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The Eighth International Mediterranean Symposium
on Medicinal and Aromatic Plants
October 20-22, 2022 / Ephesus, İzmir - TÜRKİYE



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**The Eighth International Mediterranean Symposium on
Medicinal and Aromatic Plants**

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October 20th – 22th, 2022

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Dear Colleagues,

Having 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 Faculty of Pharmacy, Eastern Mediterranean University (EMU) joint with AMAPMED (Association of Medicinal and Aromatic Plants of the Mediterranean).

MESMAP-2 Symposium was held on April 22-25, 2015 in Antalya – TURKEY, which was organized by academicians from Gazi University (TURKEY), Gaziantep University (TURKEY), Kilis 7 Aralık University (TURKEY), Yüzüncü Yıl University (TURKEY), 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 high impact factor from 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) – Turkish Republic of Northern Cyprus (TRNC), was the third event of MESMAP symposium series on Medicinal and Aromatic Plants. After scientific evaluation selected full papers published in Indian Journal of Pharmaceutical Education and Research (IJPER), indexed with THOMSON REUTERS. MESMAP-4 Symposium, which was held on April 18-22, 2018 in Sherwood Breezes Resort Hotel Antalya – Turkey, was the fourth event of MESMAP symposium series on Medicinal and Aromatic Plants. Then, the fifth one was MESMAP-5 symposium, which was organized as joined meeting with ISPBS-5 at Cappadocia on April 24-28, 2019. After scientific evaluation selected full papers of MESMAP-5 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. MESMAP Symposiums provide a platform for herbal medicines, biology, chemistry, plant biotechnology, botany, ethnobotany, phytopharmacology, pharmacognosy, food, agriculture and forestry, phytochemistry and aromatherapy. Afterwards, MESMAP-6 Symposium was organized on October 15-17, 2021 and this symposium was supported TÜBİTAK 2223-B National Scientific Meetings Grant Program. After scientific evaluation selected full papers of MESMAP-6 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. Last year, MESMAP-7 was organized on November 18-20, 2022 and hosted by Dokuz Eylül University and Torbalı (Izmir) Chamber of Commerce-Turkey. This symposium was the eighth meeting series of MESMAP, and you can find abstracts of all the scientific works presented in MESMAP-8 in this ABSTRACTS & PROCEEDINGS BOOK. We would like to encourage MESMAP-8 participants to submit the full papers to the contracted journals. After scientific evaluation, selected full papers will be published in 'Molecules', 'Brazilian Journal of Pharmacognosy', 'Annals of Phytomedicine', 'International Journal of Agriculture, Environment and Food Sciences' and 'Current Perspectives on Medicinal and Aromatic Plants (CUPMAP), after scientific evaluation. We are also proud to announce that MESMAP international symposiums are indexed by Web of Sciences Conference Proceedings Citation Index-Science (CPCI-S) / Scopus Index. Furthermore, MESMAP-8 has been supported by TÜBİTAK 2223-B National Scientific Meetings Grant Program. We would like to thank for their sincere supports of Turkish General Directorate of Forestry, TURKISH AIRLINES, Gaziantep University, Khon Kaen University, Kumamoto University, Aegean Exporters' Associations, Rural Federal University of Rio de Janeiro (UFRRJ)-Brazil, AMAPMED, Association of Pharmaceutical Teachers of India, Cosmetic Producers and Researchers Associations (KUAD), Talya Herbal Company, Laber Cosmetics Company, NS Herbs Company and all the other supporters. Organizing Committee hope that MESMAP-8 Symposium participants would have an amazing experience and unforgettable memories to take back their homes. We would like to thank to all our participants from almost all over the world for their valuable attendance and scientific contribution to MESMAP-8. We are planning to organize the ninth meeting series of MESMAP in 2023 spring and it will be a big honor for us to see you again at MESMAP-9 symposium.

Sincerely,
Symposium Chair

Prof. Dr. Nazım ŞEKEROĞLU
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KEYNOTE
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SPEAKERS



KEYNOTE SPEAKER

CHEMISTRY, TECHNOLOGY AND PHARMACOLOGY OF
DITERPENOID ALKALOIDS

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Abstract

The development of alkaloids chemistry in Uzbekistan is associated with the name of outstanding scientist academician S.Yu. Yunusov. On his initiative, in 1956, the Institute of Chemistry of Plant Substances (ICPS) has been established.

The fundamental works of S.Yunusov on the chemistry of alkaloids are the basis for the creation of drugs. Nowadays 300 plant species belonging to 60 genera and 35 families have been studied, about 1300 individual alkaloids have been isolated.

Comparative pharmaco-toxicological characterization of diterpenoid alkaloids (DAs) differ in the nature and location of functional substituents in the lycotoxine, heteratizine, and perhydrophenanthrene skeletons, has been investigated for the first time. More than 180 diterpenoid alkaloids (100 of them novel) have been isolated from 32 species of *Aconitum* L., 20 species of *Delphinium* L., and 5 species of *Consolida*.

The unique representatives of C₂₀-diterpenoid, C₁₉-norditerpenoid, and C₁₈-bisnorditerpenoid bases have been investigated. Of these, actaline, arcutine, zerakonine, corifine, and thalatisamine are the first representatives of new types of C₂₀-diterpenoid bases. The sum of C₂₀-DAs of some plants of the *Aconitum* genus contains up to 80% of lappaconitine, the main metabolite of which is N-deacetylappaconitine. It has been established that DAs exhibit a pronounced antiarrhythmic and antifibrillatory effect and belong to fast Na⁺-channel blockers. A new direction has been formed for the creation of highly effective antiarrhythmic drugs based on DAs with various spectra and mechanisms of action. For practical medicine, lappaconitine, N-deacetylappaconitine, 1-benzoylnappeline and other drug candidates significantly superior to antiarrhythmics quinidine, novocainamide, ethmosine, aimaline, rhythmylene, lidocaine, and mexityl were proposed. Research on lappaconitine molecule modification is continue.

Novel and improved technologies for complex processing of alkaloid-containing raw materials using new methods of liquid-liquid extraction-ultrafiltration, water-alcohol extraction with organic solvents limitation etc. were developed. Original antiarrhythmic drugs Allapinin, Aklesin, Aksaritmin, and Antiaritmin have been introduced into medicine now.

Key Words: C₂₀-diterpenoid alkaloids, lappaconitine, antiarrhythmic activity, technology



KEYNOTE SPEAKER

SIMILARITY AND DISSIMILARITY OF SESAMIN, SESAMOLIN AND
SESAMOL

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Abstract

Sesamin, sesamol, and sesamol are secondary metabolites of the same origin, sesame oil. Phenylalanine is a precursor of their synthesis pathway. Sesamin is a major metabolite. Sesamol is hydrolyzed into sesamol, which is in trace amounts. Dissimilarity in their chemical structures led to their different physicochemical properties, intracellular absorption, and bioactivity. Sesamin and sesamol are lipophilic compounds, while sesamol is a hydrophilic one [1]. Their topological polar surface area, number of rotatable bonds, number of hydrogen bond acceptors, Log P, and Log D_{7.4} are different [1]. The three sesame compounds were taken up inside human melanoma cells (SK-MEL-2) at a greater rate than in noncancer Vero cells. Sesamol was intracellularly uptaken in the SK-MEL-2 via a carrier-mediated pathway, while sesamin and sesamol were via passive transport [1]. These three compounds possessed anticancer activity in a cell-based assay [1–7], antioxidation [8–11], protective effect from ultraviolet rays [9], and inhibitory effect on tyrosinase enzyme *in vitro* [8–10]. These biological activities were in different degrees. Sesamol can act either as an oxidant or pro-oxidant compound depending on concentration. Despite the lowest intracellular uptake of sesamol compared to sesamin and sesamol, sesamol is the only compound that exerted antiproliferation in melanoma cells [1]. The low aqueous solubility of sesamin and sesamol limited the assessment of their cytotoxicity and any other activities [12]. In conclusion, the differences in their respective structures affect intracellular drug absorption and bioactivity, which are keys for further design and development of these three compounds for functional uses.

Keywords: sesame, physico-chemical properties, cellular uptake, transportation, melanoma, oxidant, and prooxidant.

