancing the development of capillary capillaries and fibroblasts. Wound healing is enhanced by several natural products, including plant items that include active chemicals like flavonoids, alkaloids, triterpenes, and biomolecules (Singh Pawar & Toppo, 2012).

The most commonly utilized medicinal plant species for treating skin illnesses include *Chelidonium majus* L., *Ficus carica* L., *Juglans regia* L., *Malva sylvestris* L., *Ecballium elaterium* (L.) A.Rich., *Urtica dioica* L., *Juniperus oxycedrus* L., *Rosa canina* L. and *Dracunculus vulgaris* Schott. Furthermore, the plant families that are most frequently utilized for treatment are as follows: The plant families mentioned are Asteraceae, Rosaceae Lamiaceae, Euphorbiaceae, and Polygonaceae (Erarslan, Ecevit Genc, & Kultur, 2020).

Especially some species such as *Aloe vera, Brassica oleracea, Hypericum perforatum, Plantago major, Sambucus nigra, Sesamum indicum* have shown significant burn healing activities. *Hypericum perforatum*, olive oil, *Origanum* and *Salvia* genera are herbal sources used in inflammatory skin disorders and wound healing in our country (Göç & Mat, 2019).

Discussion

Herbals have gained attention in the hunt for natural therapies for common disorders such as wounds. Herbals have long been used to treat wounds throughout history, and their use in traditional medicine has persisted in many countries to this day. Although many well-established herbal medicines may be successful, it is frequently the case that patients possess more knowledge about this type of therapy than their physicians. Despite the numerous obstacles faced in the process of discovering drugs from medicinal plants, natural products derived from plants will continue to be a crucial element in the quest for novel medications. The strategic utilization of these resources and tools in bioprospecting will undeniably expedite the identification of groundbreaking lead compounds from plants by employing sophisticated drug discovery methodologies and fostering interdisciplinary collaboration.

Conclusion

Wound healing is a basic reaction to tissue injury that leads to the restoration of tissue integrity. This is mostly accomplished through the formation of the matrix that is composed of connective tissue.

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Collagen, a significant protein found in the extracellular matrix, is the main component responsible for improving the strength and resilience of wounds. The utilization of plants for therapeutic reasons in India has been extensively described in ancient literature due to its important role in human life. Wild plant intake, management, and appraisal are fundamental components of traditional knowledge in numerous human groups. Therefore, the act of plants collecting and preserving knowledge within a community are long-standing customs that have greatly contributed to the survival of numerous cultures. There is a great amount of potential for the management and treatment of wounds that can be found in plants and their extracts. Hence, it is crucial to thoroughly investigate and analyze all possible alternatives to enhance wound management. Nevertheless, it is imperative to conduct standardization, scientific validation, and safety evaluation of traditional medicinal herbs prior to its endorsement for wound healing purposes. This review focuses on the examination, depiction, and experimental exploration of native medicinal plants and their biological properties in relation to wound healing. This field is founded upon the principles of botany, biochemistry, chemistry, pharmacology, and various other disciplines that collectively contribute to the exploration and identification of naturally occurring substances with biological properties.

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THE EFFECT OF POMEGRANATE (*Punicagranatum* L.) PEEL EXTRACT ON OSTEOARTHRITIS

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Abstract

This review was conducted to describe the effect of pomegranate (*Punica granatum* L.) peel extract on osteoarthritis.

Osteoarthritis (OA) is a disease characterized by focal (initially nonuniform) loss of articular cartilage accompanied by hypertrophic reaction (sclerosis) in the subchondral bone and new bone formations (osteophytes) on the joint surface. In the early stage of osteoarthritis, chondrocytes proliferate and increase the synthesis of DNA, RNA, proteoglycan, prostaglandin, and collagen. This is the compensation period since protective factors are in effect. If the strain and load on the joints continue, damage-causing factors are active instead of repair mechanisms. Matrix metalloproteinases (MMPs) and proinflammatory stoichins secreted by synovial cells and chondrocytes are essential mediators of cartilage damage. Many studies investigate the beneficial properties of pomegranate in the prevention and treatment of osteoarthritis. The activities of pomegranate peel can be attributed to high amounts of phenolics and tannins (especially punicalagin). Punicalagin is one of the main active compounds in *Punica granatum* peel. This compound has multiple bioactivities, such as antioxidant, antibacterial, and antitumor activities. Rafrah et al. (2017) found a significant reduction in OA pain levels when using pomegranate peel hydroalcoholic extracts orally for eight weeks compared to the control group. In a different study, *P. granatum* peel had an antiosteoarthritic effect *in vivo*. In the survey by Shivnath et al. (2019), OA mice were given *Punica granatum* L. peel extract orally for 30 days. As a result of the study, it was determined that the experimental group had a significant decrease in serum alkaline phosphatase, MMP-3, and COX-2 levels compared to the control group.

Pomegranate (*Punica granatum* L.) peel extract has a solid antiosteoarthritic effect and improved disease symptoms.

Key Words: Osteoarthritis, Pomegranate, Metalloproteinases, Extract

1.Indroduction

1.1. Definition of Osteoarthritis

Osteoarthritis (OA) is a chronic rheumatic disease used interchangeably with the most frequently seen osteoarthrosis, gonarthrosis and degenerative joint disease characterized by new bone formations causing symptoms such as mechanical corrosion in joints and cartilage loss, intraarticular narrowing, pain and impairment in functionality (Hafez AR, Alenazi AM, Kachanathu SJ, et al.,2014; Biçer, 2019). Osteoarthritis is characterized by the focal loss of the joint cartilage (with the coexistent hypertrophic reaction in subchondral bone (sclerosis) and new bone growing on the joint surface (osteophyte) (Parlar Kılıç, 2016).

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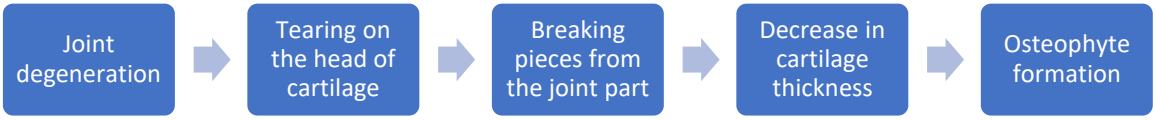

Figure 1. *Osteoarthritis Pathogenesis*

1.2. Osteoarthritis Physiopathology

In the early stages of osteoarthritis, chondrocytes proliferate and increase DNA, RNA, proteoglycan, prostaglandin and collagen synthesis. Cartilage thickening occurs in the first stage. Since protective factors are in effect, this period is defined as the compensation period. If the stress and load on the joints continues, damaging factors become more effective instead of repair mechanisms. Matrix metalloproteinases (MMP) and proinflammatory cytokines secreted from synovial cells and chondrocytes are among the most important mediators that cause cartilage damage (Parlar Kılıç 2016; Ölmez, 2011; Bilge Aİ, Ulusoy RG, Üstebay et al., 2018). New bone formation occurs in the subchondral bone through osteoclasts under the influence of these factors. These structures are harder than normal bones but have less endurance. Osteophytes develop through the metaplasia of peripheral synovial cells. Osteophytes cause movement restriction and joint deformation in patients (Parlar Kılıç 2016; Ölmez, 2011; Bilge Aİ, Ulusoy RG, ÜstebayS, et. al, 2018; Ansari, 2020). While cartilage fibrillation occurs superficially at the beginning of the disease, degeneration proceeds to deeper layers as the disease progresses (Parlar Kılıç 2016).

1.3. Osteoarthritis Treatment

There is no proven effective treatment that reverses or prevents the structural changes that occur in the disease. The aim of the treatment is to control pain and stiffness, prevent or correct disability, maintain and improve muscle strength and prevent complications. Pharmacological and non-pharmacological treatments are carried out together in the disease. Pharmacological treatments include paracetamol, COX-2 inhibitors and nonsteroidal anti-inflammatory (NSAII) drugs. Rheumatology Society, European League Against Rheumatism (EULAR) and Osteoarthritis Research Society International (OARSI) indicates non-pharmacological methods such as training, exercise, nutrition, acupuncture and herbal medicine in the guides they have issued. There are many herbal medicines and practices used in this context (Biçer, 2019; Parlar Kılıç 2016; Çeliker, 2008; Shivnath,2021; Lee, 2018; Haghighian,2021; Tuna et al., 2018).

1.4 PunicaGranatum L. and Osteoarthritis

Pomegranate is a medicinal plant that belongs to the Punicaceae family and can grow in various climates (Jurenka,2008). A pomegranate includes three main parts as peel, seeds and core and each part contains different nutrients and photochemicals. Pomegranate has many properties such as antioxidant, anti-inflammatory, antidiabetic and lipid lowering agents. Phenolic compounds in *P. granatum* are important substances responsible for therapeutic effect. Various experimental and clinical studies have investigated

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the beneficial properties of pomegranate in the prevention and treatment of osteoarthritis (Shivnath,2020; Lee, 2018; Haghighian,2021). The activities of pomegranate peel can be associated with the presence of high amount of phenolic compounds and tannin (especially punicalagin). Punicalagin is one of the main active compounds in *P. granatum*. This compound has multiple bioactivities such as antioxidant, antibacterial and antitumor activities (Tang et al 2022). In the study conducted by Shivnath et al (2019), *Punicagranatum* L. peel extract was given to mice orally for 30 days and it was determined that there was a significant decrease in serum alkaline phosphates, MMP-3 and COX-2 levels in the treatment group when compared to the control group (Shivnath,2020). In addition, the anti osteoarthritis effect of *P. granatum* peel extract was investigated and it was stated that it had the potential of preventing OA with the high level of bioactive content and strong antioxidant activity (Rawat et al., 2021).
In the study by Haghighian et al (2021), women with osteoarthritis were given pomegranate peel extract capsules having hydro alcohol orally for 8 weeks. As a result of the study, it was found that the MDA levels of the treatment group increased significantly when compared to the placebo group. In the study by Haghighian et. Al. (2016), the women with osteoarthritis were given 1 g of pomegranate peel extract capsules having hydro alcohol orally per day for 8 weeks and it was determined that the CRP levels of those in the treatment group decreased significantly when compared to the placebo group (Haghighian et al., 2016). Ghoochani et al (2015) determined in their study that drinking 200 ml pomegranate juice per day for 6 weeks decreased the serum MMP-1 and MMP-13 concentrations of those in the treatment group when compared to the placebo group (Ghoochani,2015).

2. Conclusion

A low level of inflammation and oxidative stress is important in the onset and progress of OA. Proinflammatory cytokines, especially IL-1β, IL-6 and TNF-α, are produced in OA joints highly and they have a critical function making the deformation of joint cartilage matrix a therapeutic target (Ansari, 2020). The phytochemicals in pomegranate (punicalagin, etc.) are substances providing the strongest contribution to the anti-inflammatory effect of pomegranate (Jurenka, 2008). Another possible mechanism for the benefits of pomegranate in OA is its antioxidant and free radical scavenging properties. Pomegranate shows high antioxidant activity due to the bioactive compounds it contains (Noda, 2022). These agents remove free radicals and repress in vitro lipid oxidation. Pomegranate improves inflammatory and oxidative stress parameters as well as clinical symptoms in OA patients. In addition, in vitro and in vivo studies state that pomegranate is useful in improving clinical symptoms in OA and decreasing inflammatory and oxidative indicators (Ghoochani, 2015). Consequently, pomegranate has benefits on OA by improving biochemical indicators such as clinical symptoms, inflammatory and oxidative stress and apoptotic parameters and it is thought that it may have the potential of helping the treatment of OA.

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Conflict of Interest
The authors declare they have no conflict of interest**.**

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DETECTION AND CHARACTERIZATION OF PHYTOPLASMAS AND THEIR POSSIBLE VECTORS IN THYME (*THYMBRA SPICATA* L.) IN HATAY PROVINCE OF TÜRKIYE

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Abstract

Thyme is an important crop and export product of Turkiye and accounts for 70% of world trade. Although there are some reports on fungal and viral diseases of thyme plants, limited information is available on phytoplasmas and their potential vectors. Recently some symptoms resembling phytoplasmas on thyme in Hatay province of Turkiye were observed like proliferation, paleness, leaf curling, dwarfing, reddening, yellowing drying of plants, and shoot-tip deformations (resembling dahlia). In the present study, 85 thyme, 10 bindweed (*Convolvulus arvensis*) and 34 potential phytoplasma vector insects belong to family collected from thyme fields in Hatay province tested by Nested-PCR/RFLP and sequencing analysis for phytoplasma presence and characterization. Among 85 tested thyme plants, 20 were found positive for phytoplasmas as well as 2 bindweeds and 15 insects by nested PCR. Based on RFLP analysis of 16S rDNA sequences, all phytoplasma positive samples were found related to *'Candidatus* Phytoplasma solani' (16SrXII-A) and 'Ca. P. asteris' (16SrI). Although '*Ca*. P. solani' was previously detected in thyme plants, this study is the first report for the presence of '*Ca*. P. asteris' worldwide. Detection of the same group of phytoplasmas in thymes, weeds and possible vector insects collected from the thyme fields indicates that these phytoplasmas are most likely transmitted to thyme from different host plants in the environment. Due to phytoplasma diseases of medicinal and aromatic plants severely reduce yield, quality of crops and causing changes in the composition of secondary metabolites, the impact of phytoplasma infections on medicinal and aromatic plants should be taken into account in promoting good agricultural pratices for cultivation and propagation of these plants.

Key Words: Thyme, phytoplasma, weeds, vectors, nested-PCR, RFLP

1. Introduction

Türkiye has a great agricultural potential due to its rich flora and location in the world. The production and market volume of medicinal and aromatic plants is increasing in Turkey as well as all over the world (Gül et al., 2016).There are many aromatic plant species belonging to the family Lamiaceae including thyme naturally grown in Türkiye (Bayram, 2003). Among these species, *Thymus*, *Origanum*, *Satureja*, *Thymbra* and *Coridothymus* genera have great importance both in terms of distribution and economic aspects. Thyme is one of our important export products and holds approximately 70% of the world thyme trade. Denizli province ranks first in thyme production in Türkiye followed by Uşak, Aydın and Hatay provinces (TUİK, 2023). Although thyme is a very important crop worldwide, there are not many studies on diseases and pests, affecting its production. Some fungal pathogens causing root and crown rot in thyme plants were reported as *Fusarium solani* , *F.oxysporum, R. solani, Phytophthora tentaculata* and *Verticillium spp*. (Martinez and Garcia 2007, Sato et al. 2010). During a survey study in Denizli and Manis province of Türkiye *R. solani* and *Macrophomina phaseolina* were reported as main pathogens causing root rot on thyme (Ağaner and Cer, 2017). Although not any bacteria and virus were detected in thymes, stolbur phytoplasma (*Candidatus* Phytoplasma solani-16SrXII) was detected in thymes around stolbur infected grapevine plants in Spain (Battle et al., 2000). Phytoplasmas are phloem-inhabiting bacteria discovered by Japanese scientists in 1967. They are pathogens that do not have cell walls and a specific morphological shape and cannot develop in culture media (Lee et al.,

1992). They are transmitted from plant to plant by phloem-feeding insect hosts and cause a variety of symptoms and considerable damage in many plant species. Although phytoplasmas were hosted different woody and herbaceous plants, there is not any detailed study on thymes.

The aim of this study was to investigate any phytoplasma presence in thymes related to symptoms of excessive shoot formation, leaf shrinkage, star-shaped shoot formation and as well as in weeds and possible vectors to understand transmission ways of any phytoplasmas by Nested PCR/RFLP analysis in Hatay province of Türkiy,

2. Material and Methods

a. Collection of Plant Samples and Vector Insects

A total of 85 thyme plants (*Thymra spicata* L.), 10 bindweeds (*Convolvulus arvensis*) showing phytoplasma-like symptoms, and 34 potential phytoplasma vector insects belong to *Cicadellidae* family were collected from thyme fields in Hatay province. All plant samples were immediately crushed in liquid nitrogen and insects were put in Eppendorf tubes containing 96% alcohol and kept at -20C until total DNA was extracted.

b. DNA Isolation from Plant and Insects

The total DNAs from the leaves and shoots of thymes and bindweeds were extracted using the DNA extraction kits (DNeasy Plant Mini Kit, Qiagen, Germany). DNA extraction of morphologically identified insects belonging to the family *Cicadellidae* collected from the thyme fields was performed using the Tris-NaCl-EDTA-SDS (TNES) method with some modifications according to Wasko et al., (2003).

c. Polymerase Chain Reaction (PCR) Analyses

The extracted DNA samples were amplified using nested polymerase chain reaction (PCR) using the universal primers P1/P7 in direct PCR followed by fU5/rU3 primers (Lorenz et al., 1995) in nested PCR. M1/M2 internal primer pair was also used for testing the specificity and sensitivity (Gibb et. al., 1995). For the characterization of phytoplasmas, based on the *vmp1* gene, STOLH10F1/R1 and TYPH10F/TYPH10R primers (Fialova et.al., 2009) and a group specific primers for 16SrI (R16(I)F1/R1) (Lee et al. 1995) were used. All PCRs were conducted in a thermal cycler (SimpliAmp Thermal Cycler, Thermo Fisher Scientific) in a total volume of 25 µl reaction volume containing approximately 20 ng of template, 0.5 µM of each primer, 0.2 mM of each dNTP and 0.05 U/µl Taq DNA polymerase in the buffer supplied by the manufacturer (Thermo Fisher Scientific). One microliter of the 10-fold diluted P1/P7 PCR products was used in the nested PCR (Gundersen and Lee, 1996). Thirty-five PCR cycles were performed for both P1/P7 and R16F2n/R16R2 PCR assays. PCR cycling conditions for P1/P7 primers were 40 s (3 min for the initial denaturation) at 94°C, 40 s at 56°C and 1 min and 50 s (10 min for the final extension) at 72°C. For primers R16F2n/R16R2, one cycle at 94°C for 2 min was followed by 30 cycles of 94°C for 30 s, 53°C for 1 min, and 72°C for 1 min and 30 s; and one cycle at 72°C for 10 min. A DNA template from a healthy thyme was used as negative control and RNase free water served as non-template control. PCR products were analyzed by electrophoresis through 1.2 % agarose gel, stained with ethidium bromide and DNA bands were visualized using a UV transilluminator.

d. Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) analysis of the amplified phytoplasmas 16S rRNA gene fragments was performed with *Rsa*I and *Ssp*I (Fermentas, Vilnius, Lithuania) restriction enzymes

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and patterns were compared with phytoplasma reference strains. Ten-µl of the secondary PCR amplification products were individually digested overnight at 37°C with restriction endonuclease *Rsa*I (Fermentas, Germany). The digested products were analyzed by 5% polyacrylamide gels in 1 X TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0) and DNA was visualized on a UV transilluminator after gels were stained with ethidium bromide.

3. Results and Discussion

a. Field survey

Thyme fields were observed for the presence of phytoplasma diseases in Altınözü, Yayladağı and Antakya districts of Hatay province where thyme production is very important in Türkiye. The samples were randomly taken from the thymes and weeds showing symptoms resembling phytoplasma diseases and as well as from symptomless plants around them. Insects were also collected from the same fields where symptomatic thymes were observed. The most common symptoms were redness on the stem, dwarfing, yellowing, discoloration, drying, shrinkage, curling of the leaves, as well as abnormal shape of shoot-tips, called 'dahlia' by the growers. Some symptomatic bindweeds (*Convolvulus arvensis*), were also observed as showing yellowing and leaf deformation (Fig1).

Fig 1. The symptoms of yellowing and bushy shoots in thyme plants (*Thymra spicata L.*) (on the left) deformation of shoot tips, which are popularly described as 'dahlia' (in the middle) and yellowing in bindweeds (*Convolvulus arvensis*) (on the right).

b. PCR/RFLP Analyses

In Nested-PCR analyses, 20 out of 85 thyme, 2 of 10 binweed plants and 15 insects of 34 were found to be positive for phytoplasma. Nested-PCR amplification with M1/M2 and fU3/rU5 primers produced the expected amplicon length of 509 bp and 865 bp, respectively (Fig 2).

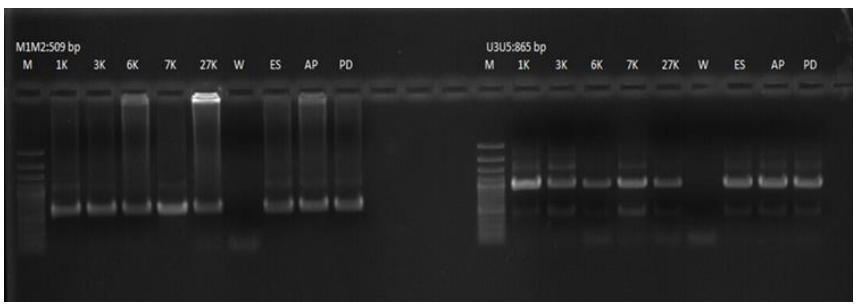

Fig 2. Gel electrophoresis of Nested-PCR analyses performed by using M1/M2 and fU3/rU5 primer pairs after the P1/P7 universal primers. M: Marker; 14K, 15K, 27K, 28K, 29K, 38K, 52K, 30K, 32K, 33K, 34K, 35K: Thyme samples ; 31K: Bindweed; ES, Ap, PD: Positive controls of European Stone Fruit Yellows, Apple proliferation and Pear decline phytoplasmas, respectively.; W: Water control

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In the Nested PCR analyses performed on P1/P7 direct PCR products using the R16IF1/R1 primer pair specific to Group I, a fragment 1100 bp long was obtained and it was determined that 3 of 85 thymes and 2 of 10 bindweeds were infected with Group I phytoplasma (Figure 3).

Fig 3. Gel electrophoresis of Nested-PCR analyzes performed by using R16IF1/R1 primer pairs after P1/P7 direct PCR. M: Marker; +C: positive control; W: Water control; 2K, 3K, 4K, 5K, 6K, 8K, 15K, 24K, 27K, 32K, 33K, 34K, 35K, 36K, 40K, 58K: Thyme samples ; 1K, 7K: Bindweeds, 20B: İnsect samples.

Direct- and Nested-PCR were performed using several primers to confirm the presence of '*Candidatus* Phytoplasma solani' ('*Ca*. P. solani') belongs to group 16Sr XII. STOLH10F1/R1 primer was used in direct-PCR to determine the *vmp1* gene, followed by TYPH10F/TYPH10R primers, which were expected to give 3 different bands between 1189 and 1438 bp in Nested-PCR. Amplicons belong to one out of 85 thyme samples and 1 out of 10 bindweeds were found identical to '*Ca*. P. solani' positive controls.

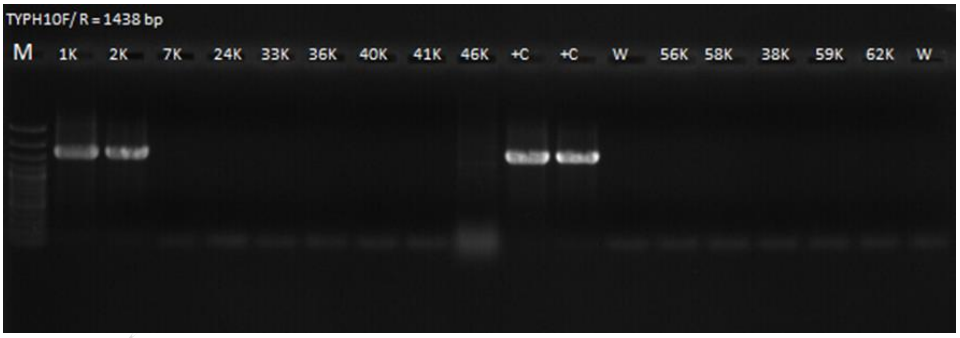

Fig 4. Gel electrophoresis of Nested-PCR analyzes performed by using TYPH10F/ TYPH10F R primer pairs after STOLH10F1/R1 Direct-PCR. M: Marker; +C: positive control; W: Water control; 2K, 3K, 4K, 5K, 6K, 8K, 15K, 24K, 27K, 32K, 33K, 34K, 35K, 36K, 40K, 58K: Thyme samples ; 1K, 7K: Bindweeds.

The conventional RFLP analyses of the amplicons obtained with U3f/U5r primers showed that all phytoplasma isolates obtained from thyme, bindweeds and insects were differentiated from ESFY, AP and PD but identical with 16SrI phytoplasma group when digested by *Rsa*I restriction enzyme (Fig 5).

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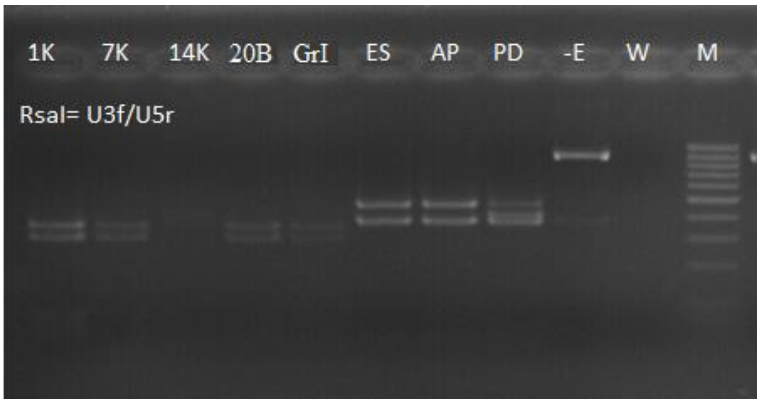

Fig 5. Restriction fragment length polymorphism (RFLP) analysis of U3f/U5r PCR product of 16S rRNA genes by using enzyme *Rsa*I. M: Marker; 1K,7K: Bindweeds, 14K: Thyme, 20B: Insect; GrI: Positive control of 16SrI group phytoplasma, ES: Positive control for European Stone Fruit Yellows; AP: Positive control for Apple proliferation; PD: Positive control for Pear decline ; -E: No enzyme ; W: water control

As a result of these molecular analyses, 16SrI ('*Ca.* P. asteris') and 16SrXII ('*Ca.* P. solani') phytoplasmas in both thyme, bindweed plants and insects collected from thyme fields were detected and characterized. It is the first report for the presence of '*Ca.* P. asteris' in thyme plants. Although there is not any detailed study on the presence of phytoplasmas in thyme plants all over the world, there is one report from Spain on the determination of stolbur phytoplasma in one thyme plants collected from the vicinity of vineyards infected with stolbur phytoplasma (Battle et al., 2000). It is also known that bindweeds are a good host for different phytoplasma groups including 16SrI-B, 16SrVII-A ve 16SrXII-A (Longone et al., 2011) however this study first time showed that stolbur (16Sr XII) and aster yellows (16SrI) phytoplasmas are hosted by thyme, bindweeds and vector incests (Cicadellidae family) in the same environment.

4. Conclusion

In this study, phytoplasmas belong to 16SrI-B and 16SrXII-A groups were detected and identified in thyme, bindweed plants and also possible vector insects of these phytoplasmas. Thyme was found a new host for aster yellows phytoplasma first time with this study. In these thyme fields, production is mostly performed based on traditional methods collecting mother plants from the nature and propagated by cuttings. Therefore, if the mother plants used are infected with phytoplasma, there is a high possibility that the newly established production areas will also be infected with phytoplasma. On the other hand, lack of weed and insect control makes it difficult to control phytoplasma diseasest in thyme plantations. Although the first evidence on the correlation of 'dahlia' symptoms in thyme plants and phytoplasmas were obtained in this study, more detailed studies are needed to confirm this hypothesis.

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Conflict of Interest
There is no conflict of interest.

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CYTOTOXIC POTENTIAL OF *DORYCNIUM SANGUINEUM* EXTRACTS

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Abstract
In recent years, many researchers have focused on medicinal plants as they are natural resources that represent significant potential in the development of new and pharmacologically active compounds. A wide range of disorders, from diabetes to cancer, are treated and/or prevented by using plant extracts/phytochemicals obtained from different plant parts (leaves, flowers, bark or others) via various techniques. There are 13 species of the genus *Dorycnium* worldwide, including 7 species in Turkey, and it is a member of the Fabaceae. There are relatively limited investigations on *Dorycnium* species, even though numerous studies on the various biological activities of Fabaceae species have been conducted. This study aims to investigate the cytotoxic potential of endemic *Dorycnium sanguineum* extracts using different extraction methods. For this purpose, the extracts obtained by maceration and infusion techniques were applied to different cancer cell lines (HL-60 and DLD-1). The cytotoxic potential of the extracts was evaluated via the MTT test and their cytotoxic potentials were determined by comparing the absorbance values of the control group. It was found that the extracts had dose- and time-dependent cytotoxic effects and the methanol maceration extract had the highest potential. Although this is a pioneering study evaluating the cytotoxic capacity of *D. sanguineum* extract, further research is required to elucidate the molecular basis of this potential.

Key Words: Endemik, MTT, Kızıl Kaplanotu.

1. Introduction

Treatment with plants dates back to ancient times. Herbal extracts and various phytochemicals isolated from plants are frequently preferred in treating many diseases. The most well-known and deadly of these diseases is cancer. In cancer treatment, plants are used as complementary and/or alternative. Today, many drugs actively used in cancer treatment are of plant origin and continue to be used successfully. Examples of these are vinca alkaloids, taxol and flavopyridol. Natural product derivatives account for up to 60% of anticancer candidates that have demonstrated noteworthy efficacy in clinical utilization for cancer treatment [1]. They are good sources of lead compounds and reasonably less-priced ingredients for modern drug development. Plant-based natural compounds are less likely to generate adverse effects, perhaps because they mimic substances found in the human diet that are very prone to cause tolerance. They start, stimulate, or alter the metabolic pathways that impact cancer cells' capacity to divide, move, and undergo apoptosis through a range of biological processes. Consequently, it should not be surprising that phytoconstituents are the primary sources of chemotherapeutic drug research in preclinical and clinical cancer investigations.

From the beginning to the end of the drug research process, which can take 12 to 15 years and cost up to $2.8 billion, [2-4]. The cytotoxicity of natural products and isolated compounds is one of the primary problems in pharmaceutical research and development [5,6]. Numerous research has looked at the in vitro and in vivo cytotoxic capabilities of plant extracts or isolates against different cancer cell lines. Therefore, a comprehensive investigation is usually required while looking for safer natural medicines. The preclinical and clinical phases are crucial, required stages in the drug development process for novel chemical entities. They evaluate the test molecule's safety and efficacy and try to forecast any possible side effects that could result from therapy.

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Toxicology studies are also necessary for predicting toxicokinetic profiles, identifying genetic anomalies, identifying potential side effects, and establishing the relationship between a dose and important organs. The family Fabaceae (Leguminosae) includes the genus *Dorycnium*, which is found across Asia and Europe [7,8]. There are a total of thirteen species within the genus *Dorycnium*, with seven of them naturally occurring in Turkey [8]. Fabaceae family members have long been employed in traditional medicine to address various ailments such as, arthritis, rheumatism, inflammation, , hemorrhoid, neoplasm, , asthma, bronchitis, liver diseases and urinary tract infections [9-11]. Research indicates that the Fabaceae family is known for its abundant presence of phenolic acids and flavonoids [12,13]. *Dorycnium* plants, belonging to the *Dorycnium* genus, have been found to produce numerous biologically active compounds. These compounds have demonstrated various properties such as antimicrobial, anti-inflammatory, antioxidant and cytotoxic effects [7,9,14]. Over time, *Dorycnium* species have been utilized in traditional medicine for their medicinal properties [9-11]. The presence of polyphenolic compounds in these plants is believed to be responsible for their beneficial activities, including the aforementioned properties [7,9,14]. *Dorycnium sanguineum* Vural is a naturally distributed and endemic species in Türkiye and according to our literature review, there is no study evaluating the cytotoxic effect of the species. The aim of this study was to determine the cytotoxic potential of *Dorycnium sanguineum* which are prepared with different extraction techniques, on leukemia and colorectal cancer cell lines.

2. Material and Methods

2.1. Plant material collection and extract preparation

The plant material was collected from Karaman Bucakkışla, Çukur village, forest clearings and identified by Prof. Dr. Kuddisi ERTUĞRUL. The sample specimen was stored at KNYA herbarium (KE-7049). The plant material was gathered underwent a thorough cleaning process and was subsequently dried away from direct exposure to sunlight. Following this, the material was finely ground and subjected to two distinct extraction methods, maceration and infusion. Methanol and water were used as a solvent in maceration and the infusion method was used to determine whether it could be used as tea. The solvents were separated via a rotary evaporator. Crude extracts were stored at -20°C till use.

2.2. Extract yield calculation

The % efficiency calculations for extracts will be made according to the formula below;

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

A1, as indicated in the formula, represents the weight of the dried extract after the solvent has been removed, while A2 represents the dry weight of the plant material.

2.3. Cell culture and MTT Assay

HL60 (human leukaemia) and DLD1 (human colorectal cancer) cell lines were used to determine the cytotoxic activity of the extracts. Cells were routinely cultured in RPMI 1640 medium containing 1% (v/v) penicillin-streptomycin and 10% (v/v) heat-inactivated fetal bovine serum (FBS) and were grown under conditions at 5% CO_2 and 37 °C. The extracts were administered to the cell lines at varying concentrations (ranging from 0 to 1 mg mL^{-1}) and time durations (24h and 48 h). The cytotoxicity of the extracts was assessed through the MTT assay. The plates were then analyzed using an ELISA reader at a wavelength of 540 nm. The impact of the extracts on cell viability was determined by comparing the absorbance values with the control group (untreated). The experiments were conducted in triplicate, with a minimum of two replicates per plate. The mean values for cell viability were taken into consideration. Statistical analysis was carried out using Graph Pad Prism 9.

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2. Results and Discussion

3.1. Extract yield calculation

The extracts obtained were coded according to the solvent and extraction method. Then extraction yields were calculated and given in Table 1.

Table 1. % yields of *D. sanguineum* extracts

Solvent-Extraction method	Code	Extraction yield (%)
Methanol-Maceration	DSMM	11,1
Water-Maceration	DSWM	16,3
Water-Infusion	DSWI	11,4

When the extracts were evaluated in terms of extraction yield, the highest yield was determined in the water maceration extract and relatively lower yields were calculated in methanol maceration and water infusion extracts.

a. MTT Assay Results

In this work, D. sanguineum extracts' cytotoxic properties were evaluated against the DLD-1 and HL-60 cell lines during a 24- to 48-hour incubation period. There have been several different extract concentrations employed, ranging from 0.0625 to 1 mg mL−1. The cytotoxic activity of the extracts was assessed using the MTT test.

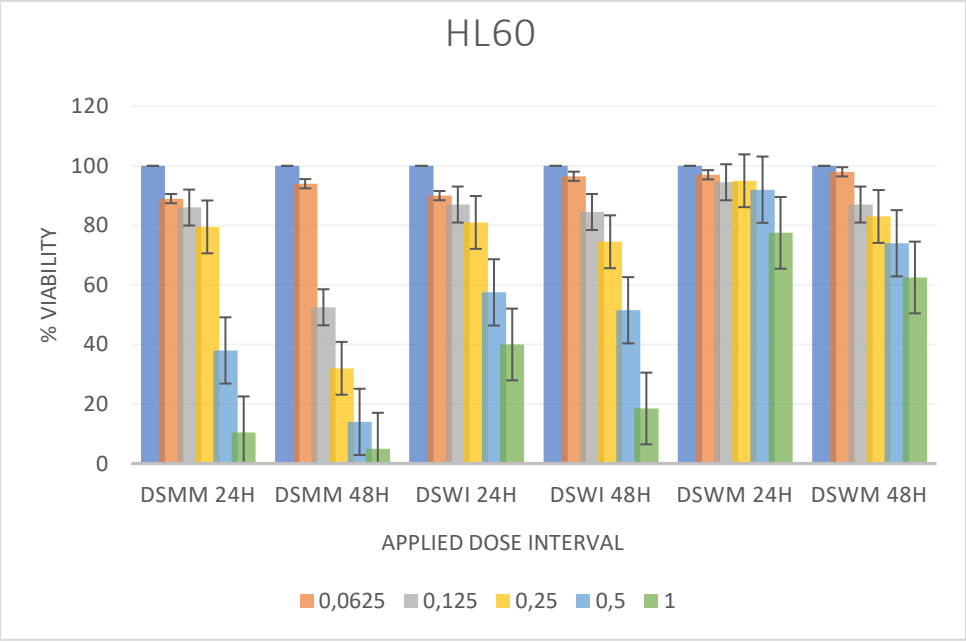

Figure 1. MTT assay graphs of *D.sanguineum* extracts on HL-60 cell line (DSMM: *D. sanguineum* Methanol-Maceration; DSWM: *D. sanguineum* Water-Maceration; DSWI: *D. sanguineum* Water-Infusion).

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The results showed that both extracts exhibited dose- and time-dependent cytotoxic action against all applied cancer cell types (Figure 1). The administered extracts had varying effects on various cell lines. Based on the extracts' 24- and 48-hour IC50 values, we can conclude that the HL-60 cell line has the lowest values.

First of all, if we evaluate the cytotoxic effects of the extracts on the HL-60 cell line, we can say that the methanol maceration extract is more effective than other extracts. On the other hand, the least cytotoxic effect was observed in the water maceration extract, which can be explained by the difference in solvent. The extracts did not show a cytotoxic effect on the DLD-1 cell line within the applied dose and time range (IC_{50} greater than 1 mg/ml). The extracts were also applied to the L929 fibroblast cell line to see how the obtained extracts act on the healthy cell line and to evaluate the selectivity status, and it was determined that the cytotoxic effect was very low at the applied dose and time interval (Figure 2). While a cytotoxic effect was observed on cancer cells, no cytotoxic effect was observed on healthy cells, which is preferable and desirable.

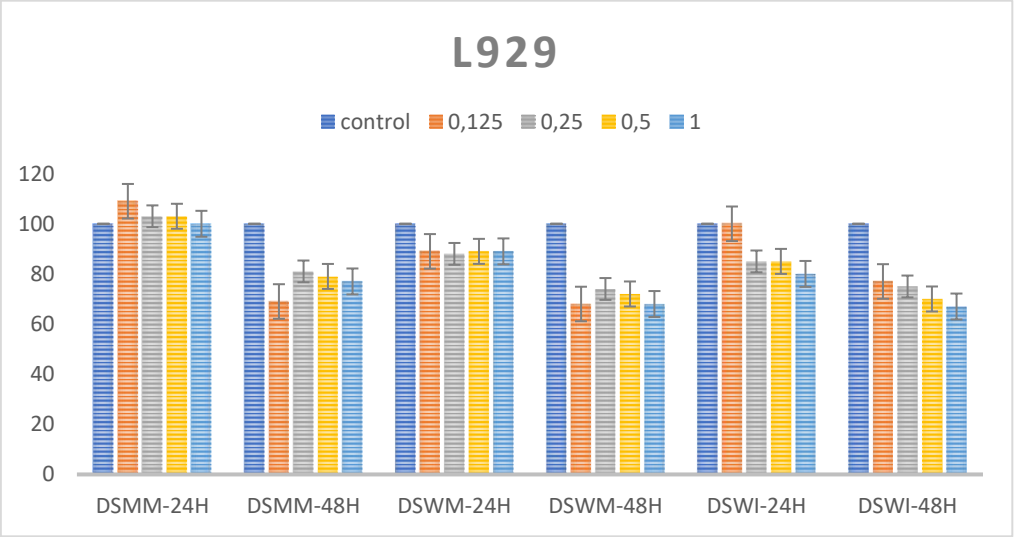

Figure 2. MTT assay graphs of *D.sanguineum* extracts on HL-60 cell line (DSMM: *D. sanguineum* Methanol-Maceration; DSWM: *D. sanguineum* Water-Maceration; DSWI: *D. sanguineum* Water-Infusion).

Two recent publications reported on the cytotoxic characteristics of *D. pentaphyllum* extract; however, there is no cytotoxic activity investigation about *D. sanguineum* species in the literature. The cytotoxic activity of this plant on human breast, liver, and lung cancer cells was reported [15]. In the cancer cell lines that were investigated, the IC_{50} values were found to range from 100.4 to 298.5 ug/mL. The cytotoxic impact was examined on human cervical (HeLa) and colon (WiDr) cancer cells in a second investigation by Demir et al. [16]. The IC_{50} values were discovered to be 46.5 and 84.5 ug/mL, respectively. In another report, the cytotoxic effects of *D. pentaphyllum* extracts on human cervical cancer cell lines were investigated and it was reported that the extract showed a selective cytotoxic effect (6.5-fold) on HeLa cells compared to normal fibroblast cells [17]. Scientific research on the cytotoxic, antiproliferative, and molecular effects of *Dorycnium* species in preventing the formation of tumours in different cancer cell lines is still lacking, nevertheless.

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5. Conclusion

In conclusion, in this study, which is still in its early stages, more cell lines need to be used to fully reveal the cytotoxic effect caused by *D. sanguineum* extracts. The results clearly show that further research on the species included in the study is required. In our further research, we will identify the molecular cause of cell death. From now on, our attention will focus on the identification of the active ingredient.

Conflict of Interest

The authors declare no conflicts of interest.

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THERAPEUTIC POTENTIAL AND CHEMICAL COMPOSITION OF *TANACETUM POLYCEPHALUM* SUBSP. *ARGYROPHYLLUM*

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Abstract
The purpose of the investigation was to determine the tyrosinase, elastase and cyclooxygenase enzyme inhibition activities and chemical constituents of above-ground of *Tanacetum polycephalum* subsp. *argyrophyllum* gathered from Van province of Turkey in June 2021. The plant extracts were prepared with *n*-hexane, ethyl acetate and ethanol using the maceration method. The essential oil was extracted using the Clevenger apparatus. The chemical profile of the extracts was evaluated by LC-MS/MS, and the chemical profile of the essential oil was evaluated by GC and GC-MS. The inhibitory activity of the samples against tyrosinase was tested at concentrations ranging from 100 to 500 µg/ml, and the extracts showed higher inhibition activity than the essential oil. While the extracts had no inhibitory effect on elastase, the essential oil had a weak effect at 500 µg/ml. None of the samples had a significant inhibitory effect on cyclooxygenase enzymes at 100 µg/ml. The yield of essential oil was found to be 1%. Thirty-five compounds were detected in the essential oil. Camphor (29.68%), di-tert-butyl(methylamino)borane (24.25%), chrysanthenyl acetate (17.71%), bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl acetate (16.64%) and cyclopentan-1-al, 4-isopropylidene-2-methyl (7.22%) were defined as main constituent of the essential oil. Cyranoside, quinic acid, chlorogenic acid, cosmosiin, luteolin, acacetin and protocatechuic acid were the major compounds of the extracts.

Key Words: *Tanacetum polycephalum* subsp. *argyrophyllum*, anti-tyrosinase, anti-elastase, anti-cyclooxygenase, LC-MS/MS, GS-MS

1. Introduction

Tanacetum genus is third-largest genus of Asteraceae family. *Tanacetum* species have been found all over Europe, the temperate zone of Asia and North America, with 160 species worldwide (Behjou et al., 2022; Khatib et al., 2023). *Tanacetum* species have been used for many years for a number of health problems such as diabetes, migraines, gall bladder inflammation, high blood pressure, gastrointestinal disorders, ringworm and sexually transmitted diseases (Khatib et al., 2023). In literature studies, *Tanacetum* species have been reported to have anti-diabetic, anti-oxidant, anti-cancer, anti-inflammatory, anti-helmintic, hepatoprotective and immunomodulatory activities (Khatib et al., 2023). More than 240 secondary metabolites have been recognised in *Tanacetum* species, consisting of volatile constituents, phenolic acids, flavonoids, fatty acids, coumarins, ceramides, vitamins and carbohydrates (Kisiel and Stojakowska, 1997; Gören et al., 2001; Hussain et al., 2010; Eyol et l., 2017; Yapıcı et al., 2021; Khatib et al., 2023).

Tanacetum polycephalum subsp. *argyrophyllum* was called Borzhan and the infusion of its capitulum was used in the region of Iraq for the treatment of colds (Kawarty et al., 2020). The water decoction of the above-ground of the plant has been used together with *Thymus* and *Achillea* as a treatment for gastroenteritis (Mosaddegh et al., 2012). It has also been utilised as a traditional folk remedy in Iran for the cure of haemorrhoids and inflammation (Ghasemi et al., 2013). In a previous paper, the plant was reported to have anti-cancer activity against MCF-7, HepG-2, A-549, and HT-29 cells (Naghibi et al., 2014).

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Free radicals are produced by a number of environmental factors such as pollution, smoking and UVA and UVB radiation. These products trigger the activity of tyrosinase and elastase, which are responsible for skin dryness, wrinkles, hyperpigmentation and inflammation (Castro-Jácome et al., 2019). COX enzymes, which have two isoforms, COX-1 and COX-2, participate in synthesising prostaglandins by metabolising arachidonic acid. Excessive synthesis of prostaglandins leads to a number of inflammatory diseases such as asthma and neurodegenerative disorders. Therefore, inhibition of these enzymes will protect against the occurrence of inflammation-related diseases (Wenzel, 1997; Choi et al., 2009).

The goal of the current work was to reveal the tyrosinase, elastase and cyclooxygenase inhibition potential of extracts prepared with solutions of different polarities and essential oil of the aerial part of *Tanacetum polycephalum* subsp. *agyrophyllum* and to characterize their chemical compounds.

2. Material and Methods

2.1. Plant material

T. polycephalum subsp. *argyrophyllum* was harvested from Tuşba, Van, Turkey in June 2021. The plant material was identified by Assoc. Prof. Dr. Hüseyin Eroğlu (Yuzuncu Yıl University, Faculty of Science, Department of Biology). The specimens were preserved in Yuzuncu Yil University Herbarium.

2.2. Essential oil extraction by hydro-distillation

50 g of dried aerial part of the plant was distilled with water for three hours using the Clevenger apparatus. The essential oil was maintained at +4°C until the experimental studies began.

2.3. GC and GC-MS analysis conditions

Chemical characterization analysis of essential oil was executed simultaneously using the Agilent 6890N GC system and the Agilent 5975 GC-MSD system with GC and GC-MS/MS. The compounds separated in the column of the GC system were detected with the FID detector, the relative percentages of the constituents were calculated, and the mass spectra of the compounds separated in the column of the GC/MS system were recorded one by one in the mass spectrometry section. The evaluation procedures of the essential oil were carried out at Van Yuzuncu Yil University Science Application and Research Centre.

2.4. Preparation of extracts

15 g of dried above-ground of the plant have been finely ground and macerated separately with 200 mL of *n*-hexane, ethyl acetate and ethanol for 48 hours and then filtered through filter paper. The filtrates were combined and evaporated using a rotary evaporator. The samples were kept at -18°C.

2.5. LC-MS/MS analysis conditions

n-Hexane, ethyl acetate and ethanol extracts of above-ground of *T. polycephallum* subsp. *agryphyllum* were characterised by LCMS/MS method. Chemical characterization of the extracts was analyzed quantitatively and qualitatively with 56 standard compounds, and the analysis conditions were described in detail with the procedure of Yılmaz (2020).

2.6. Tyrosinase enzyme inhibition assay

The tyrosinase inhibition experiment was adapted from the procedure developed by Chang et al. (2007). Kojic acid was selected to be a reference substance. Enzyme kinetics were examined in a 96-well microplate. Kinetic absorbance readings were taken at 30-second intervals with a microplate reader to determine the linear change in absorbance at 475 nm during dopachrome formation.

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2.7. Elastase enzyme inhibition assay

The elastase inhibition test of extracts was performed by modification of the method of Melzig et al. (2001). Epigallocatechin gallate was tested as a control agent. Spectophotometric measurements of the samples were taken at 405 nm.

2.8. Cyclooxygenase enzyme inhibition assay

The samples were screened for COX inhibitory potential by adaptation of the Cayman COX Colorimetric Inhibitor Screening Test Kit assay. After applying the kit procedure, the samples were read at 590 nm in a microplate reader. Standard inhibitors used for COX-1 and COX-2 enzymes were SC-560 and rofecoxib, respectively.

3. Results and Discussion

3.1. GC and GC-MS/MS analysis findings

The yield of essential oil was calculated to be 1%. The chemical characterization of the essential oil was 100% elucidated (Table 1).

Table 1. GC and GC-MS/MS characterization of essential oil

RT	Compound	%
4.10	2-Dehydro-1,8-cineol	0.07
4.17	1,3,5-Trimethylbenzene	0.31
4.29	2,6-Dimethylstyrene	0.04
4.35	α-Terpinen	0.13
4.58	di-Tert-buthyl(methylamino)borane	24.25
4.75	γ-Terpinene	0.24
4.88	dl-Limonene	0.09
5.02	4-(Methoxymethyl)benzaldehyde	0.10
5.23	Cyclohexene, 1,2-dimethyl-	0.44
5.43	Cyclopentane-1-al,4-isopropylydene-2-methyl	7.22
5.83	Camphor	29.68
6.46	Chrysanthemyl acetate	17.71
6.73	Verbenyl acetate	16.64
6.98	Endobornyl acetate	0.69
7.27	*	0.03
7.32	(1S)-6,6-Dimethylbicyclo(3.1.1)hept-2-ene-2-methanol acetate	0.05
7.67	trans-Chrysanthemyl acetate	0.09
8.25	Aromadendren	0.04
9.11	cis/trans-7-Bicyclo[4.1.0]hept-7-ylidene-bicyclo[4.1.0]heptane	0.04
9.22	Bicyclo[3.1.1]hept-2-ene, 7,7-dimethyl-2-(1-hydroxyethyl)	0.22
9.39	2,2,6-Trimethyl-1-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]heptan-3-ol	0.04
9.63	(+) spathulenol	0.89
10.43	Cedr-8-ene	0.81
10.98	Patchoulene	0.03
12.33	(-)-Caryophyllene oxide	0.02
13.74	Andrographolide	0.01
13.94	Hexadecanoic acid	0.03
14.77	Methyl eicosa-5,8,11,14,17-p-entanoate	0.01
16.61	α-Podocarpene	0.01
19.20	Ethyl linoleolate	0.02
20.15	1-Hydroxy-6-(3-isopropenyl-1-cyclopropen-1-enyl)-6-methyl-2-heptanone	tr
21.52	17-Hydroxy-3-oxoandrost-4-ene-17-carbonitrile	tr
25.74	11-n-Decyldocosane	0.01
28.64	n-Dotriacontane	0.01
31.38	Lucenin 2	0.02

RT, Retention time, % Percentage calculated from FID data, *, not detected tr, trace amounts

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Thirty-five compounds were recognised in the oil. The principal constituents of the essential oil were: camphor (29.68%), di-tert-butyl(methylamino)borane (24.25%), chrysanthenyl acetate (17.71%), bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl acetate (16.64%) and cyclopentan-1-al, 4-isopropylidene-2-methyl (7.22%) (Table 1). Najafi et al. (2007) compared the essential oils of pre-flowering and flowering above-ground of *T. polycephalum* subsp. *argyrophyllum* collected in Iran. They calculated the yields of these oils to be 0.22% and 0.47%, respectively. They found that the major components of the oils before and during flowering were camphor (36.1% and 18.5%), pinocarvone (20.1% and 31.4%), *α*-pinene (8.6% and 9.5%), *p*-cymene (9.2% and 0.5%) and bornyl acetate (8.8% and 5.9%). In the same report, 1,8-cineol (18.5%) was detected only in whole flower oil (Najafi et al., 2007). Based on literature data, the principal chemical constituents of the essential oils of *Tanacetum* species were mostly rich in oxygenated monoterpenes and included the following compounds; camphor, bornyl acetate, *α*-phellandrene, chrysanthenyl acetate, *α*-terpinene, *p*-cymene, terpinen-4-ol, *α*-terpineol, tetradecane, caryophyllene oxide, *α*-thujene, *α*-pinene, camphene, *β*-pinene, *p*-cymene, limonene, *γ*-terpinene, benzaldehyde, sabinene, pinocarvone, borneol myrtenal, *β*-caryophyllene, (e)-*β*-farnesene, valensene, *β*-bisabolene, thymol and carvacrol (Kumar and Tyagi, 2013). The diversity in the chemical content of the essential oil of *Tanacetum* species may be correlated with the analysed part, the collection region, the vegetation period and the environmental conditions.