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INVITED SPEAKER

PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF
MEDICINAL PLANTS FROM KAZAKHSTAN

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Abstract

The strategic policy of the Republic of Kazakhstan is aimed to create high-tech technologies based on pharmaceutical production by reducing planned dependence on imported medicines, i.e. domestic production forces, raw materials, the scientific and technical potential of the country. Today, one of the main directions of development of the Republic of Kazakhstan is the development of the pharmaceutical industry at the expense of domestic raw materials. Plants are important not only for food but also for medicine. Understanding the taxonomy, ecology and conservation of herbs, as well as the associated secondary metabolites, their biological properties and pathways of their synthesis are important for drug development. Natural compounds play an important role in treating human diseases, and in recent years have received much attention of scientists and pharmaceutical industries. For thousands of years, medicine and natural products have been closely linked through the using of traditional medicines. The flora of Kazakhstan is characterized by its wide variety of different types of medicinal plants, many of which can be used on an industrial scale. The Traditional Kazakh Medicine (TKM) was developed during centuries based on the six elements of ancient Kazakh theory, associating different fields such as, pharmacology, anatomy, pathology, immunology, and food nursing as well as prevention diseases, diagnosis, and treatment techniques of common diseases.

Our research team main focused on interestingly medicinal plants' resources of Kazakhstan, to investigate natural bioactive complexes, determine of their chemical profiles, and isolate of unique natural products leads. This will followed by studying their structures and pharmacological activity relationships. Recently, the research center within the framework of national and international research projects has been analyzed and determined chemical composition and bioactivities of various medicinal plants such as *Artemisia absinthium*, *Artemisia albicerata*, *Artemisia sublessingiana*, *Artemisia pauciflora*, *Artemisia heptapotamica*, *Artemisia transiliensis*, *Dracocephalum nutans*, *haloxylon ammodendron*, *Ligularia narynensis*, and *Bergenia crassifolia* from Almaty and East Kazakhstan regions. In which the most promised, selected plant materials were investigated comprehensively. Main goal of us is to develop safe and effective phytomedicines of defense against cancer, influenza, diabetes, hypertension, obesity and other future human health threatened pandemics.

Keywords: Medicinal plants, phytochemistry, natural product lead, pharmacological activities, TKM, Kazakhstan

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INVITED SPEAKER

**THE CHEMISTRY OF BRAZILIAN PITCH (BREU) OIL-RESINS:
CHEMISTRY IN THE LIGHT OF ETHNOPHARMACOLOGY**

Suzana GUIMARÃES LEITÃO

*Departamento de Produtos Naturais e Alimentos, Faculdade de Farmácia, Universidade Federal do
Rio de Janeiro, Rio de Janeiro, BRAZIL*

Abstract



INVITED SPEAKER

**BIOACTIVE TRITERPENOID SAPONINS FROM ALGERIAN
MEDICINAL PLANTS**

Zahia KABOUCHE

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Substances Thérapeutiques (LOST), Campus Chaabet-Ersas, 25000 Constantine, ALGERIA*

Abstract

Phytochemical investigations of Algerian medicinal plants belonging to Lamiaceae, Caprifoliaceae, Caryophyllaceae and Primulaceae led to the isolation of bioactive triterpenoid saponins which were identified by analyses of 1D and 2D-NMR (^1H - ^1H COSY, TOCSY, HSQC-TOCSY, HSQC, ROESY or NOESY, and HMBC) spectroscopic data and mass spectrometry (HR-ESI-MS), and by comparison with those of related metabolites. A contribution to the knowledge of their chemotaxonomy is given here.



INVITED SPEAKER

**PHYTOCHEMICAL AND BIOLOGICAL ACTIVITY STUDIES ON
ULMUS SPECIES GROWING IN TURKEY WITH UNTARGETED
METABOLOMICS STUDY**

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Abstract

The genus *Ulmus* belonging to the Ulmaceae is represented by four species in Turkey (*U. glabra* Hudson, *U. minor* Miller., *U. canescens* Melville, *U. laevis* Pallas) (Browicz and Zielinski, 1982). In India, China, South Korea, and Turkey, different *Ulmus* species have been used as folk medicine. *U. glabra*, *U. minor*, and *U. canescens* are used as wound healing, inflammation reliever, and pain reliever, especially in the treatment of bone fractures in "folk medicine studies" in Turkey (Altundağ and Ozturk, 2011). Additionally, *U. rubra*, known as slippery elm, takes part in the American pharmacopoeia (American Botanical Council, 2012). Although it has traditionally been used in the treatment of several places in the world, there are very few biological and phytochemical studies on *Ulmus* species. The aim of this study is to study phytochemical studies of all *Ulmus* species and compare the metabolite profiles of the species to reveal the differences, as well as to investigate their biological activities. Ethanolic (maceration) and aqueous (decoction) extracts have been prepared from the inner bark of four *Ulmus* species. The extract obtained from *U. rubra* was used as a reference plant because it is included in the pharmacopoeia. Metabolic profiling of the extracts has been performed LC-qTOF-MS system (Gonulalan et al., 2020). As a result of the analysis, especially 123 secondary metabolites that are common in all species and a totally of 290 different metabolites have been determined in five *Ulmus* species. In addition, they were found to be quite active in *in vitro* enzyme inhibition tests (such as collagenase, elastase, tyrosinase, α -amylase, α -glycosidase inhibition tests). In this study, the dereplication of the metabolites of the species was performed. Therefore, it is suggested that analysis profiles can be used in distinguishing the extracts in the future.

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INVITED SPEAKER

EFFECT OF THE ETHANOLIC EXTRACT OF OROXYLUM INDICUM SEED ON DEPRESSION IN UNPREDICTABLE CHRONIC MILD STRESS-INDUCED MOUSE MODEL AND ITS MECHANISM

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Abstract

Major depressive disorder (MDD) is a common and debilitating psychiatric disease characterized by persistent low mood, lack of energy, hypoactivity, anhedonia, decreased libido, and impaired cognitive and social functions. However, the multifactorial etiology of MDD remains largely unknown due to the complex interaction between genetics and environment involved. *Oroxylum indicum* (Phe-ka) is a Thai herbal medicine that has been used as one of the components in Thai folk medicines. The plentiful constituents, reported in different parts of *Oroxylum indicum*, are flavonoids. The major types are B-ring unsubstituted flavones and their glycoside, such as baicalein, baicalein 7-O-glucuronide (baicalin), baicalein, chrysin, oroxylin A, and scutellarein. These main flavonoids are responsible for biological activities. This research was aimed to investigate the antidepressive effect of *O. indicum* seed extract in unpredictable chronic mild stress (UCMS)-induced mouse model and clarify its mechanism. Daily administration of *O. indicum* extract to UCMS mice ameliorated both anhedonia, by increasing 2% sucrose intake, and hopeless behavior, by reducing immobility times in the forced swimming test (FST) and tail suspension test (TST) without any effect on locomotor activity. The mechanism of *O. indicum* activity was multi-modal. *O. indicum* extract protected the brain oxidative damage by inhibiting the lipid peroxidation and increasing the catalase and superoxide dismutase activities in both frontal cortex and hippocampus. Daily treatment with *O. indicum* significantly reversed UCMS-induced hypothalamic–pituitary–adrenal axis (HPA) axis dysregulation by reducing of corticosterone serum level and upregulating the glucocorticoid receptor (GR) while downregulating serum- and glucocorticoid-inducible kinase 1 (SGK1). *O. indicum* extracts also exhibited an inhibitory effect in vitro on monoamine oxidase (MAO) A and B. The multiple antidepressant actions of *O. indicum* emphasize its potential as an effective, novel treatment for MDD.

Key Words: *Oroxylum indicum*, chronic stress, oxidative stress, hypothalamic–pituitary–adrenal axis, glucocorticoid receptor.

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INVITED SPEAKER

HPLC-ESI-MS CHEMICAL ANALYSIS, ANTIOXIDANT, ALPHA-AMYLASE AND PANCREATIC LIASE INHIBITORY EFFECTS AND ANTI-INFLAMMATORY ACTIVITIES OF *EPHEDRA ALTISSIMA*

Hamada Haba^{1*}, Waffa Bouafia^{1,2}, Amel Hamdi^{3,4}, Soumia Mouffouk¹, Rocío Rodríguez-Arcos³, Ana Jiménez-Araujo³, Rafael Guillén-Bejarano³

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Abstract

The important therapeutic applications of plants belonging to the genus *Ephedra* of the family Ephedraceae motivated us to study the species *Ephedra altissima* Desf. This endemic species is common to the Sahara of Algeria (Hoggar and Neighboring massifs, Tefedest). *Ephedra altissima* is used in folk medicine for the treatment of various diseases such as vascular hypertension and respiratory diseases and also for diabetic diseases. The present investigation focuses on the chemical composition, α -amylase and pancreatic lipase inhibitory activities and anti-inflammatory and antioxidant properties of the extracts (petroleum ether, ethyl acetate and *n*-butanol) prepared from *Ephedra altissima* aerial parts. The characterization of the phenolic compounds in extracts was performed by HPLC-DAD-ESI/MSⁿ, and the *in vitro* α -amylase and pancreatic lipase inhibitory activities were assessed using starch and *p*-nitrophenyl butyrate as substrates, respectively. Furthermore, the anti-inflammatory activity was carried out by bovine serum albumin denaturation method and the antioxidant capacity was evaluated using five different assays including DPPH scavenging activity, Total antioxidant capacity by phosphomolybdate (PPM), Ferric thiocyanate assay (FTC), Hydrogen peroxide scavenging assay and β -carotene bleaching activity. Flavonol and flavone glycoside derivatives, and phenolic acids, especially isovitexin-2-*O*-rhamnoside and vicenin II were the major compounds detected in the *n*-butanol extract. Furthermore, the ethyl acetate extract contained kaempferol-3-*O*-rhamnoside, quercetin-3-*O*-rhamnoside and protocatechuic acid as its main phytochemicals. The *n*-butanol extract exhibited the highest content of total phenolic compounds. The results for antioxidant activity indicated that EtOAc extract possesses the strongest activity in all the tested methods. Moreover, the EtOAc extract showed the best anti-inflammatory, α -amylase and pancreatic lipase inhibitory activities with IC₅₀ values of 126.43, 9.02 and 289.11 μ g/ml, respectively. These results bring evidence for the use of the species *Ephedra altissima* in traditional medicine for diabetic diseases.