3.2. LC-MS/MS analysis findings

The components found in the *n*-hexane extract of *T. polycephalum* subsp. *argyrophyllum* were luteolin, apigenin and acacetin in trace amounts (<0.1). Major compounds of the ethyl acetate extract (mg/g extract): quinic acid (2.988), luteolin (0.623), cyranoside (0.574), acacetin (0.416), protocatechuic acid (0.368), quercetin (0.236), apigenin (0. 228), cosmociin (0.163) and traces (<0.1) of salicylic acid, chlorogenic acid, protocatechin aldehyde, caffeic acid, vanillin, p-coumaric acid, hesperidin, naringenin, hesperetin, kaempferol. The most abundant compounds present in the ethanol extract (mg/g extract) were cyranoside (10.716), chlorogenic acid (2.872), cosmociin (2.656), luteolin (2.182), acacetin (2.072), protocatechuic acid (1.138), quercetin (0.871), apigenin (0.63), caffeic acid (0.651), protocatechuic aldehyde (0.343), 4-OH-benzoic acid (0.284), (0.63), caffeic acid (0.651), protocatechuic aldehyde (0.343), 4-OH-benzoic acid (0.284), vanillic acid (0.229), vanillin (0.241), p-coumaric acid (0. 235), salicylic acid (0.388), isoquercitrin (0.251), naringenin (0.197), hesperetin (0.114) and trace amounts (<0.1) of gentisic acid, astragalin, kaempferol, and hesperidin (Table 2). The present study was consistent with the results of previous chemical composition studies on *Tanacetum* species (Yur et al., 2017; Attia et al., 2023).

Table 2. LC-MS/MS characterization of extracts

No	Analyte	*n*-Hexane extract (mg analite/g extract)	Ethyl acetate extract (mg analite/g extract)	Ethanol extract (mg analite/g extract)
1	Quinic acid	-	2.998	2.995
2	Fumaric aid	-	-	-
3	Aconitic acid	-	-	-
4	Gallic acid	-	-	-
5	Epigallocatechin	-	-	-
6	Protocatechuic acid	-	0.368	1.138
7	Catechin	-	-	-
8	Gentisic acid	-	-	0.035
9	Chlorogenic acid	-	0.041	2.872
10	Protocatechuic aldehyde	-	0.072	0.343
11	Tannic acid	-	-	-
12	Epigallocatechin gallate	-	-	-

13	Cynarin	-	-	-
14	4-OH Benzoic acid	-	-	0.284
15	Epicatechin	-	-	-
16	Vanilic acid	-	-	0.229
17	Caffeic acid	-	0.04	0.651
18	Syringic acid	-	-	-
19	Vanillin	-	0.03	0.241
20	Syringic aldehyde	-	-	-
21	Daidzin	-	-	-
22	Epicatechin gallate	-	-	-
23	Piceid	-	-	-
24	p-Coumaric acid	-	0.039	0.235
25	Ferulic acid-D3-IS[h]	-	*	*
26	Ferulic acid	-	-	-
27	Sinapic acid	-	-	-
28	Coumarin	-	-	-
29	Salicylic acid	-	0.019	0.388
30	Cyranoside	-	0.574	10.716
31	Miquelianin	-	-	-
32	Rutin-D3-IS	*	*	*
33	Rutin	-	-	-
34	isoquercitrin	-	-	0,251
35	Hesperidin	-	0.029	0.031
36	o-Coumaric acid	-	-	-
37	Genistin	-	-	-
38	Rosmarinic acid	-	-	-
39	Ellagic acid	-	-	-
40	Cosmosiin	-	0.163	2.656
41	Quercitrin	-	-	-
42	Astragalin	-	-	0.031
43	Nicotiflorin	-	-	-
44	Fisetin	-	-	-
45	Daidzein	-	-	-
46	Quercetin-D3-IS	*	*	*
47	Quercetin	-	0.236	0.871
48	Naringenin	-	0.083	0.197
49	Hesperetin	-	0.038	0.114
50	Luteolin	0.002	0.623	2.182
51	Genistein	-	-	-
52	Kaempferol	-	0.021	0.052
53	Apigenin	0.003	0.228	0.63
54	Amentoflavone	-	-	-
55	Chrysin	-	-	-
56	Acacetin	0.184	0.416	2.072

IS: Internal standard, -: Not detected, *: Not applicable

3.3. Tyrosinase enzyme inhibition findings

Samples were tested at concentrations of 100, 200, 400 and 500 µg/ml. The tyrosinase inhibition values of extracts (100-500 µg/ml) were as follows: *n*-hexane extract, 16-67%; ethyl acetate extract, 0-53%; ethanol extract, 2-62% and essential oil, 0-13%. It was observed that *n*-hexane extract had better tyrosinase inhibition at both low and high concentrations compared to other extracts (ethanol and ethyl acetate extracts). Kojic acid exhibited 75% inhibition at a concentration of 100 µg/ml (Table 3).

In previous publications, extracts obtained from various parts (capitulum, aerial part, leaf-root part, leaf, root, flower parts) of *Tanacetum haussknechtii* Bornm. Grierson, *Tanacetum poteriifolium* Grierson, *Tanacetum vulgare* L., *Tanacetum balsamita* L., *Tanacetum parthenium* L., *Tanacetum audibertii* (Req.) DC., and *Tanacetum macrophyllum* (Waldst. et Kit.) Schultz. Bip. were reported to have anti-tyrosinase activity. Based on these publications, it was concluded that extracts with abundant flavonoids

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and phenolic compounds prepared with water, ethanol and methanol solvents have significant tyrosinase inhibitory potential (Yur et al., 2017; Chiocchio et al., 2018; Zengin et al., 2019; Zengin et al., 2020; Gevrenova et al., 2020; Ak et al., 2021; Gevrenova et al., 2022). Cyranoside (syn. luteolin-7-O-glycoside) was determined in the present study to be the most abundant in the extract, and luteolin-7-O-glycoside was reported to have 32% inhibition of tyrosinase at concentration of 100 µg/ml (Sezen Karaoğlan et al., 2023). In previous studies, luteolin derivatives were reported to have photoprotective activity against UV-ABC (Fischer et al., 2011). Furthermore, gels prepared from cyranoside-rich plants were reported to have anti-inflammatory and anti-allergic effects (Szekalska et al., 2020).

There were not adequate studies on the anti-tyrosinase potential of essential oils of *Tanacetum* species. To date, only *T. haussknechtii* essential oil, which is rich in monoterpenoids, has been determined to have a weak inhibitory effect on tyrosinase (Yur et al., 2017).

Table 3. Anti-tyrosinase activity results of extracts and essential oil

Sample	% Inhibiton			
	100 µg/mL	200 µg/mL	400 µg/mL	500 µg/mL
n-Hexane extract	16.61±1.56	60.95±2.00	64.41±1.10	67.05±1.49
Ethyl acetate extract	-	10.00±0.88	33.82±1.90	53.52±0.41
Ethanol extract	2.05±0.72	14.11±1.10	49.70±1.39	62.05±2.16
Essential oil	-	-	-	13.04±0.79
Kojic acid	75.33±1.24	*	*	*

-: No activity, *: Not tested

3.4. Elastase enzyme inhibition findings

While the extracts were unable to inhibit elastase, the essential oil had a weak effect (13%) at 500 µg/ml.

3.5. Cyclooxygenase enzyme inhibition findings

The inhibitory effect of the samples on cyclooxygenase enzymes was studied in triplicate at a concentration of 100 µg/ml. Since all samples (<5%) did not have a significant inhibitory effect on cyclooxygenase enzymes.

4. Conclusion

Based on the findings of the present study, *T. polycephalum* subsp. *argyrophyllum* can be recommended as a source of natural pharmaceutical raw materials for skin improvement products. As far as we researched, the inhibition potency of *T. polycephalum* subsp. *argyrophyllum* on tyrosinase, elastase and COX enzymes and its chemical constituents have been characterized for the first time from Turkey.

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Conflict of interest

No conflict of interest between authors.

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MORPHOLOGICAL DIVERSITY AMONG MEDICINAL WILD BARBERRY (*BERBERIS VULGARIS* L.) FRUITS

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Abstract
Berberis vulgaris L., is a shrub which produces berries. In the present study, 9 wild individuals of this fruit species from different regions of Isfahan province from the center of Iran were evaluated using 18 morphological characters. For some morphological characters, variability found ranged from 0.5 to 1.6 cm in fruit length, 0.4 to 0.9 cm in fruit width and 0.12 to 0.44 g in fruit weight. In addition, color of mature fruit were varied from light yellow to dark red. Barberry fruit weight has a positive and significant correlation with seed length(r = 0.5). Barberry color showed negative correlation with fruit length (r = -0.79) and positive correlation with leave color (r= 0.74). Furthermore, Number of fruits per panicle showed negative correlation with seed width(r = -0.73). Also fresh fruit weight has positive correlation (r= 0.79) with blade length. The findings from the study highlight the necessity of conserving the individuals under examination as precious genetic assets. Moreover, enriching the genetic diversity of wild barberry is crucial for identifying numerous valuable, well-adapted specimens suitable for production.

Key Words: Diversity, Morphological traits, Barberry

1. Introduction

The Berberis genus, a member of the Berberidaceae family, encompasses a vast array of approximately 650 species distributed across 15 genera. This botanical diversity is primarily found in regions spanning Asia, Europe, North Africa, and South America (Ahrendt, 1961). Among these species, barberry stands out as a small fruit with remarkable medicinal properties. Its prevalence is particularly notable in the natural habitats of Asia and Europe, where it has been cultivated and harvested for centuries, contributing significantly to traditional medicinal practices (Chauhan et al., 1999).

The historical significance of barberry in folk medicine spans over two millennia, tracing back to ancient civilizations (Sun et al., 2021). For instance, records indicate that the ancient Egyptians ingeniously combined barberry with fennel seeds to ward off pests, showcasing early experimentation with its versatile properties. Furthermore, historical documents from Babylonia dating back to 650 BCE highlight the recognition of barberry's medicinal virtues, illustrating its longstanding reputation as a therapeutic agent (Arayne et al., 2007),

By the 7th century CE, barberries had firmly established themselves as a common element in traditional medicine across the Middle East. From combating infectious fevers to addressing the scourge of typhus, these fruits became integral components of therapeutic regimens, attesting to their enduring relevance in medical practices throughout history (Ebrahimi-Mamaghani et al., 2009). The present study was conducted to investigate significant disparities in morphological traits among various wild barberries, commonly referred to as Seedy barberry in Iran with the aim of evaluating its potential for domestication and and also use the medicinal properties of those in future.

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2. Material and method

2.1. Plant material

Totally, 9 wild individuals of *B. vulgaris* were obtained from Zarin Gol Feridunshahr company (Longitude:49°55'35.8"E, Latitude: 32°56'19.2"N) of Isfahan province from the center area of Iran. The studied areas are near each other and have similar climates.

2.3. Morphological evaluation

Eighteen morphological variables were examined (Table 1).Leaves and fully mature fruits were harvested from each shrub. Random samples were collected consisting of 30 fruit bunch of each shrub. Samples of adult fruit bunches were randomly harvested from several parts of shrubs. Some variables were measured by laboratory equipment. Leaf length, leaf width, leaf tail length fruit length, fruit width, fruit tail length, seed length, seed width, was measured with a digital caliper. Fresh fruit weight, dry fruit weight, fresh leaves weight, dry leaves weight and seed weight were measured by an electronic balance with 0.01 g of precision. Thirty experimental units were tested for each variable examined. Number of leave, number of fruits per panicle was also recorded. Leaves color, fruit color was considered according to scoring and coding. (Table 1).

2.3.Statistical analysis

Data analysis was performed using the XLSTAT software v5.03 (2016). Statistical analysis was carried out using the ANOVA procedure and the means were compared with a Least Significant Difference (LSD) test at $p \leq 0.05$. The p-values of less than 0.05 were considered statistically significant. Simple correlation (Pearson) between traits were performed using Microsoft Excel (version 2013)

3. Result and discussion

The obtained results showed that there is a significant difference ($p<0.01$) in terms of morphological traits (Table 1). The highest average traits of Number of fruits per panicle (17), was observed in 1 genotype with dark red fruit. fruit width (0.76 cm), fresh fruit weight (0.39 g), Seed length (0.7 cm), seed width (0.3 cm), was observed in fifth genotype. The average mean comparison showed that in terms of product yield, the investigated habitats from the highest production to the lowest production included 1> 3> 2> 7> 5>6>4 genotype. Comparison in terms of color of fruit, the dark red to yellow included 2>1>3&4>5&6>7 genotype (Table 4). Trait correlation analysis is used to investigate and establish meaningful relationships between traits (Table 2). Establishing this relationship between traits leads to studying traits that may be difficult to measure (Rezaei et al., 2020).

Simple correlation coefficients between traits showed that some measured traits have a significant positive or negative correlation. Barberry fruit weight has a positive and significant correlation with seed length($r = 0.5$). Barberry color showed negative correlation with fruit length ($r = -0.79$) and positive correlation with leave color ($r= 0.74$). Furthermore, Number of fruits per panicle showed negative correlation with seed width($r = -0.73$). Also fresh fruit weight has positive correlation ($r= 0.79$) with blade length. Increasing number of fruits per panicle will be decreased the seed weight. Many studies investigated and evaluated significant difference between morphological traits which depends on studied region (Khodabandeh et al. 2022, Khoshandam et al., 2023) Wild germplasm of barberry plants were assessed on their morphological parameters.

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Table 1. Mean comparison of morphological of wild barberry (*Berberis vulgaris* L.) fruits genotype.

Sample	leaf length	leaf width	leaf tail length	fruit length	fruit width	fruit tail length	Number of fruit per of panicle	Number of leave per panicle	Fruit color	leaves color	fresh fruit weight	fresh leaf weight	Blade length	dry leaf weight	dry leaves weight	Seed length	seed width	seed weight
1	2.30 ^{a}	1.30 ^{ab}	0.75 ^{bcd}	0.75 ^{c}	0.65 ^{ab}	0.62 ^{cd}	17.00 ^{b}	16.00 ^{a}	5.00^{b}	3.00^{b}	0.25 ^{bc}	0.037 ^{bc}	1.97 ^{bc}	0.06 ^{b}	0.02 ^{a}	0.60 ^{a}	0.22 ^{bc}	0.04 ^{a}
2	2.50 ^{a}	1.72 ^{a}	0.70 ^{bcd}	0.60 ^{d}	0.47 ^{c}	0.42 ^{e}	13.00 ^{cd}	9.00 ^{c}	6.00^{a}	4.00^{a}	0.14 ^{d}	0.060 ^{abc}	1.85 ^{c}	0.03 ^{de}	0.02 ^{a}	0.37 ^{b}	0.22 ^{bc}	0.02 ^{c}
3	2.20 ^{a}	1.47 ^{ab}	0.67 ^{bcd}	1.07 ^{a}	0.57 ^{abc}	0.72 ^{c}	14.50 ^{bc}	10.50 ^{b}	3.00^{d}	2.00^{c}	0.20 ^{cd}	0.068 ^{abc}	2.25 ^{b}	0.09 ^{a}	0.02 ^{a}	0.57 ^{ab}	0.22 ^{bc}	0.01 ^{d}
4	3.12 ^{a}	1.15 ^{bc}	0.97 ^{bc}	1.07 ^{a}	0.55 ^{bc}	0.70 ^{cd}	9.50 ^{e}	8.75 ^{c}	3.00^{d}	3.00^{b}	0.19 ^{d}	0.050 ^{abc}	2.10 ^{bc}	0.06 ^{b}	0.02 ^{a}	0.56 ^{ab}	0.32 ^{a}	0.033^{b}
5	2.97 ^{a}	1.55 ^{ab}	1.72 ^{a}	0.95 ^{b}	0.72 ^{a}	0.60 ^{cde}	11.00 ^{de}	8.00 ^{cd}	4.00^{c}	3.00^{b}	0.41 ^{a}	0.093 ^{a}	2.70 ^{a}	0.09 ^{a}	0.02 ^{a}	0.57 ^{ab}	0.27 ^{ab}	0.03 ^{bc}
6	2.25 ^{a}	1.05 ^{bc}	0.50 ^{d}	1.02 ^{ab}	0.65 ^{ab}	0.60 ^{cde}	10.20 ^{de}	7.00 ^{d}	4.00^{c}	2.00^{c}	0.26 ^{b}	0.033 ^{bc}	2.10 ^{bc}	0.05 ^{c}	0.02 ^{a}	0.47 ^{ab}	0.22 ^{bc}	0.03 ^{bc}
7	3.15 ^{a}	0.70 ^{c}	0.95 ^{bcd}	0.95 ^{b}	0.57 ^{abc}	0.95 ^{b}	14.75 ^{bc}	4.75 ^{e}	2.00^{e}	2.00^{c}	0.18 ^{d}	0.086 ^{ab}	1.97 ^{bc}	0.04 ^{cd}	0.01 ^{a}	0.01 ^{c}	0.01 ^{d}	0.01 ^{e}
8	2.80 ^{a}	1.20 ^{b}	1.12 ^{b}	1.05 ^{ab}	0.72 ^{a}	1.20 ^{a}	25.75 ^{a}	7.25 ^{d}	3.00^{d}	3.00^{b}	0.15 ^{d}	0.034 ^{bc}	2.20 ^{b}	0.06 ^{b}	0.02 ^{a}	0.01 ^{c}	0.01 ^{d}	0.01 ^{e}
9	3.15 ^{a}	1.45 ^{ab}	0.60 ^{cd}	0.95 ^{b}	0.52 ^{bc}	0.52 ^{de}	12.25 ^{cde}	7.50 ^{d}	1.00^{f}	2.00^{c}	0.20 ^{d}	0.022 ^{c}	2.05 ^{bc}	0.03 ^{e}	0.02^{a}	0.47 ^{ab}	0.17 ^{c}	0.02 ^{cd}

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Table 2. Correlation coefficients between all measured morphological traits in the wild barberry genotype.

	leaf length	*leaf width*	*leaf tail length*	*fruit length*	*fruit width*	*fruit tail length*	*Number of fruits per panicle*	*Number of leave per panicle*	*Fruit color*	*leaves color*	*fresh fruit weight*	*fresh leaf weight*	*Blade length*	*dry leaf weigh t*	*dry leaves weight*	*Seed length*	*seed width*	*Seed weight*
leaf length	1.00																	
leaf width	-0.27	1.00																
leaf tail length	0.47	0.08	1.00															
fruit length	0.23	-0.45	0.15	1.00														
fruit width	-0.09	-0.18	0.59	0.37	1.00													
fruit tail length	0.23	-0.59	0.28	0.52	0.50	1.00												
Number of fruits per panicle	-0.10	-0.11	0.11	0.03	0.43	0.76	1.00											
Number of leave per panicle	-0.56	0.39	-0.17	-0.39	0.05	-0.31	0.10	1.00										
Fruit color	-0.61	0.44	0.03	-0.68	0.05	-0.39	-0.03	0.51	1.00									
leaves color	-0.04	0.53	0.31	-0.65	-0.07	-0.19	0.14	0.31	0.74	1.00								
fresh fruit weight	0.00	0.15	0.59	0.12	0.60	-0.28	-0.37	0.14	0.13	-0.08	1.00							
fresh leaf weight	0.19	-0.02	0.61	-0.04	0.06	0.03	-0.23	-0.23	0.11	0.08	0.36	1.00						
Blade length	0.15	0.22	0.78	0.46	0.67	0.10	-0.08	-0.12	-0.10	-0.09	0.79	0.44	1.00					
dry leaf weight	-0.23	0.17	0.54	0.45	0.55	0.19	0.05	0.29	0.09	-0.05	0.55	0.46	0.77	1.00				
dry leaves weight	-0.49	0.67	0.04	-0.17	-0.03	-0.41	-0.22	0.05	0.62	0.46	0.13	0.11	0.26	0.27	1.00			
Seed length	-0.34	0.53	-0.10	-0.07	-0.12	-0.76	-0.63	0.60	0.28	0.05	0.50	-0.07	0.25	0.37	0.37	1.00		
seed width	-0.23	0.52	-0.01	-0.10	-0.20	-0.76	-0.73	0.44	0.39	0.25	0.44	0.02	0.22	0.31	0.51	0.93	1.00	
seed weight	-0.28	0.34	-0.08	-0.33	-0.03	-0.71	-0.53	0.67	0.49	0.30	0.46	-0.22	0.01	0.09	0.20	0.84	0.84	1.00

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4. Conclusion

In the current study, wild barberry genotypes was collected from Isfahan provinence areas of Iran, and individuals were assessed on their morphological characters. The results showed information on the variability of some the morphological properties of barberry individuals. The high variability found in their traits could be helpful for genetic improvement and further evaluation for preservation of these genetic resources. The barberry fruit is a good source of various nutrients, especially carbohydrates, protein, fat, fiber and minerals. Considering their zero cost of production, easy availability, hardy nature and abundant production, they need to be popularized and exploited on a commercial scale. This will help in conserving and managing the natural environment.

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Conflict of Interest
The authors declare no conflicts of interest.

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THERAPEUTIC POTENCY OF *Pistacia Terebinthus*: FROM TRADITIONAL APPLICATIONS TO MODERN INSIGHTS

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Abstract
Pistacia terebinthus, commonly known as the terebinth or turpentine tree, has been a subject of growing interest due to its extensive medicinal properties. This paper explores the multifaceted therapeutic potential of *P. terebinthus*, integrating traditional knowledge with contemporary scientific evidence. Through a comprehensive analysis of its phytochemical composition and pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, this paper elucidates the diverse therapeutic benefits offered by *P. terebinthus*. Furthermore, recent studies have highlighted its potential role in specific medical conditions. Investigations suggest that *P. terebinthus* resin may induce cell death in MDA-MB-231 cells through caspase-independent apoptosis pathways, indicating its potential as a supportive treatment for breast cancer. Additionally, observations reveal that terebinth oil exhibits healing and protective effects in the treatment of ovarian ischemia/reperfusion injury, attributed to its modulation of radixin protein expression. Moreover, *P. terebinthus* extract has shown promising results in reducing bacterial load and accelerating wound healing processes. The chemical composition of *P. terebinthus*, rich in organic acids, sugars, essential oils, resins, proteins, tannins, and flavonoids, contributes to its diverse therapeutic effects. Traditional uses of *P. terebinthus* in treating peptic ulcers, asthma, gastralgia, rheumatism, skin inflammation, and other ailments are supported by scientific evidence. However, its potential applications extend beyond medicine, including its use in food, beverage production, and biodiesel production. The utilization of *P. terebinthus* resin as a yeast immobilization support in alcoholic fermentations demonstrates its potential in the beverage industry. Additionally, the genetic diversity of *P. terebinthus* populations and its suitability for biodiesel production highlight its significance in agriculture and environmental sustainability. Overall, this paper provides a comprehensive overview of the medicinal properties of *P. terebinthus*, emphasizing its potential as a valuable resource for healthcare, agriculture, and industry.

Key Words: Terebinth, Folk Medicine, Phytochemical Composition, Pharmacological Activities

1. Introduction

Aromatic plants have long been essential components of the pharmaceutical and cosmetic industries, and in the last ten years, their importance has significantly increased. Essential oils derived from these plants have positioned them as key candidates for analysis within the realm of medicinal plants. The increasing intrigue surrounding these plants stems from a collective recognition of their health benefits and a shift towards natural alternatives over synthetic medications, which are often associated with adverse effects. The increasing interest in these plants derives from a collective recognition of their health benefits and a shift towards natural alternatives over synthetic medications, which are often associated with adverse effects. Technological advancements have streamlined the investigation of plant chemical properties, ushering in an era of novel drug discoveries and plant-based treatments for diverse health issues. Moreover, the market for herbal supplements has flourished due to increased consumer desire for organic products, highlighting the medicinal plants' economic feasibility. Additionally, the evaluation of multipurpose medicinal plants is crucial, as species that exhibit strong drought resilience and adaptability to harsh environments not only contribute to biofuel production but also support agricultural breeding initiatives. Among the vast array of valuable plants, the species *Pistacia*

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terebinthus has emerged as a significant player, offering extensive pharmaceutical benefits that potentially alleviate symptoms of numerous chronic diseases.

Terebinth (*Pistacia terebinthus*) is a deciduous species within the *Pistacia* genus, Anacardiacea family This tree is native to the Canary Islands and the Mediterranean region, extending from Morocco and Portugal to Greece and Turkey. It is also found in North Africa, the Arabian Peninsula, Western Asia, and southern Kosovo (Tastekin et al., 2014; Pulaj et al., 2016). Menengiç trees grow well from sea level up to 1600 meters, especially in the Taurus Mountains. The plant prefers dry, rocky slopes or is sometimes found within pine forests. Growing at altitudes up to 1600 meters, particularly in the Taurus Mountains, the Menengiç tree is commonly found on dry, rocky slopes or within pine forests (Baytop, 1984; Baytop, 1994; Kavak et al., 2010). It is a dioecious plant, meaning it bears separate male and female flowers on different individuals and typically grows as a shrub or small tree ranging from 5 to 10 meters in height (Pulaj et al., 2016). Interestingly, monoecious forms of *P. terebinthus*, which carry both male and female flowers on the same plant, have been identified in the Rhodope Mountains (Avanzato, 2003).

The leaves of the Menengiç tree are compound, spanning 10–20 cm in length, and consist of five to eleven opposite, glossy, oval leaflets. These leaflets measure 2–6 cm in length and 1–3 cm in width. The tree produces small, spherical, hard-seeded fruits that mature from red to black, each fruit measuring 5 to 7 mm in diameter. Its flowers, which appear in early spring alongside new leaves, exhibit a reddish-purple hue (Topçu et al., 2007). Further adding to its ecological value, research has shown that *P. terebinthus* plays a critical role in supporting local biodiversity, offering habitat and food sources for various insect species and birds (Gülsoy, 2013). Additionally, recent studies have highlighted the potential medicinal properties of the tree, particularly its bark and leaf extracts, which have been found to possess anti-inflammatory and antioxidant activities (Uysal et al., 2022; Bozorgi et al., 2013). This underscores the Menengiç tree's importance not only as a part of natural landscapes but also in traditional and modern medicine.

P. terebinthus showcases remarkable adaptability in Türkiye, thriving in both the humid and rainy Mediterranean climate as well as the dry, less rainy continental climate. Therefore, Türkiye serves as a key genetic hub for the *Pistacia* species, with its favorable ecological conditions. This species exhibits a high capacity for adaptation, successfully growing in rocky, calcareous, stony, and poor soils. Resistant to drought and cold, these trees are often used in otherwise unusable lands by grafting, thus maximizing the use of these areas (Ak, 2014). *P. terebinthus* L., along with two other wild species, *P. khinjuk* Stocks and *P. atlantica* Desf., are commonly used as rootstocks for cultivating pistachio trees, which is a critical agricultural practice for enhancing yield and resilience (Özçağıran et al., 2005).

Terebinth has a rich history in folk medicine across various cultures, particularly in the Mediterranean and Middle Eastern regions where it is indigenous. Traditionally, the resin, also known as Chios turpentine, derived from this tree has been used for its antiseptic, diuretic, and anti-inflammatory properties. It has been applied to treat ailments such as ulcers, respiratory issues, and skin infections (Couladis et al. 2003; Özcan et al. 2009). The leaves and fruits of the terebinth are also utilized in traditional remedies; decoctions made from the leaves are reputed to help with digestive problems and to promote wound healing, while the oil extracted from the fruits has been used to alleviate joint pain and to treat gallbladder issues. The versatility of terebinth in folk medicine highlights its potential pharmacological importance and supports ongoing research into its bioactive compounds (Bozorgi et al. 2013). These traditional uses underscore the need for further scientific exploration to validate and potentially expand the therapeutic applications of terebinth.

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Given the significant characteristics of this species and the need to develop breeding studies, this study aimed to comprehensively investigate recent research on the biologically active compounds and pharmaceutical aspects of *P. terebinthus* L.

2. Secondary Metabolites isolated from *P. terebinthus* L.

Previous research has shown that terebinth (*P. terebinthus*) is a rich source of biologically active secondary metabolites, making it a valuable resource for pharmacological substances. These secondary metabolites include phenolic derivatives, flavonoids, flavones, glycosides, and terpenes. Additionally, terebinth also contains essential primary metabolites such as essential oils, resin, proteins, and tannins. This comprehensive range of compounds highlights terebinth's potential in various therapeutic applications and underscores its significance in both traditional and modern medicine (Pulaj et al., 2016; Álvarez et al., 2009; Durmaz and Gökmen, 2011).

2.1. Essential oils isolated from *P. terebinthus* L.

Essential oils from different parts of Pistacia terebinthus, also known as terebinth, demonstrate a complex and variable composition. In the leaves and twigs of Turkish Pistacia terebinthus, the essential oils contain significant concentrations of α-cadinol (6.9%), phytol (5.4%), δ-cadinene (5.1%), α-terpineol (5.0%), and bornyl acetate (4.4%), indicating a diverse chemical makeup (Kıvçak et al., 2004). From the twigs and leaves in Sardinia, α-pinene emerges as the predominant compound, accounting for 66.0% in twigs and 16.4% in leaves, with β-pinene also present at 22.5% in twig oils and 13.5% in leaf oils (Usai et al., 2006). The essential oils from fruits collected across various Turkish regions exhibit a notable range of α-pinene (5.3% to 51.3%), limonene (up to 39.0%), and β-pinene (1.4% to 22.5%), proving influence of regional differences and fruit maturity on the chemical profiles of Pistacia terebinthus essential oils, pointing to their potential for diverse applications in pharmaceuticals and food preservation (Özcan et al., 2009). Moreover, the essential oil extracted from the leaves also displays antibacterial and insecticidal properties, with α-pinene (19.97%), sabinene (15.43%), β-pinene (8.57%), and terpinen-4-ol (9.65%) as major components (Ulukanli et al., 2014). These findings underscore the potential of Pistacia terebinthus essential oils for diverse applications across the pharmaceutical and food industries, given their potent and varied phytochemical content. Fidan et al., (2023) focused on the essential oil composition of *P. terebinthus* L., revealing notable variations between unripe and ripe fruits. The research identified monoterpenoids as the predominant chemical compounds in the essential oils of unripe fruits, while monoterpenes dominated in ripe fruits. Among these compounds, α-pinene was the main component, present at 22.8% in unripe fruits and increasing to 27.3% in ripe fruits, along with a significant presence of sesquiterpenes. This study highlights the dynamic changes in essential oil composition as the fruits mature, emphasizing the potential for varied applications based on these phytochemical properties. Serkan et al. (2022) explored the influence of environmental factors on the essential oil yields and compositions of ripe fruits of Pistacia terebinthus L., a medicinal and aromatic plant native to the Mediterranean Region of Turkey. Collecting fruit samples from 34 different locations in the Lake District, the researchers employed hydrodistillation and GC-MS methods to determine the essential oil yields and component ratios. The yields varied notably, ranging from 0.05% to 0.19% by volume/weight, with α-pinene (41.01%) and limonene (14.28%) emerging as the primary components in the oils. The study revealed significant environmental influences on essential oil yields and compositions. Longitude and sand content at 10-30 cm soil depths were significant factors affecting essential oil yields. Soil silt content and pH influenced the concentrations of α-pinene and Ocimene, respectively. Longitude and total annual precipitation were key determinants for the sabinene and limonene levels, while soil lime content at various depths predominantly affected Myrcene and p-Cymene concentrations. This research underscores the complex interaction between environmental conditions and the chemical profiles of *P. terebinthus* fruits' essential oils.

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2.2. Phenolic compounds isolated from *P. terebinthus L.*

Extensive research into the phenolic composition of *Pistacia terebinthus* has unveiled a comprehensive spectrum of bioactive compounds. A study by Özcan et al. (2020) highlighted the significant enhancement in the levels of quercetin and catechin through sonication; quercetin increased from 129.09 to 467.28 mg/100 g and catechin from 5.58 to 21.33 mg/100 g in fruits treated for 30 minutes. Further exploration by Uysal et al. (2022) revealed that the total phenolic content in the extracts ranged from 65.51 to 210.54 mg GAE/g and total flavonoids from 54.94 to 169.89 mg RE/g, with methanol extracts showing the highest phenolic content and ethyl acetate extracts having the maximum flavonoid content. HPLC-MS/MS analysis identified a rich array of phenolic compounds across all extracts, including quinic acid, gallic acid (3,4,5-Trihydroxybenzoic acid), myricetin-O-glucuronide, rutin (quercetin-3-O-rutinoside), quercitrin (quercetin-3-O-rhamnoside), and luteolin (3′,4',5,7-tetrahydroxyflavone). Additionally, specific compounds such as phyloquinone, pheophytin B, pheophytin A, and a pheophytin A isomer were uniquely identified in the ethyl acetate extract. The methanol and water extracts contained exclusive compounds like procyanidin B, catechin, three isomers of hexahydroxyflavan-O-gallate, and epigallocatechin-3-O-gallate (teatannin II). Notably, the presence of luteolin-7-O-glucoside and apigenin-7-O-glucoside was also confirmed through HPLC analysis. In a related study by Orhan et al. (2012), it was demonstrated that ethyl acetate and methanol extracts from terebinth fruits moderately inhibited the butyrylcholinesterase enzyme, aligning with the observed phenolic profiles and their associated bioactivities. These findings underscore the extensive and varied phenolic landscape of Pistacia terebinthus, revealing its potential for therapeutic applications.

2.3. Biological activities of P. terebinthus L.

2.3.1. ***Traditional and Medicinal Uses:*** Tastekin et al. (2014) reported that Pistacia species have stimulating, astringent, diuretic, antipyretic, antibacterial, anti-inflammatory, cough suppressant, and antiviral effects. They also reported the use of terebinth in the treatment of kidney stones, loss of movement, dermatitis, and as part of jaundice treatment. They also stated that soap derived from terebinth can be safely and effectively used in managing or treating skin toxicity caused by Cetuximab. The various uses of terebinth in traditional medicine for different purposes such as tonic, antihypertensive, sterilizing, aphrodisiac, and treatment of gastrointestinal, respiratory, dental, liver, and urinary tract disorders (Bozorgi et al. 2013). Terebinth smoke has also been used as a traditional medicine as an air purifier and disinfectant in different countries such as Iran (Pulaj et al. 2016).

2.3.2. ***Antidiabetic and Enzyme Inhibitory Effects:*** Uyar and Abdulrahman (2020) reported that terebinth oil extract shows antidiabetic activity by reducing harmful effects on serum enzyme levels and lipid oxidation, and regular treatment may be beneficial in protecting against complications associated with diabetes. Akyuz et al. (2022) focused on evaluating the antioxidant and enzyme inhibitory effects of hexane, acetone, and ethanol extracts from various parts of terebinth, targeting enzymes like α-glucosidase, α-amylase, acetylcholinesterase, and butyrylcholineesterase. The research demonstrated that all extracts were particularly effective against α-glucosidase, with significantly stronger inhibition compared to standard treatments. In contrast, their effects on α-amylase were notably weaker. Of particular interest was the acetone extract from the fruit shells, which showed the most potent effects on both α-glucosidase and α-amylase. This extract led to the isolation of luteolin, a bioactive flavonoid, which displayed strong antidiabetic properties and significant antioxidant activities in radical scavenging assays. The study highlighted luteolin's potential in managing blood sugar levels and underscored the powerful relationship between luteolin content and the biological activities of terebinth fruits, indicating promising pharmacological applications. The findings suggest that terebinth and its components, particularly luteolin, could be valuable in developing treatments for metabolic disorders and for their antioxidant benefits. Foddai et al. (2014) investigated the potential of aqueous extracts from Sardinian Pistacia lentiscus L. and P. terebinthus L. leaves and fruits for managing metabolic disorders.

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Tested in vitro, the extracts showed strong inhibition of key digestive enzymes involved in lipid and carbohydrate absorption. They effectively slowed carbohydrate digestion and glucose absorption, comparable to the drug acarbose used for diabetes management. These findings suggest Pistacia spp. components could be used as functional foods or nutraceuticals to manage conditions like dyslipidemia and diabetes.

2.3.3. ***Antimicrobial and Antiseptic Properties:*** Dhifi et al. (2012) reported the use of terebinth leaves in burn treatment in Turkey. Additionally, they emphasized that gum collected from Pistacia terebinthus branches, specifically called terebinth gum, is used as an antiseptic in bronchitis and other respiratory and urinary system diseases, highlighting its role in improving asthma. Nazım et al. (2021) also reported that terebinth galls have high antimicrobial properties and can be listed as bactericidal. Yılmaz et al. (2023) explored the use of nanoliposomes, drug delivery systems enhancing bioavailability, for encapsulating terebinth ethanol extract (TLE). Terebinth, a medicinal plant in Turkey, possesses antibacterial, antioxidant, and anti-inflammatory properties. The study investigated the antibacterial effects of nanoliposomes incorporating TLE against Escherichia coli and Staphylococcus aureus. The nanoliposome formulation, prepared through high-pressure and high-intensity homogenization techniques, exhibited desirable characteristics, including a negative zeta potential charge, small particle size, and high encapsulation efficiency. The nanoliposome-encapsulated TLE displayed antibacterial activity against S. aureus, despite containing less active substance than pure TLE. This indicates that the formulation effectively preserved the antibacterial properties of TLE. The findings suggest the potential of nanoliposomes as carriers for TLE, enhancing its efficacy in combating bacterial infections. This research highlights the application of nanotechnology in improving the delivery and efficacy of natural medicinal compounds like terebinth extract, paving the way for the development of novel antimicrobial therapies.

2.3.4. ***Wound Healing Properties:*** Sindak et al. (2024) investigated the wound healing and antibacterial effects of terebinth extract and terebinth combined with 3% oxytetracycline in back skin wounds in mice infected with Staphylococcus aureus. Groups treated with terebinth extract alone or in combination with oxytetracycline showed significantly faster wound healing compared to the control group. The combination group exhibited the shortest healing duration and lowest bacterial count. Terebinth extract alone also reduced bacterial load significantly. The study suggests that terebinth extract from the Siirt region possesses antibacterial properties and accelerates wound healing, indicating its potential as a natural remedy for wound management.

2.3.5. ***Anticancer Properties:*** Najibullah et al. (2022) examined the efficacy of Pistacia terebinthus in inhibiting the proliferation of human lung cancer A549 cell lines in a time and dose-dependent manner. The research indicated that P. terebinthus essential oil has significant potential as an anticancer agent, showcasing varying degrees of cytotoxicity, which provided a basis for its potential therapeutic use against cancer. HAcibek'roglu's 2014 study highlighted the potent anticholinesterase activity of ethanol and ethanol-water extracts of P. terebinthus, surpassing that of the standard drug galanthamine. These extracts also demonstrated strong antioxidant properties in several assays, with particularly high activity in reducing cupric ions, attributed to the rich flavonoid content of the terebinth extracts. These findings collectively suggest that P. terebinthus has notable anticancer and antioxidant capabilities, supporting its development for medical applications in treating cancer and managing oxidative stress-related diseases.
Firat et al. (2024) investigated the impact of Pistacia terebinthus (terebinth) resin extract (TRE) on MDA-MB-231 breast cancer cells. They assessed TRE's cytotoxicity using MTS analysis and its effect on apoptosis via Hoechst staining. Western blot analysis revealed higher caspase-3 expression in cells

treated with TRE, suggesting its potential to induce cell death via caspase-independent apoptosis pathways and supporting breast cancer treatment.

2.3.6. ***Antioxidant and Anti-inflammatory Properties:*** Durmaz and Gökmen (2011) reported that terebinth oil has high antioxidant capacity and that oil obtained from roasted terebinth has higher phenolic compound and antioxidant capacity compared to unroasted terebinth. Kavak et al. (2010) indicated that P. terebinthus has potential as an antioxidant, antimicrobial, and possible -glucuronidase inhibitor, suggesting a possible preventive role in reducing cancer risks by eliminating free radical attacks. Uysal et al. (2022) demonstrated the potential of P. terebinthus leaves as natural antioxidants and enzyme inhibitors. Bozorgi et al. (2013) also reported that three triterpenes isolated from P. terebinthus resin, namely mastichadienolic acid, mastichadienonic acid, and morolic acid, have anti-inflammatory activity. Moreover, they emphasized that oleanonic acid derived from P. terebinthus branches reduces leukotriene B4 formation from rodent peritoneal leukocytes. Sengul et al. (2023) studied the therapeutic effects of Pistacia terebinthus L. (terebinth) oil on experimental ovarian ischemia/reperfusion (I/R) injury in rats. Terebinth oil, administered orally for 4 weeks, exhibited protective effects, preventing epithelial degeneration, reducing inflammation, and restoring vascular integrity. Immunohistochemistry analysis revealed positive radixin protein expression in granulosa cells, indicating its healing effect. The study suggests terebinth oil's potential in treating ovarian I/R injury.

2.3.7. ***Utilization of Pistacia terebinthus Resin in Alcoholic Fermentation:*** Terebinth is known for its richness in tannins and resinous substances, along with its pleasant aroma (Aydın and Özcan, 2002). It has been proven that terebinth resin is a suitable immobilization support for yeast cells and leads to alcoholic fermentations. Furthermore, this newly immobilized biocatalyst can be used for alcoholic fermentations over a wide temperature range. Terpenes and total phenolic content derived from alcoholic beverages containing immobilized biocatalysts detected in fermented alcoholic beverages may potentially contribute to the antioxidant, antibacterial, and antifungal properties. Therefore, the use of P. terebinthus resin as a support for yeast immobilization has high commercialization potential in the beverage industry, providing unique aromatic and low-alcohol content products with low or negligible amounts of preservatives (Kallis et al., 2019, 2022; Schoina et al., 2019).

3. Conclusion

In conclusion, the multifaceted therapeutic potential of Pistacia terebinthus has been extensively explored through a variety of studies, ranging from traditional applications to modern scientific investigations. This comprehensive examination has highlighted its substantial role in traditional medicine and its emerging significance in contemporary medical and commercial applications. The wide range of biological activities associated with P. terebinthus—including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties—underscores its potential as a natural therapeutic agent. The studies discussed have not only confirmed the traditional uses of terebinth but have also provided a scientific basis for its effectiveness in treating a broad spectrum of conditions. The utilization of P. terebinthus resin as a support for yeast immobilization in alcoholic fermentations represents an innovative application that bridges traditional knowledge with modern biotechnological approaches, offering promising avenues for the beverage industry. Moreover, the ability of various extracts from P. terebinthus to modulate biochemical pathways, enhance wound healing, and exhibit antidiabetic properties opens new horizons for its use in nutraceuticals and functional foods. Future research should focus on clinical trials to validate the efficacy of P. terebinthus extracts and components in human health and disease management. Additionally, the environmental resilience and genetic diversity of P. terebinthus make it a valuable species for agricultural and ecological sustainability studies. Overall, P.

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terebinthus holds significant promise not only as a medicinal resource but also as a sustainable commercial and agricultural product, contributing to the health and well-being of populations while supporting environmental conservation and economic development.

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EFFECT OF GROWING CONDITIONS ON EMULSION CAPACITY, EMULSION STABILITY, AND PROTEIN CONTENTS OF DIFFERENT FENUGREEK GENOTYPES

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Abstract
Fenugreek (*Trigonella foenum-graecum* L.) is both a legume and medicinal and aromatic plants cultivated in different countries as Türkiye, Ethiopia, Egypt of the World. It is an annual plant and used for the medicinal, food, cosmetic industries because of including many important chemical properties such as carbohydrate, oil, protein and polysaccharide. In this study, the emulsion capacity (EC), emulsion stability (ES), and protein content (PC) of the different origin fenugreek genotypes were determined grown under different growing conditions (irrigated and dryland). The experiment was conducted according to randomized complete block design with three replications. The gums of the three local cultivars (Berkem, Çiftçi and Gürarslan) with 18 different origin fenugreek genotypes were used for the determination of the examined properties. The EC, ES and PC of the genotypes under irrigated condition changed between 78.57-92.86%, 62.50-92.31% and 10.52-17.63%, respectively. Under dryland conditions, EC, ES and PC values varied between 76.92-93.75%, 78.13-90.91%, and 10.40-15.11% among the fenugreek genotypes. The highest EC values were found from Berkem cultivar under dryland condition, and PI 215615 genotype under irrigated condition. The maximum ES and PC were obtained from PI 613633 genotype and Berkem cultivar under irrigated condition, respectively. The cluster analysis clustered into two main groups and the PCA analysis showed over 79.4% of total variations for the EC, ES, and PC values. The obtained result showed that the examined properties showed high variability among the fenugreek genotypes. In conclusion, the means of the EC and PC values were found higher in irrigated condition, however, the genotypes showed variability. So, the obtained results may be used for the fenugreek breeding programs and developing new cultivars.

Key Words: Fenugreek, Gum content, Protein content, Emulsion factors.

1. Introduction

The climate change is one of the major problems in the agricultural system. The increasing temperature effects the plant production area, and many of the plant exposes the extinction. Arid areas have the lowest water stock, and the stock evaporates fastly depending on the increasing temperatures. The plants grown in these areas struggle to survive for the continue to their vegetation period depending on the different stress conditions. Also, water scarcity impact on the yield and quality properties of plants. Fenugreek is one of the most important plants to adapted different environmental and stress conditions.

Fenugreek (*Trigonella foenum-graecum* L.) is a self-pollinating and diploid (2n=16) legume species from the Fabaceae family (Camlica and Yaldiz, 2021). It is an annual plant, and it is cultivated different part of the word such as China, Egypt, Ethiopia, Morocco, Ukraine, Greece, and Türkiye (Petropoulos, 2002). Fenugreek is used as a multipurpose plant and it is generally used in food, feed, spices, and medicinal plant, and perfumery (Srinivasan, 2006). The cultivation of the fenugreek enriches the soil properties via microorganism symbiosis fixing the atmospheric nitrogen (Bromfeild et al., 2001; Sadeghzadeh-Ahari et al., 2009). Fenugreek contains one of the most important biochemicals as saccharides used in the food industry as hydrocolloids. These hydrocolloids can be used for thickening, gelling, stabilizing, bulking, and emulsifying agents in food systems for many years (Giosafatto et al., 2007, Phillips and Williams, 2009). Also, gum of fenugreek contains mannose (M) and galactose (G)

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and has a high viscosity in aqueous solutions. In addition, the ratio of M/G was reported approximately 1 and the ratio was found high substituted compared to guar (M/G = 2), tara (M/G = 3) and locust bean (M/G = 4) gums. Previous studies reported that the M/G has impact on physicochemical properties of galactomannans, and low M/G ratio shows high solubility (Garti et al., 1997; Wu et al., 2009). The pure fenugreek gum contains 0.8% residual protein. In addition, it can reduce the surface tension and form stable emulsions with small oil (2-3 mm) droplet size (Garti et al., 1997). On the contrary, it was noted that pure fenugreek gum contents include 0.6% from the residual protein and it had lower surface activity than the unpurified gum. Similarly, the crude gum content of the fenugreek was found as 13.9%, and it was reported that the gum had very stable oil/water emulsion than 14 other hydrocolloid gums (Huang et al., 2001).

The knowledges on the cultivation applications, landrace divergences, and adaptations of fenugreek genotypes are necessary to develop the new varieties via selection or hybridization. Therefore, the gene resources of the fenugreek should be collected and some important properties as morphological, yield and quality criteria should be determined. This study could be evaluated as studied important research on the emulsion stability, capacity and protein content of the different origin fenugreek genotypes grown under irrigated and dryland conditions.

2. Material and Methods

a. Plant materials

The seeds of the different origin eighteen fenugreek genotypes obtained from the United States Department of Agriculture (USDA), and the Gürarslan, Çiftçi and Berkem cultivars obtained from Transitional Zone Agricultural Research Institute, Ankara University and Dicle University, respectively (Table 1).

Table 1. General information on the fenugreek genotypes and cultivars used in study

No	Accession code	Country	Origin/province	No	Accession code	Country	Origin/province
1	Çiftçi*	Türkiye	Türkiye	11	PI 381062	Iran	Ghazvin
2	Gürarslan*	Türkiye	Türkiye	12	PI 426971	Pakistan	Gujjo, Karachi
3	PI 173820	Türkiye	Malatya	13	PI 426973	Pakistan	Mirpur Batoro
4	PI 194020	Ethiopia	Debra Markos	14	PI 469264	Egypt	Nile Delta
5	PI 215615	India	Sirsa, Punjab	15	PI 568215	Türkiye	-
6	PI 251640	Ethiopia	-	16	PI 572538	Egypt	Nubaria, North Delta
7	PI 286532	India	Kulu bazaar	17	PI 613633	Australia	-
8	PI 296394	Iran	-	18	PI 617076	Bulgaria	-
9	PI 302448	India	Delhi, India	19	PI 639185	Armenia	Yerevan
10	PI 302449	India	-	20	PI 660995	Armenia	Yerevan

***Local cultivars**

b. Experimental design and growing conditions

The experiment was conducted during the 2020 vegetation period under irrigated and dryland conditions at research and application area of Bolu Abant İzzet Baysal University, Bolu, Türkiye (40°44′44″ N, 31°37′45″ E, 806 m above sea level). The experimental design was a randomized complete block design (RCBD) with three replications, and each experimental plot consisted of five 4-m-long rows with an inter row distance of 30 cm, and an inter-plant distance of 10 cm. The seeds of the fenugreek genotypes were sown in field conditions, on 10 April 2020.

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Six kg/da diammonium phosphate (DAP) and four kg/da ammonium sulphate (AS) were applied as fertilizers. The AS was given in two split doses in sowing time and before flowering time to the fenugreek genotypes into two applications. The soil properties of the experimental area were found clayey, 7.56 pH, 3.71% of organic matter, 0.052 kg/da phosphorus content, 108.31 kg/da potassium content and 0.0383% salty. The climatic data of the vegetation period was noted between 8.7 and 21.8 °C for temperature, 0 and 142.6 kg/m^2 for precipitation, and 56.1 and 76.7% for relative humidity. The weed control was done all the growing conditions, and the irrigation was done only irrigated area. The harvests of the fenugreek genotypes were done between 30 July and 18 August under irrigated and dryland conditions in the experiment year.

c. Isolation of the gum content of fenugreek genotypes

The gum contents of the fenugreek genotypes were isolated according to Kutlu (2015) with some modifications. Twenty g fenugreek seeds were measured and ground with a laboratory mill. Then, the ground seeds were mixed with the 1:40 distilled water. The mixture (pH 6.5) was heated in 60 °C for 3 h and filtered with Whatman No:1 paper. This stage was repeated by using the residue. The mixture was centrifuged for 10 min at 4400 rpm and the supernatant decanted. The ethanol added to a final concentration of 80% (v/v) with a 1:3 ratios to obtain fenugreek gum from the solution.

A glass banquette was used to mixing of the gum and ethanol concentration. This stage was repeated at least three times to obtain pure gum content. The obtained gum content was calculated and ground to pass through a 250-micron sieve. Finally, the moisture contents of gum were measured approximately 7% and 8% reported by Rashid et al. (2018).

d. Determination of emulsion capacity (EC) and stability (ES) of fenugreek genotypes

The EC and ES of the fenugreek genotypes were determined by and reported methods by Rashid et al. (2018). Fifteen ml hyrocolloid suspensions of fenugreek gum were mixed to 2 ml corn oil and centrifuged at 3000 rpm for a min. Then, the suspensions were centrifuged at 3000 rpm again for 5 min and the EC was calculated by using the formula:

EC = (ev × 100)/tv (ev: emulsion volume, tv: total volume).

To determine the ES, the samples were heated by an ultrasonic bath at 80 °C for 30 min and centrifuged at 3000 rpm during for 5 min. Then, ES values of fenugreek genotypes were calculated as follow:

ES: (fev × 100)/iev (fev: final emulsion volume; iev: initial emulsion volume).

e. Determination of protein content of gum

The protein contents of the fenugreek gum grown under irrigated and dryland conditions were determined according to report of (Yaldiz and Camlica, 2020). The protein contents of the fenugreek gum grown under irrigated and dryland conditions were determined according to report of (Yaldiz and Camlica, 2020).

Approximately 0.5 g ground gum hydrolysed with sulphuric acid (20 ml) and selenium catalyst tablet (3.5 g) in a hot block at 240 °C for 25 min and 380 °C for 3 h. After this process, the samples were mixed with H_2O and NaOH for the titration and neutralization. The protein contents of the fenugreek gum were calculated by using multiplying conversion factor (6.25) and the obtained total nitrogen contents from the samples.