Key Words: *Ephedra altissima*, HPLC-ESI-MS, polyphenol, α -amylase, pancreatic lipase, anti-inflammatory.



INVITED SPEAKER

**SOME COMPARATIVE CASE STUDIES ON EXTRACTION,
ISOLATION AND ANALYSIS TECHNIQUES APPLIED FOR
MEDICINAL AND AROMATIC PLANTS**

Temek ÖZEK

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Abstract

Extraction of target or active compounds from medical and aromatic plants can be performed using many different techniques. There are many different parameters that should be considered when performing extraction processes. While maintaining the studies of the researchers, it should be very well evaluated which parameters will reveal the results and should be able to make healthier decisions at the point of making the election to be applied. At this point, the results of previous experimental studies are guides for researchers. For this purpose, the examples obtained in different situations and conditions were briefly discussed and the results obtained depending on the techniques and methods applied were discussed comparatively.

In this context, the extraction applied to different types of medicinal and aromatic plants selected, the total yields obtained by isolation methods and the target compound yields were compared. In addition, the results performed with effective isolation and analysis methods applied to chiral substances as well as achiral substances were presented.



INVITED SPEAKER

**MEDICINAL PLANTS: WELFARE OF THE RURAL PEOPLE OF
SOUTH AND NORTH BENGAL, INDIA**

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Abstract

Herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. The blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. Of the 2,50,000 higher plant species on earth, more than 80,000 are medicinal. India is one of the world’s 12 biodiversity centres and having four hot spots with the presence of over 45000 different plant species. Of these, about 15000-20000 plants have good medicinal value. However, only 7000-7500 species are used for their medicinal values by traditional communities. In India, drugs of herbal origin have been used in traditional systems of medicines such as *Unani*, *siddha* and *Ayurveda* since ancient times. Plants, especially used in Ayurveda can provide biologically active molecules for the development of modified derivatives with enhanced activity and reduced toxicity.

Medicinal and aromatic plants are now on the decline due to deforestation, lack of scientific cultivation and conservation creating problems that demands our immediate attention for greater interest of nation. Survival of the forests leads to the survival of mankind. The state of West Bengal comprising plains and hills is gifted with enormous wealth of medicinal and aromatic plants due to its varied ecological conditions. These can be priceless resource with high potential for economic and ecological gains specially in the rural areas, if properly protected and promoted.

In order to preserve the natural resources of medicinal and aromatic plants in West Bengal along with their sustainable use with commercial exploitation as nontraditional cash crops for the welfare of rural people of Bengal, the present authors attempted to explore the medicinal plants flora in West Bengal under the UGC and DST projects. Present studies clearly revealed the feasibility of commercial exploitation of some target species both in north Bengal and South Bengal, widely used by the local people and can be exploited as non traditional cash crops for the upliftment of rural economy. A coordinated network has been suggested among the different stake holders involved.

Key Words: Herbal, medicines, safety, conservation, rural, economy



INVITED SPEAKER

**COSMETIC NANOFORMULATIONS WITH NATURAL
INGREDIENTS FROM LABORATORY TO PATENTS –
COSMEDICALS AS NEW ERA**

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Abstract

Natural cosmetics containing with herbal and other biological ingredients are a popular topic of cosmetic research due to amazingly increasing consumer trends worldwide. Because of side or undesirable effects of synthetic-based cosmetic ingredients have pushed R&D studies towards finding safer and more effective cosmetics, even called cosmeceuticals. In this regard, relatively new concept as “cosmetovigilance” has emerged. On the other hand, novel cosmetics/cosmeceuticals are expected to be environmentally-friendly. Besides cosmeceuticals must be able to penetrate deeper layer of the skin, passing through epidermis and treat a skin-related disorder. Hence, new cosmetic era can be said “cosmedicals”.

In our ongoing cosmetic-bases projects, we have been performing extensive screening assays on pure natural compounds as well as plant extracts in order to test for their anti-aging, antiwrinkle, and skin-bleaching effects against a number of cosmetic-related enzymes such as tyrosinase, elastase, collagenase, lipoxygenase, xanthine oxidase, etc. On the other hand, the active ingredients at molecule or extract forms were tested in cell culture assays, *e.g.* HACAT, HUVEC, human melanoma cell line (SKMEL 30), etc. Then, active molecules are progressed with *in silico* toxicity and molecular docking simulations. Not only anti-wrinkle or skin-bleaching effect, but also, we have been performing a project to prepare an anti-acne formulation based on plant extracts tested against *Propionibacterium acnes*. In addition, we have been working on a nanoformulation for wound dressing.

In conclusion, the extensive cosmetic-based screenings and following studies in our laboratory afforded promising findings from a number of plant extracts and pure natural molecules. Among some promising examples according to our outcomes, the extracts from *Cotinus coggygryia*, *Geranium glaberrimum*, *Garcinia mangostana*, etc., where we designed different formulations such as niosomes or nanoemulsions as well as flavonoids such as quercetin, fisetin, epigallocatechin gallate, etc. can be counted. The outcomes that we obtained from plants and other natural sources led us to register 3 patents, 4 patent applications, and a final product (commercialized as mouth spray). In the present talk, our recent cosmetic-related formulations will be emphasized more in detail.

Key Words: Cosmetic, cosmedical, cosmeceutical, plant extract, formulation, natural compound

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ORAL PRESENTATIONS



ORAL PRESENTATION

PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT
ACTIVITIES OF TRIKAYSORNMAS FORMULA

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Abstract

Trikaysornmas formula is one of the Thai traditional formulas commonly used in Thailand. The general indication of this formula followed Thai traditional medicine is enhancing appetite, nourish tonic and adaptogenic purpose. This formula composes of three herbs which are *Nelumbo nucifera* stamen, *Aegle marmelos* fruit and *Jatropha multifida* bark. Phytochemical constituents including total phenolic, total flavonoid, total carotenoid and total alkaloid were determined by colorimetric method. Antioxidant activities of this formula were performed by DPPH free radical scavenging assay and ABTS radical cation decolorization assay. High performance liquid chromatography using reversed-phase column was developed and validated for the determination of active constituent in the formula including imperatolin, kaempferol-3-o-glucoside, gallic acid, scopoletin and vitexin. Mobile phase system consisted of acetonitrile and 0.2% acetic acid solution using gradient elution for the analysis. The percentage yield from the extraction of this formula was 10.77 %. Total active contents in the formula were 303.19 mg gallic equivalent/g extract for phenolic compound, 20.28 mg quercetin equivalent/g extract for flavonoid compound, 1.49 mg beta-carotene equivalent/g extract for carotenoid compound and 13.06 mg atropine equivalent/g extract for alkaloid compound. The concentration at 50% inhibition by DPPH and ABTS radical scavenging assay was 77.50 and 25.62 µg/ml respectively. All validation parameters of this developed HPLC method performed the reliable results. From the results of this study can be used for the standardization and quality control aspect to be the support information to improve the potential of this Thai traditional formula.