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f. Analysis of data and statistical evaluation

This study was carried out as three replicates in randomized complete block design. Statistical differences among the means of the fenugreek genotypes were determined by the Least Significant Difference (LSD) test. The relationships of examined properties were determined by the cluster and principal component analyses performed by the JMP statistical program.

3. Results and Discussion

a. Emulsion capacity, stability and gum protein contents of fenugreek genotypes

The statistical analysis on emulsion capacity (EC) of the different origin fenugreek genotypes showed significant differences under irrigated and dryland conditions (Table 2). The EC ranged from 78.57% to 92.86% under irrigated conditions. The highest EC value was found from PI 215615 genotype, followed by PI 469264, PI 617076 and PI 296394 genotypes under irrigated conditions. The lowest EC values were noted from PI 381062 and PI 194020 genotypes. Under dryland conditions, the EC values were found between 76.92-93.75%, and Berkem cultivar had the highest EC value with the PI 426971 and PI 426973 genotypes. The lowest values were obtained from Çiftçi cultivar and PI 613633 genotype. Compared to cultivars based on the growing conditions, all cultivars were found closest values under irrigated conditions, but these cultivars showed variability under dryland conditions. Gürarslan cultivar showed stabile values in these growing conditions. The mean of the genotypes under irrigated conditions was found lower than the mean of the genotypes under irrigated conditions (Table 2).

By comparing the emulsion stability (ES) values of fenugreek genotypes grown under irrigated and dryland conditions, it was found that the ES values showed statistically significant differences (Table 2). The ES values changed between 62.50-92.31% and 75.79-90.91% under irrigated and dryland conditions, respectively. While the highest ES value was found in PI 613633 genotype, and followed by PI 296394 and PI 469264 genotypes, the lowest values were noted in PI 194020 and PI 381062 genotypes under irrigated condition. The maximum ES values were found from PI 426973 and PI 469264 genotypes, and the lowest values were obtained from PI 639185, PI 194020 and PI 572538 genotypes. The ES values of the fenugreek cultivars were found similar in the same growing conditions. In other words, ES values were found higher in irrigated condition (86.49-89.23%) compared to dryland condition (78.13-80.57%). It was clearly reported that PI 194020 and PI 381062 genotypes had the lowest EC and ES values under irrigated conditions. Also, the maximum EC and ES values were found over 90% both irrigated and dryland conditions.

The result on the EC and ES from this study were found similar with the Camlica and Yaldiz (2022) depending on the applications of different temperatures and gum content. Fenugreek genotypes showed statistically significant differences based on the protein contents (PCs) of the gums grown under irrigated and dryland conditions (Table 2). The PCs of gums changed between 10.52-17.63% under irrigated condition, and 10.40-15.11% under dryland condition. Berkem cultivar and PI 286532 genotype had the maximum protein content among the fenugreek genotypes under irrigated condition. The minimum PCs of fenugreek gums were found from PI 639185 and PI 381062 genotypes under irrigated condition and were noted from PI 251640 and PI 426971 genotypes under dryland condition.
The protein contents of the fenugreek cultivars were found to be close under dryland condition opposite of the irrigated condition. Berkem cultivar had the highest gum protein content among the fenugreek cultivars. The mean protein content of the gums was found to be higher under irrigated condition compared to dryland condition.

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Table 2. EC, ES, and PC of the different origin fenugreek genotypes grown under irrigated and dryland conditions

Genotypes	Irrigated			Dryland		
	EC (%)	ES (%)	PC (%)	EC (%)	ES (%)	PC (%)
Berkem	89.19de	86.49c-f	17.63a	93.75a	78.13ef	14.34ab
Çiftçi	89.23de	89.23a-d	11.36d	76.92k	80.00def	12.92bcd
Güraslan	89.20de	88.43b-e	16.20ab	89.79def	80.57c-f	12.29cde
PI 173820	89.23de	86.15def	14.71abc	87.50ı	78.13ef	11.64def
PI 194020	81.25ı	62.50h	12.59cd	86.15j	76.92ef	12.26cde
PI 215615	92.86a	89.29a-d	12.45cd	89.23efg	81.03c-f	12.54cd
PI 251640	88.75e	87.96b-f	16.00ab	89.23efg	80.00def	10.40f
PI 286532	89.19de	86.49cdef	16.63a	88.78gh	80.57c-f	12.63cd
PI 296394	90.02c	90.99ab	16.09ab	90.04cde	81.84cde	12.90bcd
PI 302448	89.20de	86.38c-f	15.85ab	87.69ı	86.15abc	12.76cd
PI 302449	89.76cd	86.95c-f	16.05ab	87.50ı	81.25c-f	14.95a
PI 381062	78.57j	71.43g	11.35d	88.24hı	88.24ab	15.11a
PI 426971	89.15de	86.82c-f	15.90ab	92.31b	84.62bcd	11.05ef
PI 426973	86.67g	86.67c-f	11.87cd	90.91c	90.91a	13.39bc
PI 469264	90.91b	90.91ab	11.64cd	89.23efg	89.23ab	13.01bcd
PI 568215	85.00h	85.00f	12.41cd	89.79def	80.57c-f	12.69cd
PI 572538	88.75e	85.94ef	16.42ab	89.23efg	76.92ef	12.62cd
PI 613633	87.69f	92.31a	11.70cd	86.15j	86.15abc	14.97a
PI 617076	90.91b	87.88b-f	11.82cd	90.26cd	81.03c-f	12.76cd
PI 639185	89.47cde	89.47abc	10.52d	88.90fgh	75.79f	12.62cd
PI 660995	89.20de	88.43b-e	13.43bcd	89.23efg	82.05cde	12.88cd
Mean	88.30	85.99	13.93	88.61	81.91	12.89
LSD (5%)	0.75	3.25	3.10	1.00	6.02	1.44
CV (%)	0.52	2.29	13.48	0.68	4.45	6.76

*Means within a row followed by the different letters are statistically different at p<0.05 level.

The obtained gum protein content from this study was found to be higher than Youssef et al. (2009) who reported the protein content of fenugreek gum 0.16-1.10% crude fenugreek gum. Similarly, Previous studies showed that fenugreek has a low amount of gum protein, between 0.80 and 0.95% (Garti et al., 1997) and 0.6% (Brummer et al., 2003). The differences compared to previous studies can be explained depending on the different genotypes, growing conditions and isolation methods.

b. Principal component analysis (PCA) and cluster analysis

PCA and cluster analysis were conducted to determine the relationship among the fenugreek genotypes in terms of ES, EC and PC grown under irrigated and dryland conditions as separately. The PCA analysis showed more than 79% total variations (Figures 1A and B). The figure 1A showed 94.0% total variation and the first (PC1) and second (PC2) principal components consisted of high variation as 64.3% and 30.7 % of the total variation, respectively under irrigated condition (Figure 1A).

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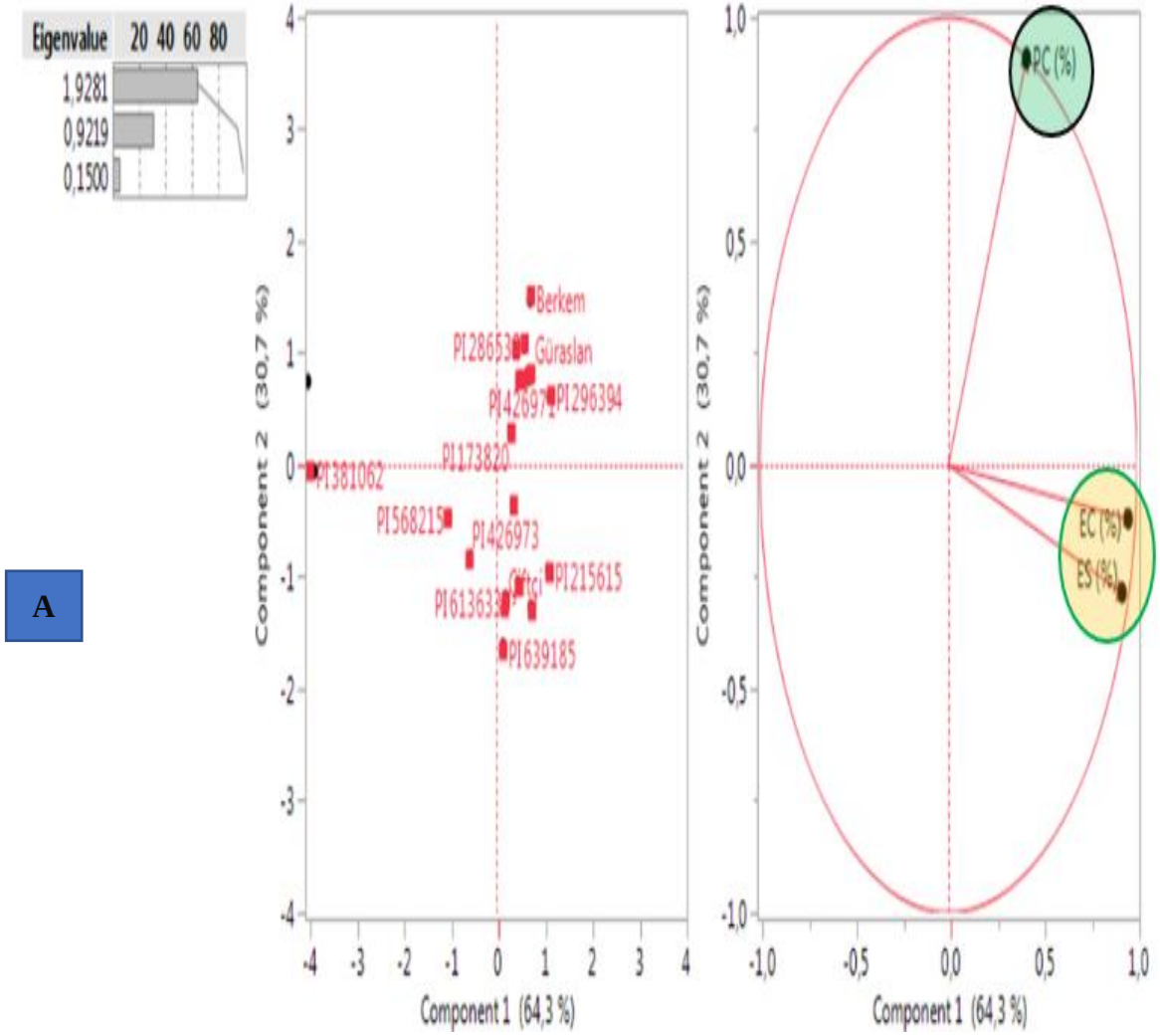
PC was found in a group as PC2 and it is found positive side of the PCA. EC and ES were found in the same group of the PC1. The fenugreek genotypes showed similarity from the each other and they are scatter in the graph according PCA graph of the genotypes used in this study under irrigated condition, except PI 381062 genotype.

Under dryland condition, the PCA analysis showed 79.4% total variation (Figure 1B). The PC1 showed 44.5% of total variation and PC2 showed 34.9% of total variation (Figure 1B). The EC was found in a group as PC2 and, ES and PC were found in the same group as PC1. Many of fenugreek genotypes showed differences from the each other and PI 426971 genotype was found highly different compared to other genotypes.



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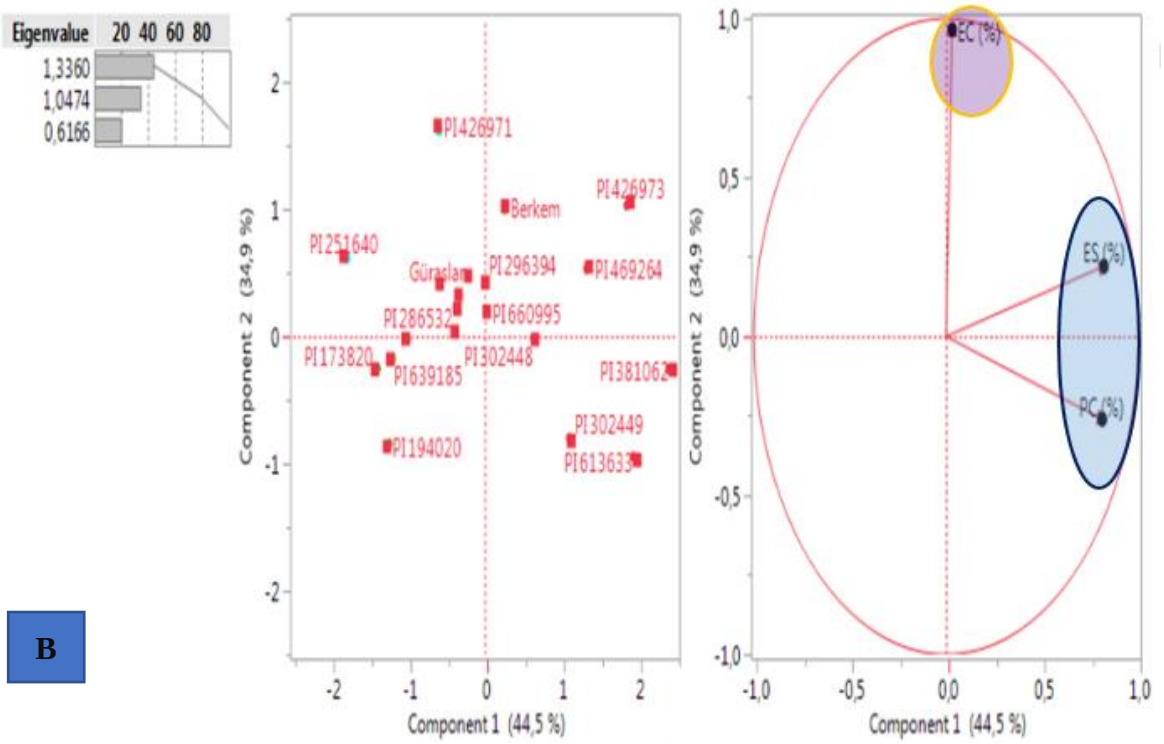

Figure 1. PCA analysis results of the examined properties and genotypes (A: irrigated condition; B: Dryland condition)

Cluster analysis divided into two main groups as A and B, and all main groups divided into two subgroups (A1, A2, B1 and B2) under irrigated and dryland conditions (Figures 2A and 2B). The figure 2A showed that the main group A contained two fenugreek genotypes as PI 194020 (subgroup A1) and PI 381062 (subgroup A2) genotypes under irrigated condition. Other genotypes and three fenugreek cultivars took place into the B main group, and nine genotypes with Berkem and Gürarslan cultivars took place in the subgroup B1. Çiftçi cultivar and seven genotypes were found in the subgroup B2 (Figure 2a). EC and ES values supported to the creation of group A.

Under dryland condition, the Figure 2B showed the results of the cluster analysis depending on the examined properties among the fenugreek genotypes. The main group A contained six genotypes and a cultivar (Çiftçi). The Çiftçi cultivar were found alone in the subgroup A1. The main group B included three cultivars (Çiftçi, Berkem and Gürarslan) and 12 fenugreek genotypes. The subgroup B2 contained two genotypes as PI 251640 and PI 426971 genotypes. ES was the main factor to distribution of the main groups. The result of the cluster analysis revealed that the genotypes were found in the different groups and subgroups depending on the EC, ES and PC values of the fenugreek gums.

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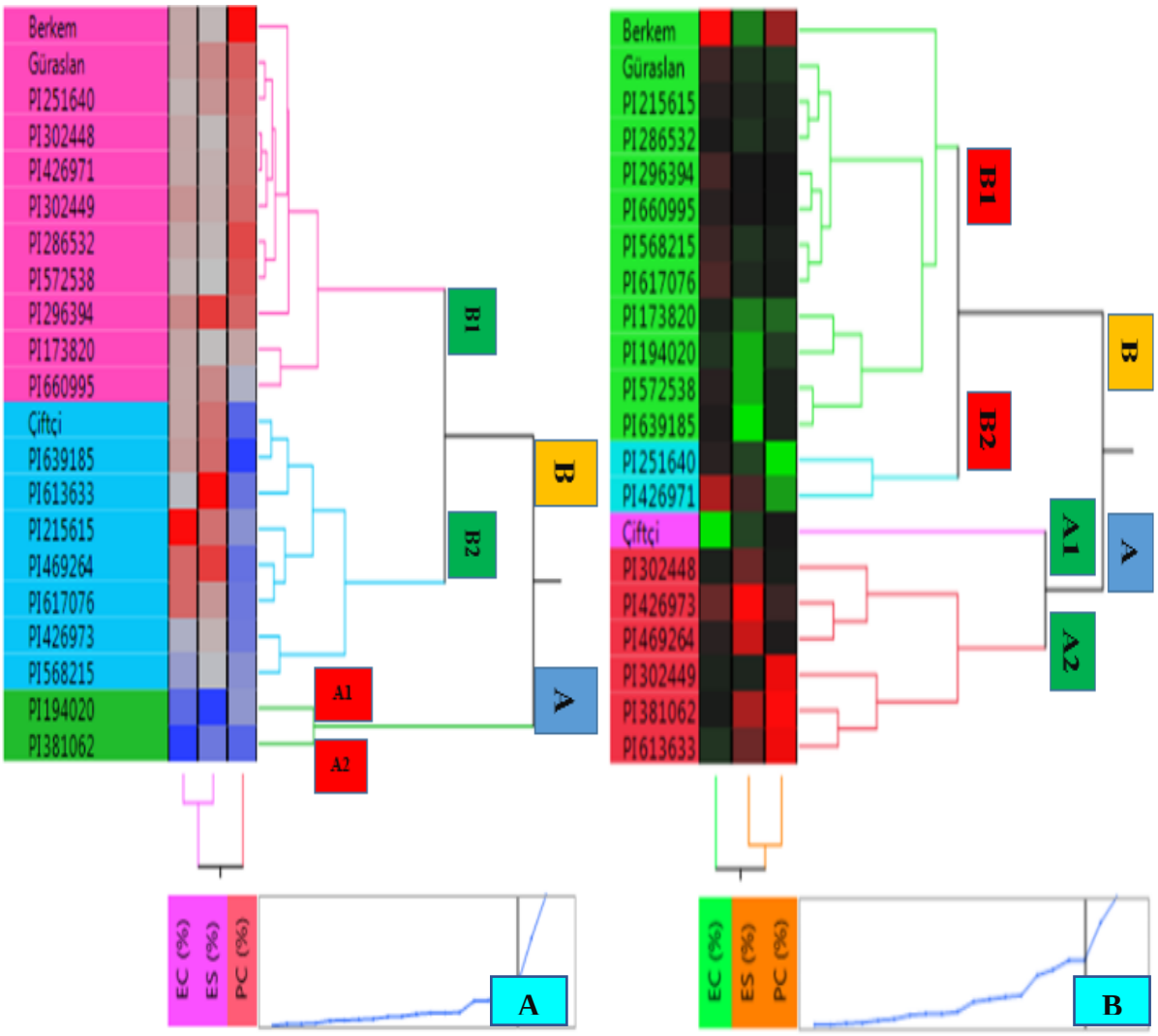

Figure 2. Cluster analysis results of the different fenugreek genotypes depending on the EC, Es and PC

4. Conclusion

In this study, we have studied to determine the potential capacities of the different fenugreek genotypes gums as EC, ES and protein contents grown under irrigated and dryland conditions. The results of the study showed that different origin fenugreek genotypes had the important values on EC, ES of gum contents grown under irrigated and dryland conditions. In this way, the examined properties showed variability depending on the genotypes and growing conditions. Consequently, the highest ES and PC values were found from irrigated condition. However, the highest EC value was noted from the dryland condition. In addition, Berkem cultivar had the maximum values for the protein content under irrigated condition, and it had also the highest EC value under dryland conditions. The PCA results showed high value over 75%, and the cluster analysis divided into two main groups depending on the examined properties. The B main group had more genotypes compared to A main group. Berkem and Gürarslan cultivars were found in the same main group both irrigated and dryland conditions.

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In conclusion, the results obtained from this study on the EC, ES and protein contents of the different fenugreek genotypes gums grown under irrigated and dryland conditions showed high potential as cheap sources of alternative uses for the food industry.

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Conflict of Interest
All authors declare no conflicts of interest.

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ALLEVIATION OF SALT STRESS CONDITION OF THE SELENIUM APPLICATIONS ON MORPHOLOGY AND YIELD PROPERTIES OF OREGANO

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Abstract
Turkish oregano (*Origanum onites*) is an aromatic plant belonging to the Lamiaceae family, which is cultured economically in Türkiye. The essential oil of oregano is preferred as a raw material in the perfume and cosmetic industries due to its distinct and characteristic aroma. At the same time, its essential oil is used for medical, antimicrobial, antiviral and antifungal purposes in the industrial sector. In sustainable agriculture, abiotic environmental stresses such as extreme temperatures, drought and salinity negatively affect agriculture. Selenium (Se) is known to have positive effects on plants under salt stress, such as preventing lipid peroxidation processes, increasing free proline accumulation, and decreasing the number of chloride ion in shoot problems. The present study was addressed to assay the effect of different Se dose fertilization on Turkish oregano under salt (NaCl) stresses. The results showed that Se treatments caused significant improvements in the fresh (3.78-6.00 g) and dry (1.30-2.18 g) weight of oregano under salt stress. The highest mean fresh and dry weight was observed in the combined treatment of 2 ppm of Se and 100 mM/L of NaCl, followed by 6 ppm of Se and 50 mM/L of NaCl respectively. Totally 31 correlations were found as positive, and negative, and PCA analysis revealed 65.7% total variation. The cluster analysis clustered into two main groups (A and B) based on the applications of different dose Se and NaCl over 63% of the applications took place in the group B and 75% of the Se applications fell into the same group. Therefore, applying Se can be effective way of improving oregano yield under salt stress conditions.

Key Words: *Origanum onites*, Salinity, Salt stress, Selenium, Growth parameters.

1. Introduction

Origanum Onites L. is an important essential oil plant belonging to the *Origanum* genus of the Lamiaceae family. *Origanum onites* L. has an important place among the *Origanum* genus. It is known that Oregano extracts were used in folk medicine in ancient times and that countries like Egypt, India, and China were pioneers in this area (Baytop, 1984). Essential oils (EOs) of oregano are among the most widely used in the world due to its high antimicrobial activity, as well as their antiviral and antifungal properties. Due to these properties, EOs of oregano are used for the pharmaceutical, food and cosmetic industries. Today, approximately 90% of the oregano supplied from Turkey, which has a large share in the worlds oregano foreign trade (Sarı and Altunkaya, 2016). Global climate change causes salinization of agricultural lands. Data from 2023 shows that salinity issues affect approximately 20% of the 38.5 million hectares of agricultural land in Türkiye (TUİK, 2024). Salinity limits plant growth and productivity by affecting physiological and biochemical parameters (Kiumarzi et al., 2022; Bistgani et al., 2019; Gontia-Mishra et al., 2014). Therefore, it is important to determine the salinity tolerance of the oregano plant, which has economic value for our country, and to find economic solutions for its cultivation in saline soils. Knowing the use of selenium in agriculture and its positive and negative returns is important for studies on selenium. Selenium uptake, transfer, and distribution depend on factors like plant species, developmental stages, form and concentration of selenium, physiological conditions (salinity and soil pH, etc.) and the presence of other substances, activity of membrane transporters, and translocation mechanisms of the plant. However, it has been reported by various

researchers that low doses of selenium protect plants from various abiotic stresses such as low temperature, drought and metal stress (Gupta and Gupta, 2017). The present study was addressed to assay the effect of different Se dose fertilization on Turkish oregano under salt (NaCl) stresses.

2. Material and Methods

2.1. Plant material and experimental design

The experimental was carried out in a climate chamber at the Agriculture Faculty of Bolu Abant Izzet Baysal University, between 06.12.2022. and 13.09.2023. The seeds of Turkish oregano were obtained from Yalova Research Institute, Türkiye. Seeds were sown in plastic pots (400 mm diameter) filled with 4 kg of field soil in 06.12.2022. Pots were placed in a climate chamber (27 °C-65 % humudity). Ten seeds were sown in each pot, after 15 days of germination; plants were tinned to 4 plants per pot. All pots were arranged under a completely randomized block design (CRD).

Se fertigation doses (2,4,8 and 16 ppm) were developed by dissolving Na2SeO4 (Sigma-Aldrich, USA) in distilled water, and NaCl treatments of 100 mM/L was developed by dissolving in distilled water. The soil used had 3.25 % organic matter, 0.85 ppm phosphorus, 127 ppm potassium, 30.71 % CaCO3, 0.03 % total soluble salts, clay loam and neutral pH value (7.2) (Yaldız et al., 2018). As base fertilizer, 40 kg/ha ammonium sulfate and 60 kg/ha diammonium phosphate were applied before the salt and selenium applications in accordance with the soil analysis. The water application was carried out according to the water needs in the field capacity through experimental period reported by Asher et al. (2002). So, each pot was watered three times weekly with 200 mL of distilled water. Cuttings were made at nearly 10 cm above the ground for fast regrowth. Three cuts were carried out on 13.03.2023, 16.06.2023 and 13.09.2023, respectively. Then, the herb was dried in a thermal drying compartment at a temperature of 35 °C, and the air-dry herb weight was determined. Treatments that were carried out can be summarized in Table 1.

Table 1. Treatments applied in experimental design.

Treatments	Selenium level (ppm)
Control	No fertilization
IOF	Chemical fertilizer
Se1	2 ppm
Se2	4 ppm
Se3	8 ppm
Se4	16 ppm
NaCl	100mM
Se1+NaCl	2ppm+100 mM
Se2+NaCl	4 ppm+00 mM
Se3+NaCl	8 ppm+100 mM
Se4+NaCl	16ppm+100 mM

2.2. Statistical analysis

The statistical analysis was conducted a one-way analysis of variance (ANOVA) by using the JMP statistical software. The statistically significant differences among the results of the examined properties were determined with Least Significant Difference (LSD) test at $p<0.05$. The mean, LSD and F values were noted and given in Table 2. Correlation, principal component (PCA) and cluster analyses were carried out to find relationships of the examined properties by using JMP statistical program.

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3. Results and Discussion

3.1. Plant development

Vegetative growth parameters were evaluated in all treatments together with the control after per plant reached the flowering stage. The data in Table 2 showed that these parameters such as plant height, branch numbers, fresh and dry weights were remarkably increased the Se treatments under salt condition. The data indicated that the highest values of these parameters were obtained from Se1+ NaCl (2 ppm+100 mM) and Se3+ NaCl (8 ppm+100 mM) application in all cuts.

Accordingly, it was observed that the effect of salt stress on these parameters of the plants was remarkably significant ($P < 0.05$). Plant heights increased up to Se4 doses under salt stress conditions. The greatest plant height (31.19 cm) was observed in the Se3+ NaCl condition followed by the only NaCl (30.93 cm) condition. On the other hand, the lowest value (24.89 cm) was obtained from Se1+ NaCl applications (Table 2).

Relationships between Se doses under NaCl condition and branch number can be seen in Table 2. The Se3 (8 ppm) application increased the branch number under salt stress as compared with the salt treatment (NaCl 100 mM). The maximum main branch number (18.02 number) were recorded in the Se3+ NaCl, while the minimum branch number (11.89 number) was determined in the Se4 treatment followed by Se4+ NaCl (12.35 number) treatment. So, the highest dose of Se (16 ppm) under salt stress significantly decreased the branch number as compared with the salt treatment and control application. When cut times were evaluated together, the maximum branch number was detected at the first cut of Se3+NaCl, followed by the first cut that had a combined treatment of Se1 and NaCl. This study clearly demonstrated that the application of Se3 significantly increased branch number of the Turkish oregano plants as compared with the salt only treatment. Salt stress and Se application remarkably affected the fresh weight of the Turkish orenago plants growing in salt stress. When the salt stress and the Se experiments were evaluated together, the Se1+NaCl (6.03 g) and Se3+NaCl (5.08 g) significantly alleviated the harmful impact of salinity and rised the fresh weight of the Turkish orenago as compared with the salt treatment. Furthermore, the Se3 (5.20 g) treatment maintained significantly higher fresh weight than the salt treatment. The Se4 treatment (3.78 g) reduced the fresh weight of the Turkish orenago as compared with the salt only treatment (Table 2). In addition, the maximum fresh weights were obtained from the first cut, followed by the second and third cuts.

Also, the combined treatments of Se1+NaCl gave the highest value of dry weight (1.20 g), followed by the Se3 control of the first cut (1.06 g), and the combined treatment of Se3+NaCl, Se2+NaCl, Se1 (0.96 g) from the first cut. The lowest values were obtained from the Se1 (0.10 g) dose from the third cut and the Se2+NaCl (0.19 g) dose during the third cut. Furthermore, the highest total dry weights were obtained from Se1+NaCl (2.18 g) application, followed by Se3+NaCl (1.92 g) application. In contrast, a significant reduction in total dry weight was noticed when the Se concentration was increased beyond 8 ppm under salt stress. Moreover, NaCl (100 mM) treatment increased total dry weight as compared with the control and Se4 treatments. So, our study showed that salt stress affected the vegatatif growth by decreasing in yield parameters in non-Se-treated Turkish oregano plants, whereas Se-treated (8 ppm) Turkish oregano plants exhibited higher yield parameters under salt stress (Table 2).

Our results were similarly with previous studies that reported that plants supplemented with Se had enhanced resistance to salt stress (Djanaguiraman et al., 2005; Filek et al., 2008; Cartes et al., 2010; Chu et al., 2010; Djanaguiraman et al., 2010; Hasanuzzaman et al., 2013; Çamlıca et al. 2019; Yaldiz and Camlica, 2021).

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Table 2. Plant height (cm), branch number (number/plant), fresh and dry weight (g/plant) of Turkish oregano exposed to selenium and salt interactions (cm).

Applications	Doses	PH (1.cut)	PH (2.cut)	PH (3.cut)	APH	BN (1. cut)	BN (2. cut)	BN (3. cut)	ABN	FW (1. cut)	FW (2. cut)	FW (3. cut)	TFW	DW (1. cut)	DW (2. cut)	DW (3. cut)	TDW
IOF	**IOF**	42.11ab	18.89klm	17.67lm	26.22abc	14.67d-j	17.00c-f	9.11k-l	13.59bcd	2.13bcd	1.21f-ı	0.82hı	4.16bc	0.75def	0.49ghı	0.25k-n	1.48c
Control	**Control**	44.33ab	21.33ı-m	23.44h-m	29.70abc	16.00d-ı	16.56c-h	11.44g-l	13.44bcd	2.22bcd	1.19ghı	0.87hı	4.27bc	0.77c-f	0.46g-j	0.24k-n	1.48c
Se1+salt	**2 ppm+100 mM**	37.44b-e	16.50m	20.72ı-m	24.89c	22.61ab	14.83d-j	12.67d-l	16.70ab	2.78a	1.27e-h	1.98cd	6.03a	1.20a	0.40h-l	0.58fgh	2.18a
Se1	**2 ppm**	41.44ab	18.33klm	22.56ı-m	27.44abc	16.89c-g	14.44d-k	9.44j-l	13.59bcd	2.18bcd	1.01ghı	0.71ı	3.89c	0.96bc	0.39h-m	0.10n	1.45c
Se2+salt	**4 ppm+100 mM**	40.11bc	16.67lm	27.61f-ı	28.13abc	18.11bcd	13.00d-l	10.78ı-l	13.96bcd	2.24bc	0.97ghı	0.91ghı	4.13bc	0.96bc	0.37ı-m	0.19mn	1.51c
Se2	**4 ppm**	33.56c-f	21.44ı-m	25.56g-k	26.85abc	11.56f-l	17.11b-e	11.67e-l	14.67bcd	1.73de	1.16ghı	1.29e-h	4.18bc	0.83cde	0.48g-j	0.27j-n	1.58bc
Se3+salt	**8 ppm+100 mM**	43.56ab	19.50j-m	30.50e-h	31.19a	27.61a	15.61d-ı	10.83ı-l	18.02a	2.47abc	1.39efg	1.21e-ı	5.08ab	0.96bc	0.62fg	0.36ı-m	1.93ab
Se3	**8 ppm**	39.11bcd	19.89j-m	26.33f-j	28.44abc	16.11d-ı	17.06c-f	13.28d-l	15.48abc	2.58ab	1.39efg	1.23e-h	5.20ab	1.06ab	0.44g-k	0.21lmn	1.72bc
Se4+salt	**16 ppm+100 mM**	37.33b-e	20.11j-m	23.89h-l	27.11abc	14.89d-j	13.33d-l	8.83l-l	12.35cd	1.98cd	1.15ghı	1.15ghı	4.28bc	0.78c-f	0.44g-k	0.31ı-m	1.53bc
Se4	**16 ppm**	32.67d-g	16.56lm	26.56f-j	25.26bc	12.00e-l	12.56e-l	11.11h-l	11.89d	1.71def	0.93ghı	1.14ghı	3.78c	0.72ef	0.42g-k	0.21lmn	1.35c
Salt	**100 mm**	47.67a	18.33klm	26.78f-j	30.93ab	22.00bc	13.00d-l	10.94ı-l	15.31abc	2.44abc	1.21f-ı	1.14ghı	4.78bc	0.89bcde	0.50ghı	0.27j-n	1.67bc
	Mean	39.94	18.87	24.69	27.83	17.50	14.95	10.92	14.45	2.22	1.17	1.13	4.53	0.90	0.46	0.27	1.63
	$F_{applications}$		1.99		1.16		2.68		2.61		4.73		3.09		3.79		3.20
	F_{cut}		195.23				32.36				130.79				222.02		
	$F_{app+cut}$		1.73				2.49				1.53				2.12		
	LSD (5%)		7.36		5.77		5.51		3.33		0.51		1.14		0.2		0.4
	CV (%)		16.07		1.18		23.16		13.54		20.63		14.74		22.83		14.35

+ PH: Plant height, APH: Avarega plant height, BN: Branch number, ABN: Averega branch number, FW: Fresh weight, TFW: Total fresh weight DW: Dry weight, TDW: Total dry weight; Means within a row followed by the different letters are statistically different at $p<0.05$ level.

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3.2. Correlation and principal component analysis (PCA)

The correlation analysis results showed that totally 31 correlations were found among the examined properties. Many of the correlations (20 correlations) were noted highly significant positive among the examined properties (Table 3). The highest significant positive correlations were found between the TFW and TDW (r= 0.0959**). It was clearly reported that TFW had the highest correlations with other properties with 8 correlations. The lowest significant correlation was found between APH and DW (2. cut) with r= 0.605*. PH (3. cut) property was showed no corretion with the other examined properties.

Table 3. Correlation analysis results of the examined properties

	PH (2.cut)	PH (3.cut)	APH	BN (1. cut)	BN (2. cut)	BN (3. cut)	ABN	FW (1. cut)	FW (2. cut)	FW (3. cut)	TFW	DW (1. cut)	DW (2. cut)	DW (3. cut)	TDW
PH (1.cut)	0.123	0.033	0.773**	0.596	0.05	-0.176	0.498	0.533	0.35	-0.354	0.162	0.074	0.389	-0.092	0.117
PH (2.cut)		0.032	0.39	-0.233	0.667*	-0.023	0.009	-0.221	0.433	-0.229	-0.129	-0.339	0.452	-0.163	-0.153
PH (3.cut)			0.594	0.333	-0.291	0.283	0.27	-0.011	0.062	0.014	0.02	0.053	0.35	-0.149	0.067
APH				0.551	0.061	0.026	0.513	0.316	0.41	-0.311	0.092	-0.013	0.605*	-0.195	0.08
BN (1. cut)					-0.117	0.148	0.882**	0.772**	0.524	0.328	0.668*	0.578	0.474	0.5	0.743**
BN (2. cut)						0.294	0.301	0.128	0.612*	0.003	0.203	0.069	0.352	0.058	0.171
BN (3. cut)							0.481	0.444	0.407	0.608*	0.617*	0.573	-0.057	0.342	0.518
ABN								0.837**	0.767**	0.445	0.813**	0.678*	0.52	0.547	0.843**
FW (1. cut)									0.644*	0.427	0.855**	0.82**	0.095	0.494	0.783**
FW (2. cut)										0.412	0.752**	0.418	0.634*	0.497	0.692*
FW (3. cut)											0.808**	0.609*	0.011	0.886**	0.823**
TFW												0.808**	0.198	0.803**	0.959**
DW (1. cut)													-0.186	0.483	0.803**
DW (2. cut)														0.204	0.275
DW (3. cut)															0.855**

Principal component analysis (PCA) was carried out to the mean values of the examined properties to determine the most substantily factors and to revelal the relationship between the variables and observations. The result of the analysis showed 5 PC axes with eigenvalues higher than 1 and 10 independent PC axes. The eigenvalues of the first five PCs (from PC1 to PC5) axes, explaining the 94.66% of the total variation, varied between 1.10-7.16 (Table 4).

Table 4. Eigenvalues, percent and cumulative percent of the examined properties

PCs	Eigenvalue	Percent	Cum Percent
1	7.1588	44.743	44.743
2	3.3646	21.029	65.771
3	2.1458	13.411	79.182
4	1.3723	8.577	87.759
5	1.1034	6.896	94.655
6	0.3472	2.170	96.825
7	0.2857	1.785	98.610
8	0.1625	1.016	99.626
9	0.0475	0.297	99.923
10	0.0123	0.077	100.000

The important first two PCs expained 65.77% of total variation, and the PC1 axis explained 44.74% of the total variation depending on the FW (1st cut), BN (1. cut) and TFW. The PC2 explained the 21.03% of the total variation depending on the PH (1, 2, and 3 cuts), and APH. BN (3. cut), FW (1 and 3. cuts),

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DW (1 and 3. cuts) and TFW were placed in the same area and have close vector lengths. NaCl, Se3 and NaCl+Se3 applications showed positive relationships many of the examined properties**.**

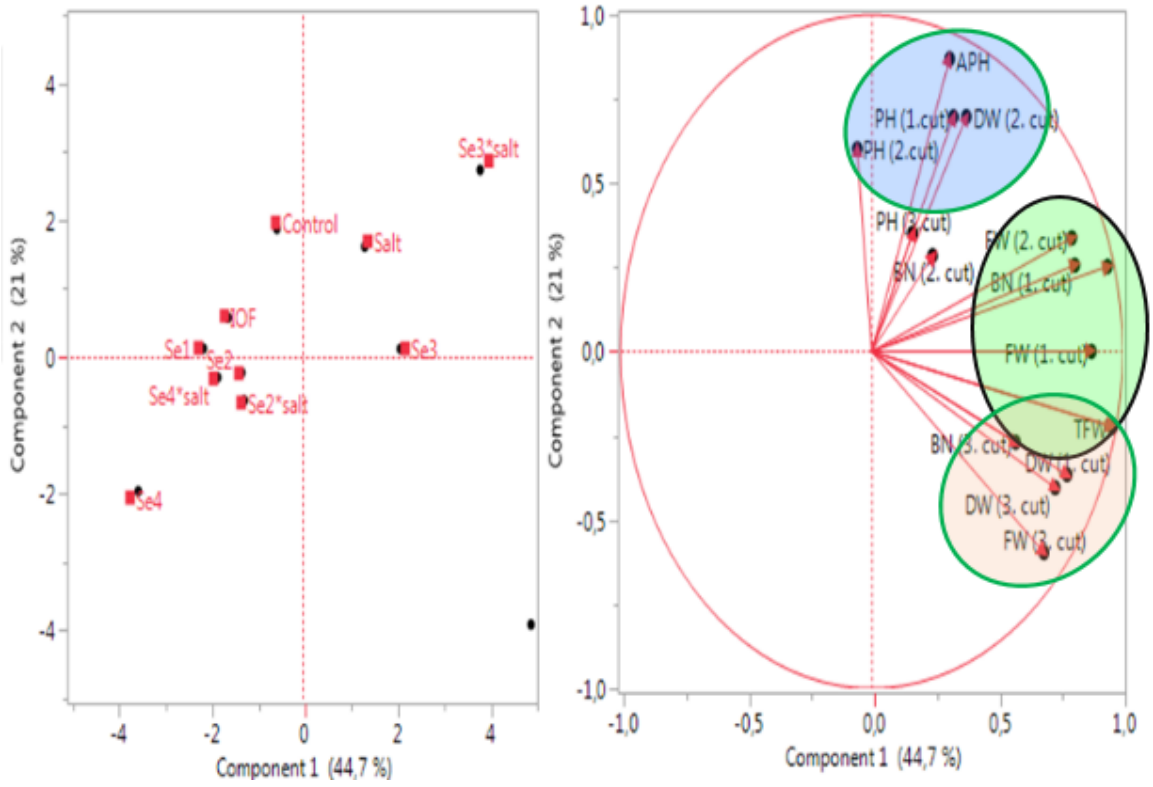

Figure 1. PCA analysis of the examined properties and applications

3.3. Cluster analysis

Cluster analysis was conducted for examined properties in accordang to the Wards method to determine the effect of the applications on the genetic diversity of oregano (Figure 2). The cluster analysis was clustered into two main (A and B) and four subgorups (A1, A2, B1 and B2) depending on the applications.

Resultant cluster analysis showed that NaCl, NaCl+Se1, NaCl+Se3 and Se3 applications were found into the main group A, and other applications were found into the main group B. NaCl+Se1 took place alone in subgroup A1. A2 subgroup was separeted from the other groups by NaCl+Se3, NaCl and Se3 applications. Se1, Se4 and NaCl+Se1 applications were found in the subgroup as B2 (Figure 2).

It was clealry noted that the cluster analysis showed that Se3 applications showed similar affect on oregano as NaCl applicaiton. The subgroup A1 was separeted from the other groups by FW (1 and 3 cuts), DW (1 and 3 cuts), TFW and TDW properties. It was also noted that the factors explaining for the separeted of subgroup A1 were found the main factors of the dividing of the main group A from the B main group.

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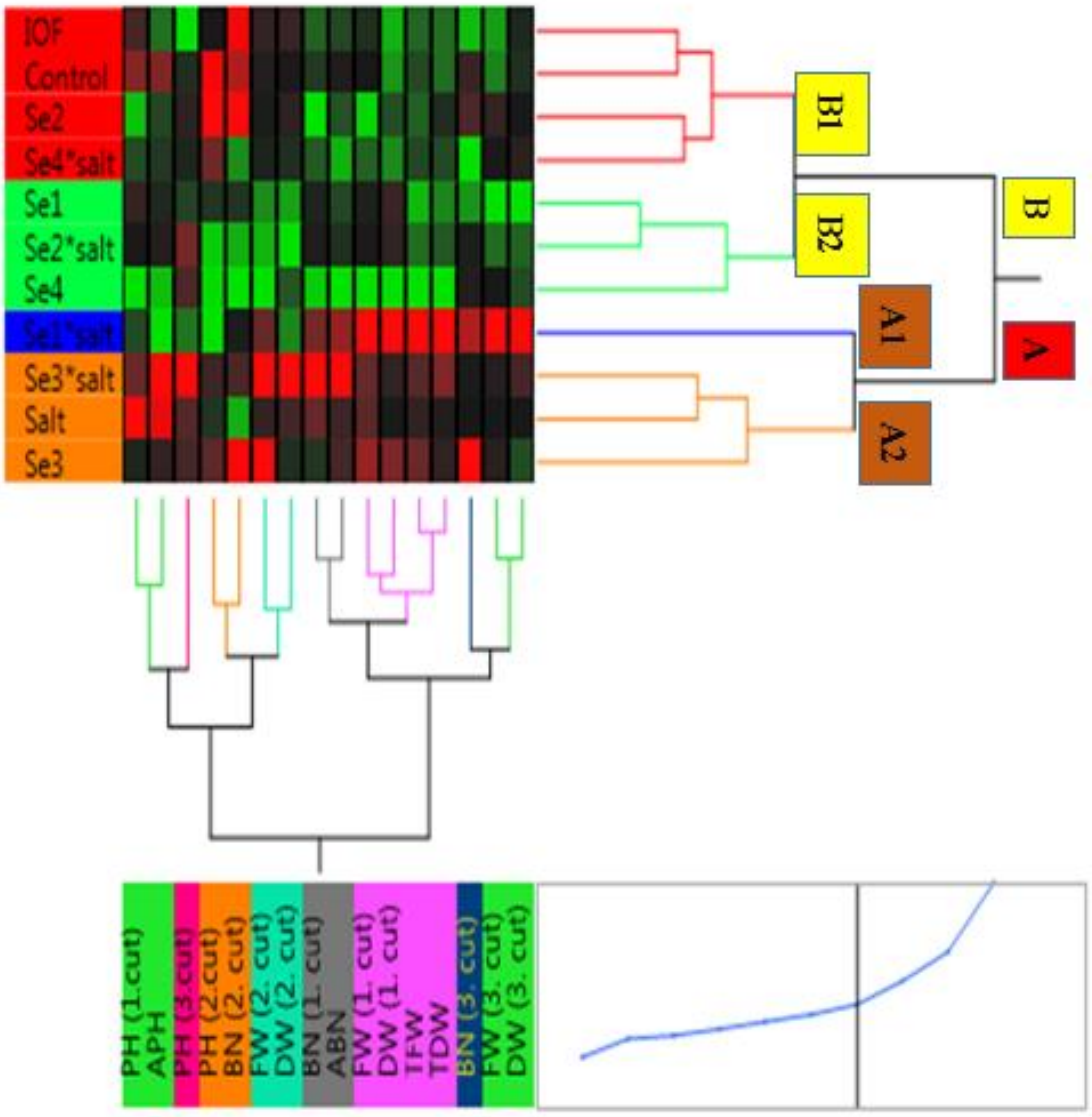

Figure 2. Cluster analysis results of the applications and examined properties

4. Conclusion

The current study exposed that the addition of Se at 8ppm dose to Turkish oregano plants growing in saline soil had better effects on all of the studied parameters. Thus, application of selenium can be considered the potential approaches to minimize the effects in Turkish oregano plants of salt toxicity growing under salt-affected soils. The multivariate analyses showed that the examined properties showed relationships, and correlation analysis showed totally 31 correlations. PCA result revealed total over 65% of total variation and cluster analysis divided into two main groups to applications depending on the examined properties.

Conflict of Interest
All authors declare no conflicts of interest.

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KARYOLOGICAL ANALYSIS OF SOME *CENTAUREA* TAXA

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Abstract
Centaurea L. (Asteraceae) is a well-known genus in ethnomedicine due to its various pharmacological properties. Although the genus *Centaurea* is taxonomically problematic, the number of taxa of the genus is increasing day by day, especially in Asia Minor, indicating that the genus is still evolving in terms of speciation. Various methods and approaches have been applied by different researchers for a long time to solve taxonomic problems in the genus *Centaurea*. One of these approaches is karyological analysis. KAMERAM program was used for karyotype measurements of the examined taxa and the taxa were compared in terms of asymmetry indices (CV_{CL}, CV_{CI}, AI and M_{CA}). In this study, chromosome morphologies of taxa are presented. The basic chromosome numbers of *Centaurea inexpectata* Wagenitz (x=11), *Centaurea athoa* DC. (x=10), *Centaurea stapfiana* (Hand.-Mazz.) Wagenitz (x=9) and *Centaurea babylonica* (L.) L. (x=8) located in different sections within the genus *Centaurea* are consistent with previous reports. The karyotypes had a predominance of metacentric (m) chromosomes. Among the taxa examined, *C. babylonica* was found to have the highest asymmetry index. Comparison of the karyotypes can provide valuable information about phylogenetic relationships and chromosome evolution between related species.

Key Words: Asteraceae, karyomorphology, Türkiye.

1. Introduction

The Anatolia is an important cross point among the three main continent and this situation of Asia Minor supply a big advantage for speciation of many plant and animal species. In this meaning, the main center of diversity of the genus *Centaurea* L. (Asteraceae) is Turkey, and the discovery of new species day by day is strong evidence supporting this situation. Chromosomal changes in total play an important role in evolution of *Centaurea* species. Therefore, it is seen too important to determined the karyotype and chromosomal indices in our understanding of chromosomal variation and evolution. According to our literature information [1-25], with the recently published new species and records, the genus is represented by 247 taxa in Turkey. 145 of these taxa are endemic to our country, and the endemism rate is 58.7%. Additionally, the *Centaurea* is used in traditional medicine for its medicinal properties, including antidiabetic antiviral, immunomodulatory, antimicrobial, neuroprotective, and anti-inflammatory biological activity [26-28]. Many phytochemical studies have been reported on the genus [29-46]. *Centaurea* is a taxonomically difficult genus due to its wide morphological variability [38,47-54]. Various methods and approaches have been applied by different researchers for a long time to solve taxonomic problems in the genus *Centaurea* [54-60]. One of these approaches is karyological analysis. It has been reported that chromosome data is important for resolving taxonomic problems or confusion in many species and genera. Additionally, it has been emphasized that karyotype analyzes contribute to the evaluation of the genetic relationship among species or populations belonging to species and to a better understanding of how they differ from each other [61]. Karyotype analyzes commonly used in cytotaxonomy of the genus *Centaurea* have been reported in the literature [54, 55, 58, 62, 63, 64]. For this purpose, the chromosome morphologies of the taxa *Centaurea inexpectata* Wagenitz [Subsection *Jacea* (L.) Garcia-Jacas, Hilpold, Susanna & Vilatersana], *Centaurea athoa* DC. [Section *Acrocentron* (Cass.) DC.], *Centaurea stapfiana* (Hand.-Mazz.) Wagenitz [Section *Phaeopappus* (DC.) O.Hoffm.)]

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and *Centaurea babylonica* (L.) L. [Section *Microlophus* (Cass.) DC.] belonging to the genus *Centaurea* were examined.

2. Material and Methods

Mature seeds were selected and germinated for karyological analysis (Table 1). Germinating root tips were pretreated with 0.002 M 8-hydroxyquinoline for 8 h at low temperature and then fixed in Carnoy's fixative for 24 h. For staining, the samples were hydrolyzed with 5 M hydrochloric acid (HCl) for 1 hour at room temperature and stained with 1% aceto-orcein. For all counts, at least five metaphase plates from different individuals were examined. After the best metaphase image was obtained, pictures were taken with light microscope. The best chromosome images of the taxa were selected and chromosome features and karyotype indices were calculated with the KAMERAM program [65, 66]. M_{CA} indices were calculated according to Peruzzi and Eroğlu [67]. Additionally, chromosome naming was done as suggested by Levan *et al.* [68].

Table 1. Localities of *Centaurea* taxa

Taxa	Localities
Centaurea inexpectata	Antalya: Gevne Valley, around Küçüklü village, 1750 m, 30 vi 2004, *T. Uysal* 598
Centaurea athoa	Balıkesir: Edremit, KazDağları, Sarıkız hill, 1650 m, 15 vii 2003, *T. Uysal* 521
Centaurea stapfiana	Diyarbakır: Diyarbakır-Silvan, Yeşilköy village, 627 m, 1 viii 2004, *T. Uysal* 900
Centaurea babylonica	Kahramanmaraş: Süleymanlı district road, 750 m, 26 vii 2003, *T. Uysal* 533

3. Results and Discussion

The chromosome morphologies of taxa are presented in the first time by this work. According to Index to Plant Chromosome Numbers (IPCN) and Chromosome Counts Database (CCDB, version web/CCDB_1.66), karyotype indices of the examined taxa were reported for the first time in this study. The chromosome features and karyotype indices of the taxa are shown in Tables 2 and 3. Furthermore, metaphase, karyogram and idiograms of the taxa are given Figure 1.

Table 2. The chromosome features of *Centaurea* taxa

Taxa	2n	R SC-LC (µm)	R- LC / SC	p (µm) mean (±SD)	q (µm) mean (±SD)	CL (µm) mean (±SD)	TCL (µm)	CI mean (±SD)	KF
Centaurea inexpectata	22	2.50 - 4.26	1.704	1.48 (±0.25)	1.80 (±0.22)	3.28 (±0.46)	36.048	45 (±0.02)	22m
Centaurea athoa	20	1.03 - 1.63	1.584	0.55 (±0.08)	0.73 (±0.11)	1.27 (±0.17)	12.75	43 (±0.03)	18m + 2sm
Centaurea stapfiana	18	5.27 - 7.65	1.452	2.82 (±0.50)	3.60 (±0.57)	6.41 (±0.91)	57.713	44 (±0.04)	16m + 2sm
Centaurea babylonica	16	3.93 - 8.93	2.272	2.31 (±0.83)	3.35 (±0.74)	5.66 (±1.51)	45.32	40 (±0.05)	12m + 4sm

R: Range. SC: shortest chromosome length. LC: longest chromosome length. p: mean length of the long short arm. q: mean length of the long arm. CL: mean chromosome length. TCL: total haploid complement length. CI: mean centromeric index. SD: standart deviation. KF: karyotype formula. m: metacentric.