Keywords: Trikaysornmas, *Nelumbo nucifera*, *Aegle marmelos*, *Jatropha multifida*



ORAL PRESENTATION

QUANTITATIVE ANALYSIS OF PHYTOCONSTITUENTS AND
SCREENING OF ANTIOXIDANT ACTIVITIES OF *COLEUS*
AROMATICUS BENTH. ORIGINATED IN CAMBODIA

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Abstract

Coleus aromaticus Benth., in the family of Lamiaceae, was widely cultivated in almost all tropical regions and used for centuries as a traditional medicine for treatment and prevention of diseases. There has not been any scientific evidence on phytochemicals and antioxidant properties of this plant originated in Cambodia yet. This study aims to determine the bioactive constituents and antioxidant activities of this plant. The leaves were collected and prepared as methanolic extract to quantitatively investigate for phenolic content via Folin Ciocalteu's method. Total chlorophyll content, total flavonoid content, and total alkaloid content were determined with spectrophotometric method. Antioxidation was screened by using DPPH radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. Results showed that this plant consisted of phenolic content as 391.8 ± 13.2 mg gallic acid equivalent/g of crude extract (CE). The content of flavonoid was 19.2 ± 2.1 mg quercetin equivalent /g of CE, while chlorophyll was 0.007 ± 0.001 mg/g CE, and alkaloid content was 0.2 ± 0.001 mg atropine equivalent /g CE. Furthermore, radical scavenging activities via DPPH assay was shown with the IC_{50} value of 86.9 ± 9.6 μ g/mL, and ABTS value was 0.4 ± 0.02 mM trolox equivalent /g CE. Moreover, the reducing antioxidant activity of FRAP assay was 0.4 ± 0.02 mM FeSO₄/g CE. Our study found the presence of alkaloids, flavonoids, and chlorophyll and this plant and may be attributed to the antioxidant activities of this plant. This evidence would be useful for further study for the development of food supplements or additives.

Keywords: *Coleus aromaticus*, Total Phenolic Content, Total Flavonoid Content, Antioxidant Activities.

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ORAL PRESENTATION

STUDIES ON CHEMICAL CONSTITUENTS OF THE SEEDS OF
LEPIDIDIUM SATIVUM L.

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Abstract

Lepidium sativum L. belonging to the family Brassicaceae (Cruciferae), also known as “garden cress”, is a fast-growing annual herb, it is a polymorphic species [1], used for its wide therapeutic application including anti-inflammatory, hypoglycemic, antihypertensive, fracture healing activity and efficacy in gastrointestinal diseases [2]. It can be assumed and cultivated for it is medicinal purpose. Eaten plants and the seed oils are used in treating dysentery, diarrhea and migraine [3]. It is also reported to be useful in the treatment of diabetes, hypertension, cough and bleeding piles [4]. They have various nutritional and medicinal attributes, and are recommended for anti-diarrheal, cardiogenic, hypotensive, antimicrobial, bronchodilator and hypoglycemic applications [5]. Seeds are also useful in hiccup, dysentery, diarrhea and skin diseases caused by impurities and toxins in blood and chronic enlargements of spleen [6]. They are useful in the treatment of asthma, coughs with expectoration, poultices for sprains, leprosy, skin disease, dysentery, diarrhea, splenomegaly, dyspepsia, lumbago, leucorrhoea, scurvy and seminal weakness [7]. The seeds are good source of protein, fat, calcium, iron and phosphorous. It mainly contains alkaloids, saponins, anthracene glycosides, carbohydrates, proteins, amino acids, flavanoids, and sterols as chief phytochemical constituents [8].

Seeds of *L. sativum* were purchased in August 2021 in an organic supermarket in Almaty and were identified by Prof. Janar Jenis (The Research Center for Medicinal Plants, Al-Farabi Kazakh National University). Dried and milled *L. sativum* seeds (10 kg) were extracted (3×24) with EtOH (95%). The extract was evaporated to remove the solvent. The EtOH extract (1300 g) was suspended in distilled H₂O and extracted successively with petroleum ether, dichloromethane, ethyl acetate and *n*-BuOH. The BuOH part (220 g) was chromatographed over a column of AB8 with Ethanol:Water 30%, 50%, 70%, 90%. The fractions that were rechromatographed over ODS columns and Sephadex LH-20 to afford compounds 1-5. The structures of isolated compounds are being identified by using 1D, 2D NMR spectroscopic analyses.

Keywords: *Lepidium sativum* L., chemical constituent, bioactivity, isolation, NMR

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ORAL PRESENTATION

**A HYDROPHILIC DEEP EUTECTIC SOLVENT-BASED METHOD
FOR THE RECOVERY OF NATURAL PRODUCTS FROM *HIBISCUS
SABDARIFFA***

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Abstract

Alternative nature antioxidant sources are of great importance for the development of novel functional food products. Citric acid-based deep eutectic solvent (DES) was integrated into automatic solvent extraction (ASE) to obtain high added-value substances from *Hibiscus sabdariffa*. Based on the earlier reports [1,2], a DES combination including citric acid as hydrogen bond acceptor and ethylene glycol as hydrogen bond donor was employed with a molar ratio ¼. This green solvent integrated extraction system was optimized by response surface method (RSM). Box-Behnken design type of RSM produced 17 experimental runs with 3 independent variables (water level in DES, extraction time and mass of the raw material) and 3 levels (20-50%, 30-60 min and 0.5-1.5 g). The ASE system was optimized to achieve the highest total phenolic content (TPC) and total anthocyanin content (TAC). The extract obtained under the optimal conditions were compared to those of conventional solvents such as ethanol, methanol and aqueous mixtures of them. Additionally, antioxidant activity of the *Hibiscus sabdariffa* extracts was also measured depending on *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazil) assay. The relationships between the TPC/DPPH findings and TAC/DPPH findings were also established.

Key Words: Green chemistry, green solvents, deep eutectic solvent, anthocyanin, antioxidant, multivariate optimization methods.

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ORAL PRESENTATION

ANTIBACTERIAL ACTIVITY OF HYDROGEL CONTAINING
PLAUNOTOL COMPLEXED WITH HYDROXYPROPYL- β -
CYCLODEXTRIN

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Abstract

Introduction: Plaunotol is acyclic diterpene accumulated in the leaves of *Croton stellatopilosus* Ohba [Euphorbiaceae]. It exhibited antibacterial activity against *Helicobacter pylori*, *Staphylococcus aureus*, *S. epidermidis*, and *Propionibacterium acnes*, for instance [1, 2]. **Objective:** This study aimed to prepare the inclusion complex of plaunotol and hydroxypropyl- β -cyclodextrin (HP- β -CD) due to increasing plaunotol's solubility. The topical hydrogel was formulated and assessed for its acne-prone bacteria. **Methodology:** Plaunotol was isolated from *C. stellatopilosus* leaves by column chromatography. Its chemical structure was confirmed by ¹H-NMR spectroscopy. The inclusion complex of plaunotol and HP- β -CD was prepared using the lyophilization technique. Investigation of the physical property of the complex was performed, including SEM, FT-IR, XRD, DSC, aqueous solubility, and thermal stability. The hydrogel was prepared from the inclusion complex using Carbopol® Utrez20 as the gelling agent. The MIC and MBC were determined. **Results and discussion:** The phase solubility study revealed the A_L type of the plaunotol and HP- β -CD inclusion complex. Ratios 1:1 and 1:2 of HP- β -CD were prepared and characterized. Aqueous solubilities of 1:1 and 1:2 ratios were increased by about 100%, while only 16% plaunotol was dissolved in water. The complexation of plaunotol with HP- β -CD significantly improved its antibacterial activity. It had a synergistic effect when combined with clindamycin. These results suggested that the inclusion complex enhanced plaunotol solubility. Therefore, the hydrogel could release plaunotol and exhibit antibacterial activity [3]. **Conclusion:** Hydrogel containing the inclusion complex of plaunotol and HP- β -CD is an alternative anti-acne gel formulation and is further applied as a cosmeceutical product.

Key Words: plaunotol; inclusion complex; antibacterial activity; hydrogel; synergistic

Acknowledgements:

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ORAL PRESENTATION

ANTAGONISTIC POTENTIAL OF LAVENDER ESSENTIAL OILS IN
COMBINATION WITH ANTIBIOTICS

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Abstract

In the post-antibiotic period, the issue of bacterial resistance refers to antibiotics. Due to increasingly common resistance to β -lactam antibiotics among *Staphylococcus aureus* the treatment of infections with these microorganisms has become more demanding. Essential oils have shown effectively synergistic effects with several antibiotics but the interactions of antibiotics with plant extracts can cause decreased effectiveness. This study aimed to verify the existence of the synergistic/antagonistic antimicrobial effect of *Lavandula stoechas*, *Lavandula heterophylla* essential oil, and linalool individually combined with amoxicillin and tetracycline towards clinical isolated methicillin-resistant *S. aureus* strains (MRSA). Previously, *L. stoechas*, and *L. heterophylla* essential oils were analyzed by gas chromatography-mass spectrometry (GC-MS) and Gas Chromatography/Flame Ionization Detector (GC-FID). Chemical analysis of *L. stoechas* essential oil contained predominantly camphor. The major components of *L. heterophylla* essential oils were linalool and linalyl acetate. Firstly, we determined the minimum inhibitory concentration (MIC) of essential oil, linalool, and antibiotics by microdilution assay. The combined effects of the tested samples were evaluated using the checkerboard method. In the assays, fractional inhibitory concentration (FIC) values were calculated. As a result, our studies showed that tetracycline has a potent antimicrobial effect on MRSA alone. However, essential oil and linalool combinations haven't shown any antimicrobial effect.