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Table 3. The karyotype indices of *Centaurea* taxa

Taxa	A_1	A_2	CV_{CL}	CV_{CI}	AI	M_{CA}
Centaurea inexpectata	0.181	0.14	14.014	5.251	0.736	9.76
Centaurea athoa	0.237	0.136	13.592	7.22	0.981	14.06
Centaurea stapfiana	0.209	0.141	14.115	9.428	1.331	12.15
Centaurea babylonica	0.32	0.267	26.687	12.605	3.364	18.38

A_1:intrachromosomal asymmetry index, A_2:interchromosomal asymmetry index, CV_{CL}:coefficient of variation of chromosome length, CV_{CI}:coefficient of variation of centromeric index, AI:karyotype asymmetry index, intrachromosomal asymmetry (M_{CA}).

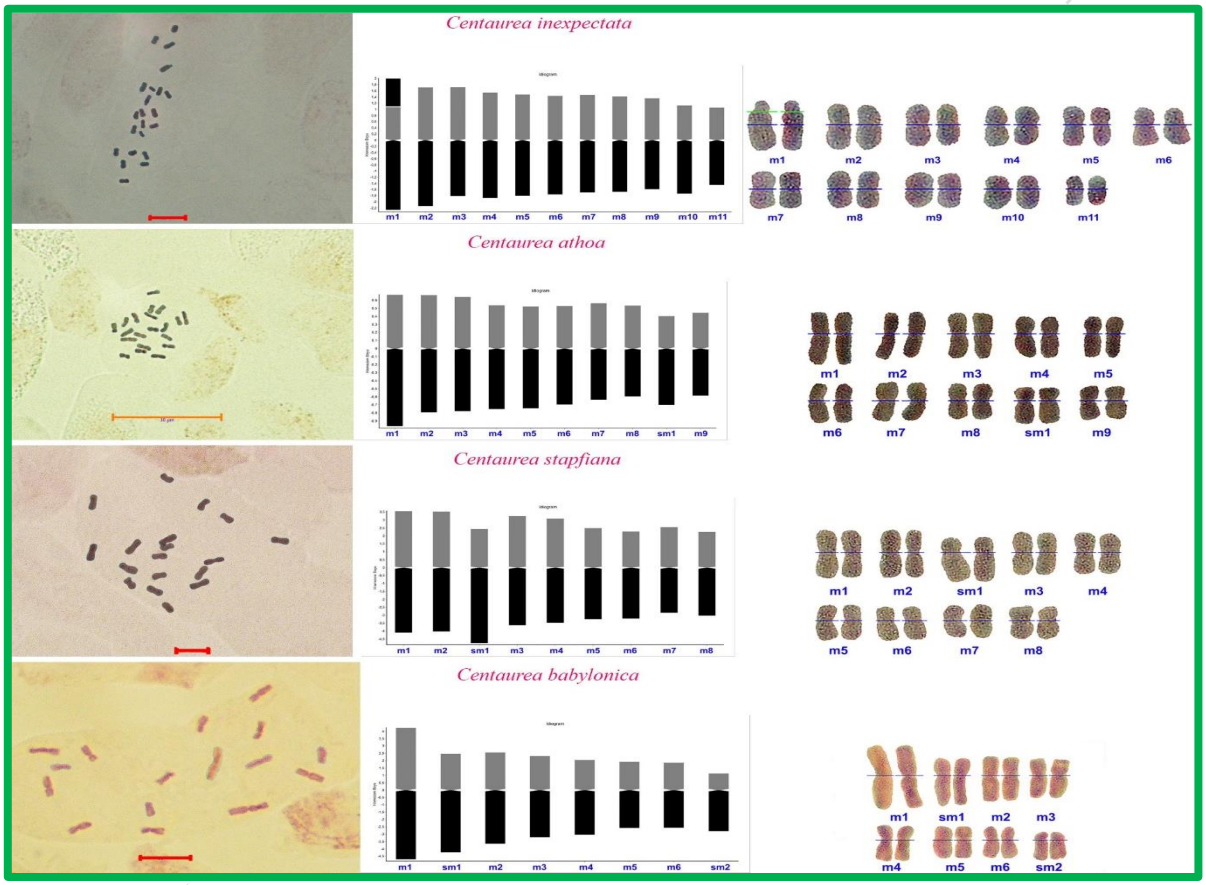

Figure 1. Mitotic metaphase chromosomes, idigrams and karyograms of *Centaurea* taxa

***Centaurea inexpectata* Wagenitz [subsection *Jacea* (L.) Garcia-Jacas, Hilpold, Susanna & Vilatersana]**

C. inexpectata is an endemic species for Turkiye. Its basic chromosome number is *x*=11. Additionally, it has a satellite located on the short arm of the first chromosome pairs. The karyotype formula of the species is 2*n*=22m (Table 2, Figure 1). The previous report is 2*n*=22 [54]. Moreover, it was emphasized that the taxa within *Jacea* have the same basic chromosome number (*x*=11) [54, 69]. Therefore, our results confirm the previous reports. The lengths of chromosomes vary among 2.50-4.26 and total haploid complement length is 36.048. When the karyotype indices (Table 3; CV_{CL}, CV_{CI}, AI and M_{CA}) of the taxa examined in the study are compared, *C. inexpectata* has the most symmetrical karyotypes.

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***Centaurea athoa* DC. [section *Acrocentron* (Cass.) DC.]**
C. athoa is a rare Mediterranean species and its basic chromosome number is *x*=10. The karyotype formula of the species is 2*n*=18m + 2sm (Table 2, Figure 1). Previous reports are 2*n*=20 [54, 70-72]. Therefore, our results confirm the previous reports. The lengths of chromosomes vary among 1.03-1.63 and total haploid complement length is 12.75. When the taxa examined in the study are compared in term of these values (Table 2), *C. athoa* has the smallest chromosomes and TCL.

***Centaurea stapfiana* (Hand.-Mazz.) Wagenitz [section *Phaeopappus* (DC.) O.Hoffm.)]**
C. stapfiana is an endemic species for Turkiye. Its basic chromosome number is *x*=9. The karyotype formula of the species is 2*n*=16m + 2sm (Table 2, Figure 1). The previous report is 2*n*=18 [54]. Therefore, our results confirm the previous report. The lengths of chromosomes vary among 5.27-7.65 and total haploid complement length is 57.713. When the taxa examined in the study are compared (Table 2), *C. stapfiana* has the greatest TCL.

***Centaurea babylonica* (L.) L. [Section *Microlophus* (Cass.) DC.]**
The basic chromosome number of *C. babylonica* is *x*=8. The karyotype formula of the species is 2*n*=12m + 4sm (Table 2, Figure 1). The previous report is 2*n*=16 [54]. Therefore, our results confirm the previous report. Different basic chromosome numbers (*x*= 8 and 9) have been reported in the *Microlepis* section containing this species [73-79]. The lengths of chromosomes vary among 3.93-8.93 and total haploid complement length is 45.32. When the karyotype indices (Table 3; CV_{CL}, CV_{CI}, AI and M_{CA}) of the taxa examined in the study are compared, *C. babylonica* has the most asymmetrical karyotypes. Stebbins [80] reported that asymmetric karyotypes were more developed than symmetric karyotypes in the phylogeny and evolutionary process, and those with a high level of symmetry were considered "primitive" (i.e. plesiomorphic). It was reported by Garcia-Jacas *et al.* [81] that the threshold value between primitive and derived is *x*=12, and those below this value are considered the most evolved group. According to the literature, *C. inexpectata* is primitive and *C. babylonica* is advanced among the taxa examined, and we can say that *C. babylonica*, the lowest basic chromosome number among the taxa, is more evolved than the others.

As a result, it was determined that the species for which chromosomal studies were carried out in different sections of the cornflower genus were not uniform in terms of basic chromosome number and morphology, and it was revealed that they showed a series of changes in terms of chromosomal variation. It was considered an important result that the *C. babylonica* species, which has a wider distribution area compared to the others among the species considered, also has high asymmetry values in terms of chromosomal variation.

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INVESTIGATION OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID SUBSTANCE AMOUNTS AND DUALEX VALUES OF BURDOCK (*Arctium lappa* L.) PLANT

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Abstract
Burdock (*Arctium lappa* L. = *Lappus officinalis* L.) is a commercially an important plant often used in traditional medicine. Burdock (*Arctium lappa*) is an edible plant from a member of the Asteraceace family. Burdock is widely used as a traditional medicinal herb due to its beneficial effects on inflammatory disorders such as arteriosclerosis, gout, and hepatitis. Additionally, it has anti-inflammatory, antimutagenic and anti-aging properties due to its rich antioxidant content. The plants were collected from Van Yüzüncü Yıl University, Medicinal and Aromatic Plants Garden in 2020. In this study; antioxidant activity, total phenolic and flavonoid substance amounts and dualex values (nitrogen balance index (NBI), chlorophyll content, flavonol and anthocyanin content) were examined. In the results of working; The amount of antioxidant substance was determined as 124.87 µmol TE/g, total phenolic substance (211.50 mg GAE/g) and total flavonoid substance amount was determined as 11.87 mg QE/100 g. The data obtained in terms of Dualex values (dx) such as Nitrogen Balance Index (NBI), chlorophyll content, flavonol and anthocyanin content are respectively; It was determined as 13.73 mg/g, 25.80 mg/cm2, 1.88 dx and 0.07 dx.

Key Words: *Arctium lappa* L., medicinal plant, antioxidant, phenolic, flavonoid, dualex value

1. Introduction

Arctium lappa plant is a member of the *Asteracea* family. It grows widely in the Balkans and Central Anatolia, in the Toros Mountains, Maraş, Ardahan Bursa and Yalova (Tanrıkulu, 2009; Gok Hündür, 2022). *Arctium lappa* species does not have a natural distribution in Van province. However, there are 4 species of the *Arctium* genus. These are; *Arctium minus* subsp. *Minus, Arctium minus* subsp. *puberty, Arctium platylepis* and *Arctium tomentosum* var. *Glabrum.*

It is a durable perennial herbaceous plant that grows on roadsides. It can grow up to 1.5 meters tall (Yalcın, 2010). Burdock is a two-year-lived plant, approximately 1 to 1.5 meters tall, with dark green leaves, brownish flowers, and small violet-colored flowers at the ends of the flowers. It is found on roadsides, on fertile land shores (in places rich in ammonia), where animals pass. It blooms in summer. The part used is mostly the root. Its root is thick spindle shaped. The leaves are used fresh. It is collected in every season (Colak, 2014). It is a biennial herbaceous plant. It grows in moist habitats, gardens, roadsides and waste areas. All plant parts, especially dried roots, are used. Its important components are lignans, flavonoids and phenolic acids. It is good for infections, skin diseases, cancer, stomach and intestinal problems (Duke 2002; Li et al. 2013). There are many plants used in the treatment of diabetes among people in Turkey. One of these plants is the *Arctium lappa* (burdock) plant. The plant is popularly used in the treatment of diabetes by boiling its root parts (decoction) (Baytop, 2009). They grow in arid and stony lands, along roads and walls. Its roots and leaves are used. All plant parts, especially the dried roots, are used for therapeutic purposes.

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Figure 1. Plant of *Arctium lappa* L.

Figure 2. Flowers of *Arctium lappa* plant

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The tea prepared from its root as an infusion is considered as a painkiller, diaphoretic, blood purifier, diuretic, and curative of inflammatory skin diseases and eczema. Infusion prepared from its leaves; It is used in intestinal diseases, ulcers and burns (by applying or washing), stomach inflammation, mental illnesses and rheumatism. It (leaves) contains inulin, acids, pectin, sugar, mucilage, sesquiterpene, lactones and tannin (Ozata, 2009). It cleans the blood. It is diuretic. It is effective against germs and fungi. It has sweat enhancing properties. It helps in rheumatism, lumbago, sciatica pain and swelling. It is used as. Kidney and bladder stones and eczema, psoriasis and thrush It is effective in the treatment of skin diseases. It is good for oily and acne skin. It cuts the dandruff in the hair. It accelerates the healing of wounds and ulcers on the skin (Ozata,2009). It cleans toxins from the body and is effective in digestive system disorders (Emre, 2012).There are many plants used in the treatment of diabetes among people in Turkey. One of these plants is the *Arctium lappa* (burdock) plant. The plant is popularly used in the treatment of diabetes by boiling its root parts (decoction) (Baytop, 2009). Since its roots are poisonous, it should not be used in high doses. To use the leaves of burdock, the plant leaves can be plucked and collected in May. Food (stuffed) is made from its leaves. It is a herbaceous plant with above-ground parts that are annual, roots that are perennial, and can grow up to 50-150 cm tall. The body is grayish in color and hairy. It has wide and fleshy leaves with an upright stem. The lower leaves are very large. It is 30 cm wide and 50 cm long. It is covered with feathers. Leaf stalk is long. While its stalks are consumed raw, they are also collected by the local people to cook the stalks as food. It blooms in June and September. Its seeds are striped. *Arctium lappa* is an important plant used for health since ancient times. This plant, which has been preferred in alternative medicine since the past, is also beneficial for health.

Figure 3. Root parts of *Arctium lappa* plant

This plant, which has a natural botox effect, has been preferred to stay young since ancient times. It is also particularly beneficial for skin and hair. It is resistant to cold weather and likes moist areas (Lushchak, 2014). It is widely found in nature and its leaves are always green. It helps reduce intestinal

worms. It is also frequently used in decorative areas (Mandal ve ark., 2009). Antidiabetic effect of *Arctium lappa* plant in certain dosage ranges has shown. However, in high doses, the plant has been shown to further increase blood sugar levels. Therefore, the plant should be used by adjusting the dosage. The public should be informed about the hyperglycemic effect of the plant in high doses. As an ideal dose, 7 grams of dried root should be used per day for an average person weighing 70 kg (Bilge, 2023). Antioxidants are classified into two groups, namely, pri-mary or chain-breaking antioxidants and secondary antioxi-dants, depending on their mechanism of action. The formerreact with lipid peroxy radicals to convert them to stableproducts; this group includes chain breakers (or free radicalinhibitors) and peroxide decomposers. Secondary antioxi-dants, such as oxygen scavengers, reduce the rate of chain ini-tiation (Gordon, 1990). In this study; It was aimed to determine the dualex values, antioxidant activity amount, and total phenolic and flavonoid substance amounts of *Arctium lappa* species, which is an important medicinal and aromatic plant.

2. Materials and Methods

The plants were collected from Van Yuzuncu Yil University, Medicinal and Aromatic Plants Garden in 2020. With the Dualex Scientific+ (FORCE-A, France) device, which can measure chlorophyll (μg/cm^2), nitrogen balance index (NBI) (mg/g), flavonol and anthocyanin content (dx) on the leaf just before the plants are collected, in real time and non-destructively, thanks to the leaf clip on the sensor was measured and recorded. Then, harvesting was done with the help of sterile scissors from the connection part where the soil and the stem part meet. For the analyzes to be performed on dry samples, plant samples were dried in an oven at 40 ^{0}C and stored under appropriate conditions.

In the study, total antioxidant activity amount, FRAP (ferrous ion reducing antioxidant power), Lutz et al (2011); total phenolic substance amount, Obanda and Owuor (1997); The total amount of flavonoid substance was determined according to the method developed by Quettier et al. (2000).

3. Results and Discussion

Antioxidants are a group of chemical substances that are produced by body cells and also taken with food (especially from herbal products). The most important antioxidants taken with food are beta-carotene and vitamins E and C. In addition, other antioxidant substances are lycopene, eugenol, resveratrol, myrcetin, silymarin and some sesquiterpene lactone, triterpene, steroid and tannin compounds (Wang et al., 1996; Aruoma, 2003). It is known that medicinal plants provide great support to the body's defense system and that the use of these plants in the treatment of liver and bile disorders, as well as their antioxidant effects, plays a preventive role in the onset of many illnesses and diseases. In this study, the amount of antioxidant capacity was determined as 124.87 μmol TE/g KA, the phenolic substance content was determined as 211.50 mg GAE/g KA, and the flavonoid substance content was determined as 12.00 mg QE/100g KA (Figure 1).

In the numerous studies, (in vitro and in vivo), burdock has been found to exhibit a plethora of biological activities and pharmacological functions, including anti-inflammatory, anti-constipation, anti-cancer , angiogenic, antioxidant (Li ve ark., 2019; Zhang ve ark., 2020; Wang ve ark., 2021; Yang ve ark., 2021). *A. lappa* has shown positive effects, especially in the treatment of acne (Miglani et al., 2014). In terms of antioxidant capacity (DPPH), they determined it to be 76.23% in the root extracts of burdock and 46.41% in the leaf extracts. Burdock contains high amounts of monosaccharides and polysaccharides, mainly consisting of mannose, glucose, fructose and galactose compounds with potent antioxidant activity (Jiang et al., 2019; Chen et al., 2020).

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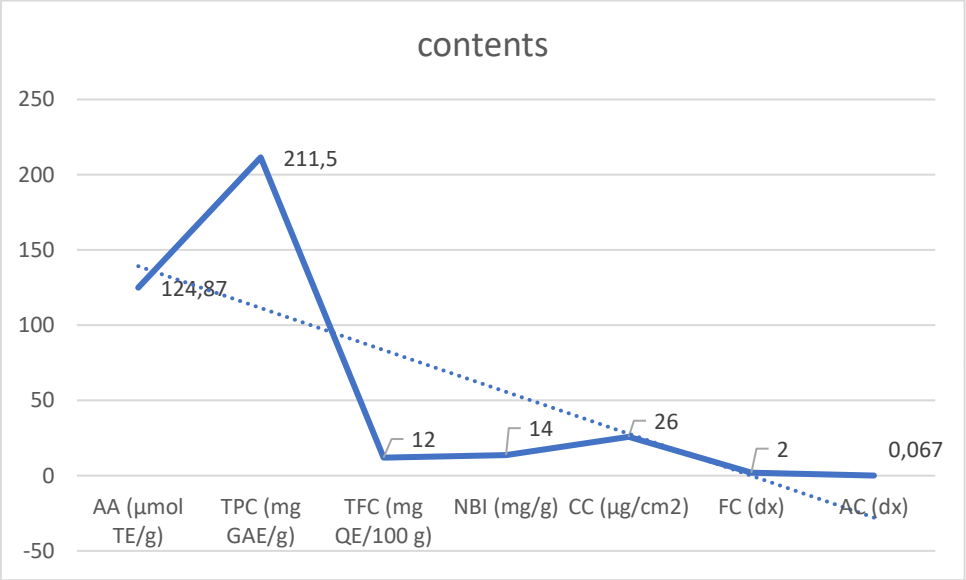

Figure 4. Total antioxidant activity, total phenolic, flavonoid substance contents and dualex values of *Arctium lappa* plant

In his study, the amount of antioxidant capacity in the flower extracts of burdock was found to be between 19.77-36.31 mg/ml (DPPH), 9.77-26.31 mg/ml, and 0.19-3.63 mg/ml in the plant fruit. Today, extracts taken from parts of medicinal plants such as roots, leaves, flowers and fruits constitute the main component of many medicinal drugs (Eren, 2011). Free radicals and reactive oxygen species (ROS) occur during metabolic events that occur in our bodies throughout our lives. Oxidative stress disrupts the balance between ROS production and antioxidant defense and causes oxidative degradation, which causes antioxidant defense mechanisms (A, C, E, glutathione, ubiquinone, flavonoids, etc.) to enzymatic (catalase, superoxide dismutase, glutathione peroxidase, etc.) It has been shown that deficiency as well as ROS and excessive activation play a role in the emergence of many diseases such as heart and nerve diseases, diabetes, asthma and rheumatism (Gok and Serteser, 2003).

It has been shown that *Arctium lappa* plant has an antioxidant effect and has effects on free radicals and active oxygen (Duh, 1998). It has been determined that the antioxidant effect of the AL plant is effective in reducing the blood sugar levels of diabetic rats and that the *Arctium lappa* plant has an antidiabetic effect at certain dose ranges. However, it has been determined that in high doses the plant increases blood sugar levels even further. Therefore, the plant should be used with dosage adjustments (Cigremiş et al., 2003). In this study; NBI (chlorophyll/phenolic) content was determined as 14.0 mg/g, chlorophyll content as 14.26 microgram/cm2, flavonol content as 2 (dx) and anthocyanin content as 0.067 (dx). The Dualex™ was developed by Goulas et al. (2004) in cooperation with FORCE-A to non-destructively measure leaf phenolic content to evaluate a correlation with the nitrogen status of plants. Cerovic et al., (2005), described that phenolics increased under nitrogen deficiency. At the same time, chlorophyll responded in an opposite manner. Cartelat et al. (2005) suggested that the chlorophyll/phen ratio expressed as NBI was a better indicator for leaf nitrogen concentration as each parameter alone (Overback et. al., 2018). Phenolics including the major group of flavonoids are most important groups of secondary metabolites and bioactive compounds in plants (Kim et al., 2003) and their synthesis is highly correlated with light because of their function as UV-protective pigments (Li et al., 1993). A rapid increase in flavonoid biosynthesis is generally observed under high light conditions, which reflects the important role of flavonoids in photoprotection (Schmelzer et al., 1988; Zoratti et al., 2014). First

results of Barthod et al. (2007) showed that the Dualex™ derived UV absorbance of leaf epidermis increased significantly with increasing light and the data from the instrument could be a good indicator for the amount of phenolics in the leaves (Overback et. al., 2018).

4. Conclusion

It has been determined that an unknown component in the fresh plant liquid obtained from burdock root has antimutagenic effect (preventing mutation, that is, an unexpected change in the cell DNA). In vitro studies have shown that burdock root extracts have a similar effect to the enzyme that neutralizes harmful radicals called superoxide dismutase in the body, and thus have an antioxidant effect by neutralizing harmful hydroxyl radicals. In this study we conducted, it was determined that burdock has rich content in terms of antioxidants in its aboveground parts. Burdock has anti-inflammatory, antimutagenic and anti-aging properties, and the infusion prepared especially from its leaves is used in intestinal diseases, ulcers and burns (applied or washed), stomach inflammations, mental disorders and rheumatism. It is an important plant where the whole plant, including the root part, is used for phytotherapeutic purposes. It grows naturally in our geography. This potential should be better evaluated.

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INVESTIGATION OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID SUBSTANCE AMOUNTS OF *Filipendula ulmaria* (L.)

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Abstract
Filipendula ulmaria (L.) Maxim. is a perennial plant belonging to the Rosaceae family. Meadowsweet plant grows widely throughout Western Asia, Europe, North America and Siberia. In our country; It has a natural distribution in the Western, Central and Eastern Black Sea, Upper Sakarya, Upper Euphrates, Erzurum-Kars, Upper Murat-Van and Hakkari sub-regions. FU contains flavonoids, tannins, phenolic glycosides (salicylate), essential oils (salicylaldehyde), minerals and vitamin C. It is a plant with anti-ulcer, anti-rheumatic, immunomodulatory and cytotoxic properties because it contains various polyphenol compounds. The aboveground parts of the plants were collected from Van Yuzuncu Yil University, Medicinal and Aromatic Plants Garden in 2020. In this study; antioxidant activity, total phenolic and flavonoid substance amounts were examined. In the results of working; The amount of antioxidant substance was determined as 136.59 µmol TE/g, the total phenolic substance (198.08 mg GAE/g) and the total flavonoid substance amount was determined as 24.71 mg QE/100 g.

Key Words: *Filipendula ulmaria*, medicinal plant, antioxidant, phenolic, flavonoid

1. Introduction

Filipendula ulmaria (FU) is a perennial plant belonging to the Rosaceae family, growing widely in Europe and Western Asia. *Spirea ulmaria L.*, and *Filipendula denudata (J.&K. Presl) Rydb*. are known as *Filipendula ulmaria.* It is a perennial, herbaceous shrub. Since it contains various polyphenol compounds, it has anti-ulcer, anti-rheumatic, immunomodulatory and cytotoxic properties and provides significant benefits in terms of biological activity (Blazic et al., 2010; Barros et al., 2013; Neagu et al., 2015). Filipendula's natural habitats are moist meadows and moist coasts. The plant prefers neutral and basic soils and enjoys moist soil (Grieve, 1982). It flowers from June to early September (Lindemann et al., 1982). The use of the goat's beard plant dates back to ancient times. In fact, it was one of the three most sacred plants, like oak, of the druids (priests from the clergy) who ensured the continuation of Celtic traditions. The leaves and flowering stems of *F. ulmaria* have anti-inflammatory, antiseptic, analgesic, aromatic, astringent, diaphoretic, diuretic, stomachic and antipyretic properties. It also has a long history of use in the treatment of gout and inflammatory diseases such as rheumatoid arthritis, diarrhoea, cystitis, irritable bowel syndrome, hyperacidity, heartburn, gastritis and peptic ulcers (Grieve 1982; Dobelis 1990; Chevalier 1996; Baytop 1999). A strong decoction of the boiled root is said to be effective in treating wounds and ulcers when used externally (Grieve 1982).

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Figure 1. Plant of *Filipendula ulmaria*

The flower head of *Filipendula* contains salicylic acid, from which the drug aspirin can be synthesized. Unlike aspirin, which can cause stomach ulcers in high doses, the combination of ingredients in this herb protects the lining of the stomach and intestines while providing anti-inflammatory benefits. This is due to the combination of salicylates found in the plant with tannins and other components (Chevallier, 1996).

Figure 2. Flowers of *Filipendula ulmaria* plant

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Studies have shown that there are numerous phenolic compounds, including salicylates, flavonols and ellagitannins, that contribute to biological activities in the plant extract (Santas et. al., 2008; Azman et al., 2014). Additionally, it shows antioxidant and antimicrobial activity in various foods. Consumers should benefit from this plant in a health-conscious manner. Because, as a result of long-term use, gastrointestinal irritation, constipation, iron deficiency anemia and malnutrition may occur. Therefore, it should not be used in people with bleeding problems. Additionally, research has shown that it causes breathing difficulties and should not be used in people with asthma (Blumenthal, 1998). In the study by Abd-Hamid et al., (2017); They determined that *Filipendula ulmaria* is an important source of antioxidants in extending the shelf life of food products. In this study; it was aimed to determine the amount of antioxidant activity and total phenolic and flavonoid substance amounts of *Filipendula ulmaria* species, which is an important medicinal and aromatic plant with cytotoxic effects.