Key Words: Essential oil, *Lavandula*, antimicrobial effect, MRSA



ORAL PRESENTATION

THE POTENTIAL OF ALCOHOLIC PLANT EXTRACTS TO
CONTROL PATHOBIONTS OF SWINE ON A LOW-INPUT FARM

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Abstract

Objective / Purpose: Plant extracts have long proven antimicrobial potential depending on both the plant and target animal species. Pathogenic bacteria expose animals to disease risk, thus their control being of utmost importance. This research aimed to investigate the natural potential of locally available traditional medicinal plants in controlling the antibiotic resistant bacterial load in swine raised on low-input outdoor farms from North Western and Central Romania.

Material and Methods: Aerobic bacterial strains (n=14) originating from the nasal cavities of extensively raised swine were subjected to biochemical identification (Vitek®2 Compact System) and further tested for susceptibility to antibiotics (n=12, antibiotic classes=6, Kirby-Bauer method). Simultaneously, the aromagram technique was applied to indigenous *Calendula officinalis*, *Saturaja hortensis*, *Coriandrum sativum*, *Artemisia absinthium*, *Cucurbita pepo*, *Allium sativum* alcoholic extracts.

Results: The antibiogram indicated a multiple antibiotic resistance (MAR) index > 0.2 in 86% of the bacteria (overall MAR=0.34). The highest average of inhibition diameters were observed with chloramphenicol (20.75±0.92 mm) and norfloxacin (20.68±1.55 mm), while the lowest was shown by cefotaxime (7.5±0.79 mm). Considering for intermediate susceptibility towards the tested antibiotics the threshold diameter of 13 mm, 12/14 strains were susceptible to *C. pepo*, 4/14 to *C. sativum* and 1/14 to *C. officinalis* extracts, while other extracts exerted insignificant antimicrobial activity. The average inhibition diameter was of 18.43±1.68 mm for *C. pepo* and of 11.11±0.68 mm for *C. sativum* extract. The lowest average was found in *Allium sativum* - 6.86±0.35 mm.

Conclusion / Discussion: Some of the tested plant extracts could display a considerable antimicrobial activity on pathobionts of swine. These plants could enhance the welfare of the animals by reducing the potentially pathogenic, antibiotic resistant bacterial load, as an alternative to classical antibiotic therapy.

Acknowledgements

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Key Words: plant extracts, low-input swine farm, bacteria, antibiotics



ORAL PRESENTATION

**BIOLOGICALLY ACTIVE SUBSTANCES OF THE STEVIA
REBAUDIANA PLANT AND TECHNOLOGY FOR THEIR
PRODUCTION**

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Abstract

Stevia rebaudiana is a plant belonging to the Asteraceae (Compositae) family, native to South and Central America. This plant is extremely rich in biologically active substances, cultivated in different countries and used as a raw material for the production of many biologically active supplements and medicines. In the scientific literature, various biologically active substances extracted from stevia have hypoglycemic, hypotensive, immunomodulatory, bacteriostatic, antioxidant, hypolipidemic, cardioprotective, hepatoprotective, nephroprotective, antiviral, anti-cancer, and anti-inflammatory activity.

In Uzbekistan, scientific studies on the cultivation of this plant have been conducted. Currently, this plant is successfully grown in large areas. As a result of research carried out in the Institute of the Chemistry of Plant Substances, AS RUz, it was found that cultivated plant is rich in diterpene glycosides and flavonoids with high biological activity. These substances were found in experiments to have natural sweetener properties close to sugar, in addition to being a general stimulant and may be used in prevention of some diseases.

So, scientific researches were carried out on the extraction of diterpene glycosides of high purity from the raw material of the aerial part of this plant cultivated in our republic. As a result, a technology for extracting a substance containing high-purity stevioside diterpene glycoside and highly active flavonoids rutin and quercetin from plant raw materials was developed. According to the developed technology, plant raw materials are crushed and extracted with water. Substances with a molecular mass above 10,000 are purified using the ultrafiltration method. The purified extract is washed with chloroform solvent, and biologically active substances are extracted with butanol solvent. The extract was dried and treated with methyl alcohol solvent to isolate the main part of stevioside diterpene glycoside. e diterpene glycoside and flavonoid-containing substances isolated from raw materials were standardized and produced for pharmaco-toxicological studies.

Key Words: *Stevia rebaudiana*, steviosid, biologically active substances, diterpene glycoside



ORAL PRESENTATION

**BINARY COMBINATIONS OF *MENTHA SPICATA* L. AND
MATRICARIA CHAMOMILLA L. AGAINST COX-1 AND COX-2
ENZYMES AND EVALUATION OF CYTOTOXICITY POTENTIAL**

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Abstract

Mentha spicata L. (Lamiaceae) and *Matricaria chamomilla* L. (Asteraceae) are plants known as ethnobotanical for their anti-inflammatory effects. In this study, 8 different plant extracts were investigated in terms of COX-1 and COX-2 enzyme inhibitions and the two extracts with the best effect observed and the best COX-2 selectivity were selected for the binary combination study. Also, anticancer activities were evaluated via COX-1 and COX-2 enzyme inhibitions, as well as their apoptosis potential through the caspase pathway.

All plants were extracted with 70% aqueous ethanol. Each extract was then standardized by HPLC over the species-specific secondary metabolites specified in the European Pharmacopoeia. MTT was used for *in vitro* cytotoxic/anticancer effects using the HEK293/A549, MCF7, PC3 cell lines. To evaluate caspase, COX-1 and COX-2 inhibition assays commercial test kits were used.

Major components of *M. spicata* and *M. chamomilla* extracts were characterized as rosmarinic acid and apigenin, respectively. The IC₅₀ values for *M. chamomilla* and *M. spicata* extract on A549, MCF7, PC3 cells were between 199.5-704.5 µg/mL range. No toxic effect on healthy HEK293 cells were observed. However, the tested extracts increased apoptosis activity and all results were statistically significant. According to the anti-inflammatory activity results, it was determined that *M. chamomilla* and *M. spicata* extracts were selective COX-2 enzyme more than COX-1.

Overall, both *M. spicata* and *M. chamomilla* extracts showed selective potential for COX-2 enzyme inhibition and apoptosis against selected cancer cell lines for the first time to the best of our knowledge with this specific mode of action. Besides, this is the first binary combination study for *M. spicata* and *M. chamomilla* extracts for their anticancer and anti-inflammatory potential.

Key Words: *Matricaria chamomilla*, *Mentha spicata*, anti-inflammatory, anticancer, binary combination

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ORAL PRESENTATION

PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF
CERATONIA SILIQUA L. SEEDS EXTRACTS

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Abstract

Ceratonia siliqua L. is a plant that belongs to the *Fabaceae* family, it's locally known as "Kharroub" and is widespread in Algeria. It is widely used in traditional medicine. The aim of this work is to valorize this plant via its organic extracts obtained by maceration using two solvents: ethanol and acetone. The respective yields were: 2% and 1.15% (w/w). The phytochemical screening showed the presence of polyphenols, flavonoids, tannins, saponosides and quinones. Total phenolic contents were determined using the Folin-Ciocalteu reagent, they were 25.33 ± 0.07 and 63.51 ± 1.94 $\mu\text{g GAE/mg DE}$ in ethanol and acetone extract respectively. The determination of flavonoid contents on one hand and flavones and flavonols on another hand was conducted according to aluminum trichloride method, it was 0.65 ± 0.02 and 12.31 ± 1.93 $\mu\text{g QE /mg DE}$ in ethanol and acetone extract respectively for the former, and 15.03 ± 0.36 and 54.11 ± 1.29 $\mu\text{g QE/mg DE}$ for the latter, while the condensed tannins content was determined according to the method using vanillin, it was estimated as 184.76 ± 3.25 and 225.92 ± 9.24 $\mu\text{g CE/mg DE}$ in the ethanol and acetone extracts respectively. The antioxidant activity was evaluated using three different methods: the DPPH free radical scavenging test, total antioxidant capacity and the reducing power of ferric iron, for the first test the IC_{50} was estimated at 1545.78% and 1095.87% for the ethanol and acetone extracts respectively, meanwhile that of BHA is 9.48%. For the second test, using one volume of extract (0.3 ml) and two standards, the results showed an antioxidant capacity of 44.58 - 52.22 % AAE and 9.96- 11.65 % QE for the ethanolic and acetic extracts respectively. Therefore, the reducing power of ferric iron is 2348.5 and 1609.33 $\mu\text{g/ml}$ for ethanol and acetone extracts respectively, and 50.06 and 38.73 $\mu\text{g/ml}$ for positive control BHA and quercetin respectively. These results lead us to conclude that the acetone extract is richer in active compounds with antioxidant potential and consequently confirms the use of these seeds, then this plant for its therapeutic virtues as a food additive (or complement).