2. Materials and Methods

The aboveground parts of the plants were collected from Van Yuzuncu Yil University, Medicinal and Aromatic Plants Garden in 2020. After the collected plants were washed with tap water, they were sterilized with distilled water and dried between drying papers, away from direct sunlight. In this study; Total antioxidant, total phenolic and flavonoid substance contents were examined in 4 replicates. In the study, the amount of total antioxidant activity, FRAP (ferrous ion reducing antioxidant power), Lutz et al. (2011); total phenolic substance amount, Obanda and Owuor (1997); The total amount of flavonoid substances was determined by Quettier et al. It was determined according to the method developed by (2000).

3. Results And Discussion

In this study, the total antioxidant content of the *Filipendula ulmaria* plant was determined as 136.59 µmol TE/g, the total phenolic substance was 198.08 mg GAE/g, and the flavonoid substance content was 24.71 mg QE/100 g (Figure 3). In the study by Abd-Hamid et al., (2017); They determined that *Filipendula ulmaria* is an important source of antioxidants in extending the shelf life of food products. The ferric reducing antioxidant power (FRAP) antioxidant activity for FU and AV extracts were 44.6 and 40.12 mmol of TE/g DW. Adb-Hamid et al., (2017). Sroka et al. (2001) and Papp et al. (2004) recorded the antioxidant activity of *F. ulmaria.*

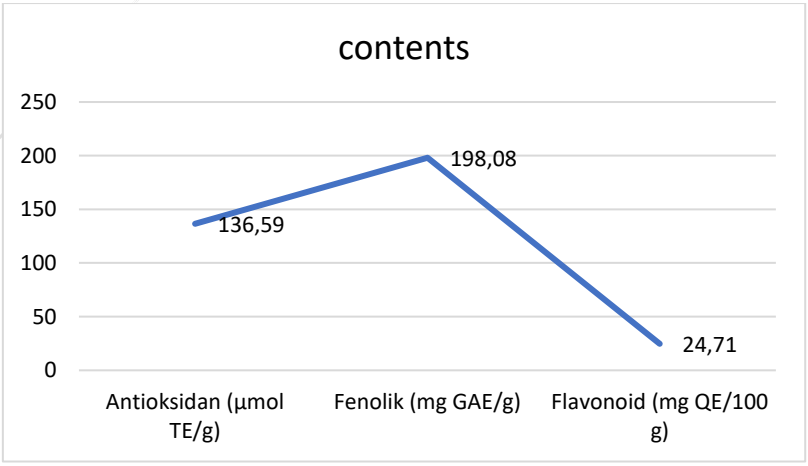

Figure 3. Total antioxidant activity, total phenolic and flavonoid substance contents of *Filipendula ulmaria* plant

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In their study by Savina et al., (2023) on the leaves (lower middle and upper), stem (lower, middle and upper), flowers, fruits and roots of the *Filpendula ulmaria* plant; While the highest amount of total antioxidant capacity (230.4 mg AsA g-1) was determined from the flower parts, the lowest antioxidant activity (30.4 mg AsA g-1) was obtained from the lower parts of the stem, as was the total phenolic and flavonoid substance content. In the study, the highest total phenolic substance content was obtained from the flower parts (64.65 mg GAE g-1), but there was no statistical difference between the fruit (63.66 mg GAE g-1) and upper and lower leaf (62.87 and 61.29 mg GAE g-1) parts. They reported that there was no difference and that they were in the same group.

They reported that the highest value in terms of total flavonoid substance was obtained from flower parts (166.78 mg RE g-1). In addition, they reported that the lowest values in terms of both total phenolic (22.40 mg GAE g-1) and flavonoid substance content (1.54 mg RE g-1) were detected in the stem parts of the plant. Modern studies conducted both in vivo and in vitro prove the anti-inflammatory, antibacterial, anticancer, antidepressant, and antioxidant properties of meadowsweet (Katanic et al., 2016; Kurkin et al., 2020; Gainche et al., 2021). As a result of this study, it was found that the leaves, flowers, fruits, and roots of the meadowsweet plant are characterized by a high total content of phenolic compounds (up to 65 mg g-1), although about two times lower than the previously determined value in meadowsweet grass (about 120 mg g-1) presented in a study by (Harbourne et al., 2009). Additionally, the flavonoid content (maximum content in flowers was about 170 mg g-1) of the samples analyzed in the present study was about 1.6 times higher than the results obtained for meadowsweet flowers in the work of Baranenko et al., (2019). The differences in the results are probably due to the different growing conditions of the plants, as well as the different extraction methods used in the studies. In a study by Harbourne et al., (2009), the total content of phenolic compounds was determined in the extracts of aerial parts (a mixture of flowers, stems, and leaves) of meadowsweet collected in Ireland. The extract was obtained using heated distilled water (100 ^{0}C) stirred on hot plate (Harbourne et al., (2009). Baranenko et al. investigated the extracts of flowers obtained using ethyl alcohol with a volume fraction of 70%. The plant material was collected from different regions of Russia (Leningrad and Yaroslavl regions, Republic of Bashkortostan) Baranenko et al., (2019). The variation in the content of phenolic compounds and flavonoids in extracts of meadowsweet (Lipinska et al., 2014; Nile et al., 2021).

Climatic conditions such as average air temperature, humidity, and soil structure and composition are known to significantly affect the phenolic compound content in wild plants (Bautista et al., 2016). The aqueous extract, FUE, used for the synthesis of FUAgNP, contained similar total phenolic content (241.86 mg GA/g of dry extract) and higher flavonoid content (235.70 mg QU/g of dry extract) when compared with methanolic extracts of *F. ulmaria* aerial parts (249.53 mg GA/g of dry extract and 45.47 mg QU/g of dry extract, respectively). Similar antioxidant activity of *F. ulmaria* methanolic extract and fue was also observed (Katanic et al., 2015). Mihaliovic et al., (2023), in their study, in DPPH and ABTS methods in FU extracts; The amount of antioxidant activity is respectively; They determined as 15.82 and 59.85 microgram/ml.

4. Conclusion

With the increasing importance of phytotherapy in today's modern medicine, medicinal plants are frequently used to support the body's antioxidant mechanism and to provide prophylaxis (preventive treatment) against diseases that may be caused by free radicals. It is known that medicinal plants provide great support to the body's defense system and that the use of these plants in the treatment of liver and bile disorders, as well as their antioxidant effects, plays a preventive role in the onset of many illnesses and diseases. With this study, it has been concluded that some of its species, which can be cultured and are naturally distributed in our country's flora, have a high adaptation to cool seasons, and also have a

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strong potential to be used as alternative medicine or food supplements due to their cytotoxic properties. The Filipendula plant contains salicin, which is used as the raw material of several drugs such as aspirin. Salicylin is the main ingredient of aspirin and has analgesic, antipyretic and anti-inflammatory properties. However, the use of the *Filipendula* plant is not common in direct aspirin production. Instead, commercial aspirin is often produced synthetically. However, salicin can be obtained from some plant sources (such as blueberries). Therefore, they may be a potential source for the production of aspirin from natural sources. Nowadays, the use of salicin obtained by reliable and standardized synthetic methods is preferred for the production of aspirin. More comprehensive research is needed to determine whether the *Filipendula* plant has a standard suitable for salicin concentration and quality for use as a pharmaceutical raw material. By achieving positive results, raw materials will be obtained through natural means and a contribution to the pharmaceutical industry will be made.

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ELASTASE AND LIPOXYGENASE INHIBITORY EFFECTS AND PHYTOCHEMICAL ANALYSES ON CULTIVATED SAMPLE OF *MOMORDICA CHARANTIA* L. IN SAMSUN PROVINCE (TÜRKİYE)

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Abstract
Momordica charantia L. is an important medicinal plant species from the Cucurbitaceae family, known in English as "bitter melon, balsam pear, or bitter gourd". It is an annual, herbaceous, and climbing plant with a stem up to 1-2 m long. The fruits, which resemble a lumpy shuttle, turn orange when ripe. The fruits are about 10 cm wide and 20 cm long, flat, and bear 20-30 seeds that turn brown as they ripen. The plant seeds are rich in fixed oil and protein in addition to high amounts of vitamins C and A, beta-carotene, alpha-carotene, potassium, magnesium, and zinc. On the other hand, the plant is also rich in components such as cucurbitane-type triterpenoids, triterpene glycosides, phenolic acids, flavonoids, volatile oil, saponins, fatty acids, and proteins. Although there are many studies on the positive effects of the fruits and oily macerate of *M. charantia* on skin wounds, the number of studies on the inhibitory effects against the lipoxygenase (LOX) enzyme is very few. According to our literature search, there is only one study on *M. charantia* growing naturally in Türkiye. On the other hand, no study on elastase inhibition of naturally grown or cultivated samples in our country has been found so far. Therefore, there is no study examining the LOX and elastase inhibitory effects on cultivated pomegranate in our country. For this reason, elastase and LOX inhibitory effects of the extracts in different polarities prepared from the seeds, seed pods, and leaves of the bitter melon cultivated in Samsun province were investigated. The inhibitory effect of all extracts on elastase was found to be below 20%, while only the leaf extract showed a 57.82 ± 2.04% inhibitory effect against LOX. The phytochemical content of the leaf extract was analyzed by liquid chromatography-mass spectrometry (LC-MS). In this paper, the elastase and LOX inhibitory effects of the plant and the results of the LC-MS analysis will be presented.

Key Words: *Momordica charantia*, bitter melon, elastase, lipoxygenase, enzyme inhibition, LC-MS

1. Introduction

Momordica charantia L. (bitter melon) is a very important medicinal plant species from the Cucurbitaceae family, known in English as "bitter melon, balsam pear or bitter gourd". It is an annual, herbaceous, climbing plant with stems up to 1-2 m long. The fruits, which resemble a lumpy shuttle, turn orange when ripe. The fruits are about 10 cm wide and 20 cm long, flat, and bear 20-30 seeds that turn brown as they ripen. The plant seeds are very rich in fixed fat and protein and contain high amounts of vitamins C and A, beta-carotene, alpha-carotene, potassium, magnesium, and zinc [1]. On the other hand, the plant is also rich in components such as cucurbitane-type triterpenoids, triterpene glycosides, phenolic acids, flavonoids, volatile oil, saponins, fatty acids, and proteins.

M. charantia grows in many parts of Asia and Africa. It grows naturally in East Africa, the Amazon basin, and the Caribbean Islands. It is also found in South America and the Far East. The fruits of the plant are grown in Yalova, Bursa, and Antalya and cultivated in different provinces in our country for consumption. The ripe fruits of the plant are kept in olive oil or sweet almond oil for 3-6 months to make

a paste. This paste-like oily mixture is used internally and externally. In addition to the healing of stomach ulcers, the oily macerate of potassium pomegranate is also directly applied to skin disorders such as wounds, burns, eczema, and psoriasis.

The use of bitter melon has been recorded in the folk medicine of various countries against diabetes, dysmenorrhea, eczema, gout, jaundice, abdominal pain, kidney stones, rheumatism, psoriasis, leprosy, hemorrhoids, pneumonia, fever, and scabies [2-10]. There are also studies on many bioactivities of the plant such as antidiabetic, anthelmintic, immunomodulatory, antimalarial, anti-inflammatory, antioxidant, antimicrobial, anticancer, and laxative [11-21]. Also known as "akçakız" in Savaştepe district of Balıkesir and "yağanda, yarhand" in the Lalapaşa district of Edirne, the maserate of *M. charantia* prepared with olive oil was reported to be used internally for stomach ulcers and externally as a wound healer [22,23]. It was also reported to be used in the same way and for the same purpose in the Marmaris district of Muğla [24]. In the Manisa region, the use of the fruits of the plant against diabetes has been recorded [25].

In this study, the elastase and 15-LOX inhibitory effects of extracts of different polarities prepared from seeds, seed pods, and leaves of the pomegranate plant cultivated in the Samsun region were investigated. The phytochemical content of the extracts determined to be active was elucidated by the liquid chromatography-mass spectrometry (LC-MS) technique.

2. Material and Methods

2.1. Plant materials and extraction

The seeds, seed pods, and leaves of the plant cultivated in Samsun were collected as study material in September, 2023. All samples were dried in the shade and ground into powder in the mill. Ethanol (85%) extracts were prepared from the seeds, seed pods, and leaves obtained from samples of the fruit of the plant at different ripening stages of the plant by maceration at room temperature followed by evaporating to dryness under vacuum. All of the extracts were stored in the refrigerator for further experiments.

2.2. Elastase inhibition assay

Elastase inhibition by the extracts was determined using the modified spectrophotometric method of Kraunsoe et al. [26] and Lee et al. [277]. In order to evaluate the inhibition of elastase activity, the amount of released p-nitroaniline, which was hydrolyzed from the substrate, N-succinyl-Ala-AlaAla-p-nitroanilide, by elastase, was assayed by measuring absorbance at 410 nm. In brief, 1.015 mM solution of *N*-succinyl-Ala-Ala-Ala-*p*-nitroanilide was prepared in a 0.1 M Tris-Cl buffer (pH 8.0) and this solution (130 µL) was added to the test sample (10 µL) in a 96 well microplate. The microplate was pre-incubated for 5 min at 25°C before an elastase (0.5 Unit mL^{-1}) stock solution (15 µL) was added. After enzyme addition, the microplate was kept at 25°C for 30 min, and the absorbance was measured at 410 nm using a microplate reader. All experiments were carried out in triplicates.

2.3. LOX inhibition assay

The 96-well microplate-based-FOX assay was carried out on the extracts according to the method described by Waslidge and Hayes [28], with minor modifications. An aliquot of 50 µL LOX in 50 mM Tris HCl buffer, pH 7.4 (final concentration, 100 ng protein/mL), was pre-incubated with 20 µL test sample (plant extracts or standard inhibitor) in each well of the 96-well microplate at 25°C for 5 min. For the control, 50 µL of LOX solution and 20 µL of buffer containing 0.2% (v/v) DMSO (final concentration) were pipetted into the wells. Blanks (background) contained the enzyme LOX during incubation, but the substrate (linoleic acid) was added after the FOX reagent. The reaction was initiated by the addition of 50µL linoleic acid (final concentration, 140 µM) in 50 mM Tris HCl buffer, pH 7.4,

and the reaction mixture was incubated at 25°C for 20 min in the dark. The final concentrations of LOX and linoleic acid were based on a total volume of 120 µL for the reaction mixture. The assay was terminated by the addition of 100µL freshly prepared FOX reagent: sulfuric acid (30 mM), xylenol orange (100 µM), iron (II) sulfate (100 µM), methanol/water (9:1). After termination, the Fe^{3+}–dye complex was allowed to develop for 30 min at 25°C before being measured at 560nm on a microplate reader.

2.4. LC-MS/MS analysis conditions

A Shimadzu-Nexera model ultrahigh performance liquid chromatography (UHPLC) coupled with a tandem mass spectrometer was used to accomplish quantitative evaluation of 53 phytochemicals using the method of Yılmaz [29]. The reversed-phase UHPLC was equipped with an autosampler (SIL-30AC model), a column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and a degasser (DGU-20A3R model). The chromatographic conditions were optimized in order to achieve optimum separation for 53 phytochemicals and overcome the suppression effects. Different coluımns such as Agilent Poroshell 120 EC-C18 model (150 mm×2.1 mm, 2.7 µm) and RP-C18 Inertsil ODS-4 (100 mm×2,1 mm, 2µm), different mobile phases (B) such as acetonitrile and methanol, different mobile phase additives such as ammonium formate, formic acid, ammonium acetate, and acetic acid, different column temperatures such as 25°C, 30°C, 35°C and 40°C were tried and applied until the optimum conditions were achieved. Consequently, the chromatographic separation was performed on a reversed-phase Agilent Poroshell 120 EC-C18 model (150 mm×2.1 mm, 2.7 µm) analytical column. The column temperature was set to 40°C. The elution gradient was composed of eluent A (water+5 mM ammonium formate+0.1% formic acid) and eluent B (methanol+5 mM ammonium formate+0.1% formic acid). The following gradient elution profile was used: 20-100% B (0-25 min), 100% B (25-35 min), 20% B (35-45 min). Furthermore, the solvent flow rate and injection volume were settled as 0.5 mL/min and 5 µL, respectively.

The mass spectrometric detection was carried out using a Shimadzu LCMS-8040 model tandem mass spectrometer equipped with an electrospray ionization (ESI) source operating in both negative and positive ionization modes. LC-ESI-MS/MS data were acquired and processed by LabSolutions software (Shimadzu). The MRM (multiple reaction monitoring) mode was used for the quantification of the phytochemicals. The MRM method was optimized to selectively detect and quantify phytochemical compounds based on the screening of specified precursor phytochemical-to-fragment ion transitions. The collision energies (CE) were optimized in order to generate optimal phtochemical fragmentation and maximal transmission of the desired product ions. The MS operating conditions were applied as: drying gas (N2) flow, 15 L/min; nebulizing gas (N2) flow, 3 L/min; DL temperature, 250°C; heat block temperature, 400°C, and interface temperature, 350°C.

3. Results and Discussion

The ethanolic extracts were of the seeds and seed coats at 1st and 2nd ripening stages; ripe seeds, and leaves obtained from the cultured sample of *M. charantia* were screened against elastase and 15-LOX. As tabulated in Table 1, all of the extracts displayed elastase inhibition lower than 50%, whereas extracts of seed coats at 2nd ripening stage and leaves had 49.93 ± 5.96% and 57.82 ± 2.04% LOX inhibition, respectively.

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Table 1. Elastase and 15-LOX inhibition results of the ethanolic extracts

Extracts	Elastase Inhibition (Inhibition % ± S.D.[a])	15-LOX Inhibition (Inhibition % ± S.D.)
Seeds at 1st ripening stage	11.59 ± 0.59	15.13 ± 2.09
Seeds at 2nd ripening stage	6.37 ± 0.88	-[b]
Ripe seeds	11.14 ± 1.06	14.47 ± 1.82
Seed coats at 1st ripening stage	11.55 ± 1.38	8.24 ± 1.11
Seed coats at 2nd ripening stage	8.73 ± 0.88	49.93 ± 5.96
Leaves	12.39 ± 1.04	57.82 ± 2.04
References	99.65 ± 0.08[c]	80.17 ± 1.17[d]

[a] Standart deviation (n: 3), [b] No inhibition, [c] *N*-Methoxysuccinyl-Ala-Ala-Pro-Val-chloromethyl ketone (1 mg/mL), [d] Baicalein (1 mg/mL)

LC-ESI-MS/MS analysis was carried out in the seed coats at 2nd ripening stage and leaves. LC chromatogram of the standards is presented in Figure 1. When the quantitative results were examined, 16 and 14 components were detected in seed coats at 2nd ripening stage and leaves samples, respectively (Table 2). The highest fumaric, quinic, and 4-OH-benzoic acids and vanillin (551.1284, 77.5486, 34.4357, and 28.7159 mg analyte/100 g dry plant, respectively) were detected in the seed coats at 2nd ripening stage sample. In the leaf sample, isoquercitrin, astragalin, rutin, and nicotiflorin components (17.2280, 4.5263, 4.3508, and 3.2280 mg analyte /100 g dry plant, respectively) were found to be the highest. Generally, it was determined that the 2nd ripening stage sample was richer in phenolic acids, while the leaf sample was richer in flavonoids.

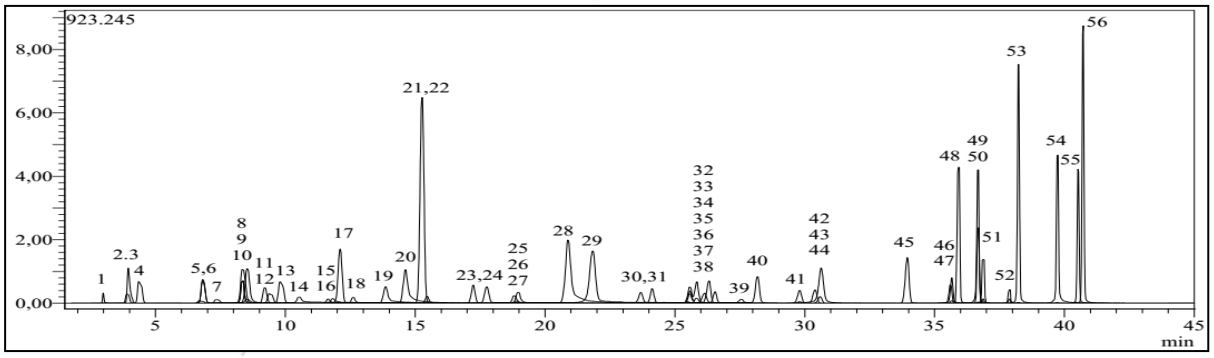

Figure 1. LC chromatogram of the standards
(1: Quinic acid 2: Fumaric acid 3: Aconitic acid 4: Gallic acid 5: Epigallocatechin 6: Protocatechuic acid 7: Catechin 8: Gentisic acid 9: Chlorogenic acid 10: Protocatechuic aldehyde 11: Tannic acid 12: Epigallocatechin gallate 13: 1,5-Dicaffeoylquinic acid 14: 4-OH-Benzoic acid 15: Epicatechin 16: Vanillic acid 17: Caffeic acid 18: Syringic acid 19: Vanillin 20: Syringic aldehyde 21: Daidzin 22: Epicatechin gallate 23: Piceid 24: *p*-Coumaric acid 26: Ferulic acid 27: Sinapic acid 28: Coumarin 29: Salicylic acid 30: Cynaroside 31: Miquelianin 33: Rutin 34: Isoquercitrin 35: Hesperidin 36: *o*-Coumaric acid 37: Genistin 38: Rosmarinic acid 39: Ellagic acid 40: Cosmosiin 41: Quercitrin 42: Astragalin 43: Nicotiflorin 44: Fisetin 45: Daidzein 47: Quercetin 48: Naringenin 49: Hesperetin 50: Luteolin 51: Genistein 52: Kaempferol 53: Apigenin 54: Amentoflavone 55: Chrysin 56: Acacetin)

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Table 2. Identification and quantification of phenolic compounds at 2nd ripening stage and leaves by LC-MS/MS

No	Analytes	RT[a]	M.I. (*m/z*)[b]	F.I. (m/z)[c]	Ion. mode	Equation	r^{2d}	Seeds at 2nd ripening stage (mg analyte /100 g dry plant)	Leaves (mg analyte/ 100 g dry plant)
1	Quinic acid	3.0	190.8	93.0	Neg	*y*=-0.0129989+2.97989×	0.996	77.5486	N.D.[e]
2	Fumaric aid	3.9	115.2	40.9	Neg	*y*=-0.0817862+1.03467×	0.995	551.1284	N.D.
4	Gallic acid	4.4	168.8	79.0	Neg	*y*=0.0547697+20.8152×	0.999	0.5447	0.5438
6	Protocatechuic acid	6.8	152.8	108.0	Neg	*y*=0.211373+12.8622×	0.957	12.6848	2.105
10	Protocatechuic aldehyde	8.5	137.2	92.0	Neg	*y*=0.257085+25.4657×	0.996	4.4747	2.8245
14	4-OH-Benzoic acid	10.5	137,2	65.0	Neg	*y*=-0.0240747+5.06492×	0.999	28.7159	N.D.
17	Caffeic acid	12.1	179.0	134.0	Neg	*y*=0.120319+95.4610×	0.999	0.5447	0.5614
19	Vanillin	13.9	153.1	125.0	Poz	*y*=0.00185898+20.7382×	0.996	34.4357	1.4912
20	Syringic aldehyde	14.6	181.0	151.1	Neg	*y*=-0.0128684+7.90153×	0.999	4.0077	N.D.
24	*p*-Coumaric acid	17.8	163.0	93.0	Neg	*y*=0.0249034+18.5180×	0.999	5.0583	2.3333
28	Coumarin	20.9	146.9	103.1	Poz	*y*=0.0633397+136.508×	0.999	1.5564	1.4561
33	Rutin	25.6	608.9	301.0	Neg	*y*=-0.0771907+2.89868×	0.999	N.D.	4.3508
34	Isoquercitrin	25.6	463.0	271.0	Neg	*y*=-0.111120+4.10546×	0.998	2.8793	17.2280
35	Hesperidin	25.8	611.2	449.0	Poz	*y*=0.139055+13.2785×	0.999	N.D.	3.1052
37	Genistin	26.3	431.0	239.0	Neg	*y*=1.65808+7.57459×	0.991	0.3891	0.2280
42	Astragalin	30.4	447.0	255.0	Neg	*y*=0.00825333+3.51189×	0.999	N.D.	4.5263
43	Nicotiflorin	30.6	592.9	255.0/284.0	Neg	*y*=0.00499333+2.62351×	0.999	N.D.	3.2280
47	Quercetin	35.7	301.0	272.9	Neg	*y*=+0.00597342+3.39417×	0.999	2.8793	N.D.
49	Hesperetin	36.7	301.0	136.0/286.0	Neg	*y*=+0.0442350+6.07160×	0.999	0.4280	N.D.
50	Luteolin	36.7	284.8	151.0/175.0	Neg	*y*=-0.0541723+30.7422×	0.999	0.1945	0.0526

[a]R.T.: Retention time, [b]MI (*m/z):* Molecular ions of the standard analytes (*m/z* ratio), [c]FI (*m/z):* Fragment ions [d]r^2: Coefficient of determination, [e]N.D.: Not detected

4. Conclusion

No study on elastase inhibition of naturally grown or cultivated samples in our country has been found so far. Therefore, in this study, the elastase and LOX inhibitory effects of extracts of different polarity to be prepared from the seeds, seed pods, and leaves of the pomegranate plant cultivated in the Samsun region were investigated. The phytochemical content of the extracts determined to be active was elucidated by LC-MS. The leaf extract has some phenolic acids and flavonoids. The most active extract, which was the leaf, as well as the seeds, seed coats, and other parts, should be studied further. The present work represents the first study on cultivated bitter melon in our country, examining its LOX and elastase inhibitory effects, as well as profiling its phenolic content.

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Conflict of Interest
We disclose no conflict of interest.

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