Key words: *Ceratonia siliqua* L., *Fabaceae*, seeds, polyphenols, organic extracts, antioxidant activity.



ORAL PRESENTATION

SYNERGISTIC COMBINATIONS OF FOUR DIFFERENT ORIGANUM ESSENTIAL OILS AND CARVACROL WITH AMOXICILLIN AND TETRACYCLINE

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Abstract

Microbial resistance is an important problem in modern healthcare systems. Moreover, some strains of *Staphylococcus aureus* have become resistant to penicillin group and other similar antibiotics. These strains are known as Meticillin Resistant *Staphylococcus aureus* (MRSA), which cannot be cured with traditional penicillin-related drugs. Instead, MRSA must be treated with alternate antibiotics. Amoxicillin (Penicillin group), and tetracyclines are a class of broad-spectrum antibiotics used in the management and treatment of a various infectious diseases. In addition to antibiotic resistance concerns, side effects of the antibiotics lead patients to alternative therapies. With the increased interest in aromatherapy and its well-known antimicrobial activities, essential oils emerge in the international markets as an alternative therapy. In this present study, combinations of amoxicillin and tetracycline with four different *Origanum* essential oils (*O. majorana*, *O. onites*, *O. minutiflorum*, and *O. vulgare*) were evaluated against MRSA. For the experiment, analytically analyzed commercial essential oils were used. The *in vitro* antimicrobial activity was determined using the broth microdilution assay and the checkerboard method was used to quantify the efficacy of essential oils in combination with amoxicillin and tetracycline antibiotics. Fractional inhibitory concentrations (FIC) were calculated and interpreted. The fractional inhibitory concentration FIC index of < 0.5 indicates synergism, > 0.5–1 indicates additive effects, > 1 to < 4 indifference and ≥ 4 is considered to be antagonism. We evaluated this *in vitro* FIC index range based on the experimental variation of the checkerboard technique using multiple replicates. As a result of our study, 80% of the tested combinations showed synergism, 10% showed additive effects, and 10% showed no difference according to their FICI values. Overall, no antagonistic effect was observed. This result may suggest that the rates of synthetic resistant developed antibiotics in treatment can be reduced by combining them with natural agents.

Key Words: Origanum, essential oil, synergism, amoxicillin, tetracycline.



ORAL PRESENTATION

COMPREHENSIVE STUDY OF THE PLANT *HAPLOPHYLLUM*
GRIFFITHIANUM

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Abstract

Genus *Haplophyllum* Juss. (*Haplophyllum*) belongs to one of the large families Rutaceae (Rutaceae), contains about 150 genera and 1600 species. In the flora of the states of the former Union, the genus *Haplophyllum* is represented by 32 species, 23 of which grow in Central Asia. Of the 32 species, 16 are endemic; they do not grow anywhere outside of Central Asia. The greatest number of species were recorded in Uzbekistan - 16, which were the center of the species diversity of these plants. Of the 23 species growing in Central Asia, the 11 species have been studied for their chemical consistent. The pharmacological studies of alkaloids and their derivatives isolated from plants of *Haplophyllum* genus, which are unique source of various alkaloids, showed that they are low toxic and have wide spectrum of pharmacological action. Most of them possess by inhibitory action on CNS, sedative, sleeping, anticonvulsant, estrogen and other effects. The plant species are considered as potential sources of biological active compounds appearing Cytotoxic, Antimicrobial and Anti-inflammatory activity and other effects.

Chemical investigation on *H. griffithianum* collected from Nilu, and Khondiz led to the isolated of 14 compounds, from the aerial part, identified to be dubinine, dictamnine, skimmianine, dubinidine, dubamine, evoxine, N-methylhaplofoline, flindersine, folimine, and nev compounds gerphitin, gerphitinin, griffithine, 1,8*-dihydro-8,8-dimethylpirano[2,3]quinolin-2-one. As a result of research total alkaloids with yields 0,4% and 0,58% respectively, were isolated from the aerial part and roots of *H.griffithianum* collected in Nilu village (Surkhandarya province). The known alkaloids skimmianine, dictamnine, dubinine, dubamine and a new base, gerfitin with m.p. 91-93°C, were isolated for the first time from the aerial part, and skimmianine, dictamnine, dubinine and sterols with m.p. 76-78°C, and 60°C – from the roots. Isolation was conducted by column chromatography of silica gel and sephadex LH-20, Flash chromatography, as well as semi-preparative HPLC. In the research were obtained 13 known and one new individual compound which exact the plant from Khondiz.

From the plant overall were isolated 14 compounds, of them which one are new and other three compounds were isolated for the first time from the genus *Haplophyllum*. So, all of them are isolated for the first time.



ORAL PRESENTATION

MICROENCAPSULATION OF CLARY SAGE (*SALVIA OFFICINALIS* L.) ESSENTIAL OIL BY COMPLEX COACERVATION: MICROCAPSULE CHARACTERIZATION AND ENCAPSULATION EFFICIENCY

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Abstract

Clary sage essential oil is obtained from *Salvia sclarea* L. (family Lamiaceae). The main components of clary sage oil are linalool and linalyl acetate [1]. It has anti-oxidant, anti-stress, antimicrobial, anticancer, anti-tumor, cytotoxic, antidepressant, and anti-inflammatory effects [2-3]. However, it is chemically unstable and susceptible to environmental degradation. In order to overcome the disadvantages and increase its application in foods, perfumery, and aromatherapy, microencapsulation technology is one of the most promising ways. Microencapsulation is a means of applying relatively thin coating materials to core materials such as very tiny droplets or particles of liquid or solid materials [4]. This study aimed to investigate the microencapsulation of clary sage essential oil by a complex coacervation method, using gelatin and gum Arabic as the coating material. In the synthesis process, clary sage oil was emulsified in a polymeric solution by ultrasonic treatment for determined times (5, 10, 15, and 20 minutes). The effect of ultrasonication time on the encapsulation efficiency of clary sage oil through the complex coacervation process was investigated. The optimal time of ultrasonic treatment for the complex coacervation between gelatin and gum Arabic was observed to be 20 minutes. The sage oil microcapsules showed excellent encapsulation ability as indicated by high total oil and low surface oil. The morphology and chemical structure of microcapsules was investigated with a Field Emission Scanning Electron Microscopy (FESEM) and a Fourier Transform Infrared Spectrometer (FT-IR). The results confirmed that clary sage oil was successfully encapsulated with gelatin and gum Arabic via complex coacervation method.

Key Words: Microencapsulation, ultrasonic treatment, clary sage essential oil, complex coacervation

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ORAL PRESENTATION

**POLYPHENOLIC PROFILE, ANTIBACTERIAL ACTIVITY AND
ANTI-INFLAMMATORY ACTIVITY OF LEAF EXTRACTS FROM
TWO TUNISIAN SPONTANEOUS SPECIES**

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Abstract

Medicinal plants offer imperative sources of innovative chemical substances with important potential therapeutic effects. Among them, *Pistacia atlantica* and *Oudneya africana* have been widely used in traditional medicine for the treatment of several diseases. The present study investigated the polyphenolic profile, the antioxidant (DPPH and FRAP assays), the antibacterial, and the anti-inflammatory properties of aerial parts of *P. atlantica* and *O. africana* extracts. The LC-ESI-MS analysis proved the identification of 19 compounds, including quinic, gallic, and protocatechuic, as major phenolic acids and high levels of flavonoids, such as catechin, rutin, and quercetrin. Interestingly, in *O. africana* rutin turned out to be the most abundant one (8003.95 µg/g). Leaves extracts of the two species also exhibited a potential *in vitro* antioxidant activity when compared with ascorbic acid in DPPH and FRAP tests. The plant extracts showed antimicrobial efficacy against *Listeria monocytogenes*, *Staphylococcus aureus*, *P. aeruginosa*, *Escherichia coli*. Plant extracts revealed the best MIC and MBC values against *candida albicans*. Moreover, *P. atlantica* and *O. africana* leaves did show an important inhibitory effect on protein denaturation.

Keys: *Pistacia atlantica*, *Oudneya africana*, MIC, MBC, antioxidant activity, bioactive compounds



ORAL PRESENTATION

ANTI-BACTERIAL ACTIVITY OF NANOPARTICLES LOADED WITH
THE EXTRACT OF TEW KHON

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Abstract

Plant extracts containing natural reducing agents can be utilized for the synthesis of metal nanoparticles (MNPs). In this study, ethanolic extract from stem of Tew Khon (*Cratoxylum formosum* ssp. *pruniflorum*) was used to synthesize the MNPs from silver. The MNPs were characterized using UV-visible spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and dynamic light scattering (DLS). The antibacterial activity was assessed by the WST-8 assay against several bacterial species and strains. Results showed that the MNPs had a spherical shape and an average diameter of less than 100 nm with the negative value of zeta potential. The MNPs showed a minimum inhibitory concentrations (MIC) of 50–100 µg/ml against different strains of *S. aureus* (i.e., Newman, R113, LUU17, USA230, POA10, and N315). Notably, the MIC of MNPs was less than ampicillin—a positive control—in *S. aureus* strain N315. Here we report the biosynthesis approach for MNPs from the plant extracts that can be further exploited as a potential candidate for antibacterial agents.

Keywords: MNPs, green synthesis, antibacterial activity

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ORAL PRESENTATION

ANTIOXIDANT CAPABILITY AND BIOACTIVE COMPOUNDS
EVALUATION OF SIX DIFFERENT YOUNG SPROUTS ORIGINATED
IN CAMBODIA

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Abstract

Sprouts have been reported to possess bioactive substances and pharmacological activities that are beneficial for the treatment and prevention of diseases. So far, there has not been any scientific evidence on the antioxidant properties and bioactive constituents of the sprouts originated in Cambodia yet. The aim of this study was to investigate the bioactive compounds and antioxidant activities of the methanolic extract from six different sprouts harvested at the edible age (4-5 days). Folin-Ciocalteu's method was used to determine the total phenolic content of sprout extracts spectrophotometrically. Calibration was done using gallic acid dissolved in methanol at a concentration range of 10–50 µg/ml. The calibration curve was used to extrapolate the concentration of phenolic compounds in sprout extract. Total anthocyanin content was determined by pH differential method. Total flavonoid and chlorophyll content were screened spectrophotometrically. The content was calculated as equivalent to the standard compound. Antioxidant activities were determined by DPPH and ABTS radical scavenging activities assays, and ferric reducing antioxidant power (FRAP) assay. Results showed that soybean sprouts exerted the highest percentage yields (7.6 % per fresh weight) and flavonoid content (98.9 ± 4.8 mg quercetin equivalent /g of crude extract (CE)). Swamp morning-glory sprouts exhibited the highest phenolic content (232.2 ± 9.3 mg gallic acid equivalent /g CE) and the strongest radical scavenging ability in the DPPH assay (IC₅₀, 283.6 ± 25.9 µg/ml). Interestingly, the highest chlorophyll content (0.03 ± 0.0 mg/g CE) and the highest FRAP power (0.08 ± 0.005 mM FeSO₄ /g CE) was in sunflower sprouts. The highest anthocyanin content was detected in black sesame sprouts (3.5 ± 0.4 mg cyanidin-3-glycoside/g CE). Moreover, the highest ABTS radical scavenging ability was found in mustard sprouts. Our study found the presence of different bioactive compounds and their antioxidant activities in different sprout species. This evidence raises the awareness of their advantages as a source of nutraceuticals, pharmaceuticals, and supplements and suggests the importance of these vegetables toward the needs and promotes the consumption of organic sprouts as a functional food for health benefits.

Keywords: Antioxidants, Anthocyanin, ABTS Radical Scavenging Ability, Young Sprouts

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ORAL PRESENTATION

COSMETIC NANOFORMULATIONS POTENTIAL EFFECT OF
PROPOLIS ON PHOTO-INDUCED SKIN AGING

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Abstract

Ultra-violet might cause premature aging and it can negatively affect the skin leading to carcinogenesis [1]. Recent strategies included the use of natural products as they can both be used as a preventive and curative treatment. Propolis is a sticky and gummy substance collected by honeybees and used as a multifunctional material in beehive [2]. It has been found to display a variety of biological properties, such as anti-oxidative, anti-microbial, anti-cancer and anti-inflammatory effects. Propolis has therapeutic potential against human skin aging, although its precise molecular target and mechanism of action are not fully understood [3]. We aimed in the present study to investigate the preventive effect of Algerian propolis in photo-induced skin aging. Propolis extracts have been screened and evaluated using a bioguided approach to isolate selective inhibitor compounds of MMP-3 (Matrix Metalloproteinase-3), a key enzyme in the collagenolytic and elastolytic cascades involved in both intrinsic and extrinsic aging. The tested propolis was found to selectively inhibit MMP-3 activity with no effects on other MMPs. This fraction also prevented plasmin-mediated proMMP-3 activation and suppresses' plasmin amidolytic activity. In addition, the tested propolis significantly inhibited UVA-mediated MMP-3 upregulation by fibroblasts

Key Words: propolis, ultra-violet, skin aging, MMP-3, plasmin, fibroblasts

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ORAL PRESENTATION

**ENCAPSULATION OF ROSESHIP SEED EXTRACT IN ALGINATE
BASED MICROBEADS: OPTIMIZATION OF IONIC GELATION
SYSTEM BY RESPONSE SURFACE METHOD**

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Abstract

Natural products obtained from various biowaste are prone to adverse effects of environmental factors such as oxidation, humidity and light. In order to deal with such drawbacks, microencapsulation is an alternative preservation method. In the current study, ionic gelation has been used for trapping the active materials of roship seed in alginate beads. The performance of the produced beads were evaluated based on encapsulation efficiency in terms of total phenolic content (EE_{TPC}) and antioxidant activity. The process parameters of the ionic gelation system was selected as gelling medium (calcium chloride) concentration (2–15%, w/v), wall material (sodium alginate) concentration (1-2%, w/v) and hardening time (15-45 min). EE_{TPC} and antioxidant activity were the response variables for the optimization of the ionic gelation system by application of Box-Behnken design of Response Surface Method (Box-Behnken-RSM). 17 experimental runs with 3 variables and 3 levels were carried out depending on Box Behnken-RSM. The maximum EE_{TPC} and antioxidant activity yield were found as 54.655% and 4.221 mg trolox equivalent per gram dried capsule under the optimal ionic gelation conditions (8% $CaCl_2$, 1.66% sodium alginate and 26 min of hardening time). Additionally, antioxidant activity of the beads was determined by DPPH (2,2-diphenyl-1-picrylhydrazil) test. Depending on the analysis of variance test (ANOVA) of Box-Behnken-RSM, the most effective process parameter was found as the calcium chloride concentration followed by the second power sodium alginate concentration ($p < 0.0001$), whereas the most significant parameter for the antioxidant activity was the second power of calcium chloride concentration, followed by sodium alginate concentration ($p < 0.0001$).

Key Words: Biopolymer, alginate beads, ionotropic hydrogel, biowaste, antioxidant activity, Box-Behnken-RSM.



ORAL PRESENTATION

QUANTITATIVE ASSESSMENT OF BIOACTIVE COMPOUNDS AND
ANTIOXIDANT ACTIVITIES OF SELECTED AROMATIC PLANT
EXTRACTS

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Abstract

Cambodian condiments are often from aroma plants and have been used for many centuries in food and in Khmer traditional medicine. Some aromatic plants have been studied on their phytochemical compounds and bioactivities in Cambodia. However, there has not been any study conducted on the leaves of *Salvia officinalis* L., *Ocimum tenuiflorum* L., *Ocimum basilicum* L., and *Heliotropium indicum* L. Thus, this study was conducted to quantify the bioactive compounds and screen the antioxidant activities of these aromatic plants. The polyphenol, total flavonoid content, total alkaloid content and total chlorophyll content were quantified by using spectrophotometry. Antioxidant activities were screened with two different mechanisms such as hydrogen atom transfer (HAT) mechanism and single electron transfer (SET) mechanism. The radical scavenging activity via HAT mechanism was screened with DPPH and ABTS assays. SET mechanism was screened with ferric reducing antioxidant power (FRAP) assay. As a result, *Ocimum basilicum* L. exhibited, alkaloid content (721.4 ± 0.6 mg atropine equivalent (AE) /g of crude extract (CE)), phenolic content (83.1 ± 23.1 mg gallic acid equivalent / g CE), flavonoid content (7.1 ± 0.8 mg quercetin equivalent (QE) /g CE) and strongest radical scavenging ability via DPPH assay (IC_{50} value of 130.7 ± 1.8 μ g/ml). Chlorophyll content was found to be high in *Heliotropium indicum* L. (42.5 ± 0.5 mg/g CE). Interestingly, *Salvia officinalis* L. extract exerted the strongest radical scavenging ability via ABTS assay (0.08 ± 0.01 mM trolox equivalent (TE) /g CE) and strong reducing power with the FRAP value of 1.07 ± 0.05 mM FeSO₄/g CE. Our study found some amounts of bioactive compositions and antioxidant activities in the selected aromatic plants and provided the basis for the therapeutic importance of these selected aromatic plants.

Keywords: Aromatic Plants, Bioactive Compositions, Antioxidant Activity, Polyphenols.

Acknowledgements

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ORAL PRESENTATION

EVALUATION OF SOME BIOLOGICAL ACTIVITIES OF *SALVIA SERICEOTOMENTOSA* EXTRACTS PREPARED BY DIFFERENT EXTRACTION METHODS

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Abstract

Since ancient times, plants, herbs and spices have been used for their several biological activities and medicinal properties. Plants' importance for contemporary life is increasingly being recognized. Therefore, traditionally used medicinal plants also represent potential sources for developing new drugs [1]. *Salvia* species, known as "sage" in Turkey, are used to treat many diseases such as colds, tooth and throat aches, menstrual problems, Alzheimer's disease and diabetes. Various biological properties of extracts and essential oils obtained from *salvia* species, such as antitumor, antioxidant, antimicrobial, antidiabetic and anti-inflammatory activities, have been reported before [2]. *Salvia sericeotomentosa* is an endemic plant for our country. In this study, the cytotoxic effects and total phenolic contents of the extracts prepared by three different methods (infusion, maceration and soxhlet extraction) using the aerial parts of the endemic *Salvia sericeotomentosa* plant were determined comparatively. Cytotoxicity was determined by the MTT test and total phenolic determination was made by the Folin Ciocalteu test. The obtained data revealed that the infusion extract was the most effective extract both in terms of cytotoxicity and total phenolic content. This study is a pioneering study for future studies, and it is very important to evaluate cytotoxicity in different cell lines in a broad perspective.

Key Words: Cytotoxicity, nurşalbası, Turkey.

ORAL PRESENTATION

ANTI-ACETYLCHOLINESTERASE AND SYNERGISTIC
ANTIFUNGAL ACTIVITIES OF SELECTED *SALVIA* SPECIES:
CORRELATION WITH METABOLIC PROFILES

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Abstract

Objectives: *Salvia* species are known for their anti-fungal and anti-acetylcholinesterase (AChE) activity [1,2]. Metabolomics is defined as a comprehensive quantitative and qualitative analysis of large scale of metabolites [3]. In this study, besides determining anti-acetylcholinesterase and synergistic antifungal activities of selected *Salvia* species (*S. cryptantha* Montbret & Aucher ex Benth., *S. tchihatcheffii* (Fisch. & C.A.Mey.) Boiss., *S. officinalis* L., *S. virgata* Jacq.), the metabolite profiles were clarified and correlation analyzes between the activity results and profiles were carried out.

Materials and Methods: Aerial parts of all plant materials were extracted by methanol to determine metabolic profile by using GC-MS (Gas Chromatography-Mass Spectrometry) and LC-QTOF-MS (Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry). Also, Ellman's spectrophotometric method for anti-acetylcholinesterase activity, and the checkerboard method (4) for synergistic antifungal activity between extracts and fluconazole were performed.

Results: 295 known and 1408 unknown metabolites were detected by using GC-MS while 346 known 69008 unknown metabolites by using LC-QTOF-MS. *S. cryptantha*, *S. tchihatcheffii*, *S. officinalis* and *S. virgata* demonstrated inhibitory activities on AChE with the ratio of 32.72%, 5.9%, 43.96%, and 12.1% respectively at the concentration of 200 µg/mL. A synergism was found for all *Salvia* sp. against *Candida tropicalis* ATCC 750 strain (FICI values of ≤0.5). In addition, primary and secondary metabolites, which are highly correlated with both activities, were determined.

Conclusions: All *Salvia* species exhibited synergic activity with fluconazole while having weak AChE inhibitory activity. Combining natural products with synthetic drugs is important in order to increase efficacy. At the same time, herbal products must be used carefully by the point of herb-drug interactions. Further studies are needed to investigate active metabolites according to correlation results.

Key Words: Acetylcholinesterase inhibitory activity, Metabolomics, *Salvia*, Synergism, *Candida*,

Acknowledgements

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ORAL PRESENTATION

CYTOTOXIC WITHANOLIDES FROM PLANTS COLLECTED IN THE
BRAZILIAN ATLANTIC FOREST

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Abstract

The present study evaluated a cytotoxicity of 478 extracts prepared from 121 trees species, native to the Brazilian Atlantic Forest biome. The extracts were prepared according to the methodology of the BIOPROS/UFV extract library [1]. Leaves and branches were collected and dried in the oven at 40°C after which it was sequentially extracted (1:5 w/v) in percolator, starting with a 1:1 mixture of CH₂Cl₂/MeOH to pure MeOH. Each extract was concentrated under reduced pressure and then lyophilized. The MTT assay was used to screen the extracts for their cytotoxicity against MCF-7, HepG2 and B16F10 cancer cells. The most active extracts were selected for further investigation to isolate and identify bioactive compounds with potential antitumor activity. Since the CH₂Cl₂/MeOH (1:1) crude extract of the leaves of *Athenaea velutina* (Solanaceae family) was found to significantly inhibit migration, adhesion, invasion and cell colony formation in B16F10 cells, it was partitioned to various solvents, i. e. hexane, CH₂Cl₂, EtOAc, and *n*-butanol. Interestingly, the withanolide-rich fraction from the CH₂Cl₂ extract showed cytotoxic activity 1.5 times higher than the original extract. By using HPLC-HRMS analysis [2], 14 withanolides were detected in the CH₂Cl₂ extract. In order to isolate the bioactive compounds, bioassay-guided fractionation of the CH₂Cl₂ extract of *A. velutina* leaves was performed, resulting in the isolation of three withanolides, namely, withacnistin, withacnistin acetate and withalutin. The structures of the three withanolides were established by spectral analysis, including 1D and 2D NMR as well as MS [4]. It is worth mentioning that this is the first report of the isolation of these three withanolides in this species [3]. The withanolides isolated from *A. velutina* reduced cancer cell viability with IC₅₀ values ranging from 1.52 to 5.39 mM. These findings revealed that *A. velutina* could be an important source of cytotoxic withanolides.

Key Words: *Athenaea velutina*, withanolides, withalutin; cytotoxic activity, Brazilian Atlantic Forest

Acknowledgements

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ORAL PRESENTATION

PHOSPHODIESTERASE 5 INHIBITORY AND ANTI-
INFLAMMATORY ACTIVITIES OF THE *IN VITRO* ROOT CULTURE
OF *EURYCOMA HARMANDIANA*

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Abstract

Eurycoma harmandiana (EH) is an aphrodisiac plant belonging to the Simaroubaceae family and is well-known for its ability to enhance male performance. The root part of EH was used as a health-supplement product, which was discovered in northeast Thailand. The present study developed an *in vitro* root culture of EH and evaluated phosphodiesterase 5 (PDE5) inhibitory by enzymatic assay [1] and anti-inflammatory activity by lipopolysaccharide-activated RAW 264.7 cells [2]. The *in vitro* root culture was obtained from the planlet of EH cultured on a hormone-free Murashige and Skoog (MS) medium. The root part of the plantlet was cut and cultured on a half-strength MS liquid medium containing 5 mg/L of naphthaleneacetic acid (NAA). On the 8th time of subculturing, the *in vitro* root was harvested for phytochemical determination by HPLC. The total quassinoids, β -carboline alkaloids, and canthin-6-one alkaloids were 0.12 ± 0.00 , 0.04 ± 0.00 , and 3.48 ± 0.08 mg/g dry weight, respectively. The half-maximal inhibitory concentration (IC₅₀) of PDE5 inhibitory activity of the extract of *in vitro* root culture was 2.34 ± 0.41 μ g/mL. The extract of *in vitro* root culture significantly inhibited nitric oxide levels and suppressed the expression of inducible nitric oxide synthase (*iNOS*) gene. The established *in vitro* root culture of EH can be used to replace the intact plant as an alternative source and shows promising activity via PDE5 inhibition and anti-inflammatory effects.

Key Words: *Eurycoma harmandiana*, erectile dysfunction, phosphodiesterase 5, anti-inflammation, *in vitro* root culture

Acknowledgements

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ORAL PRESENTATION

OPTIMIZATION OF CULTIVATED AGES ON THE CONTENT OF PHENOLICS, FLAVONOIDS, ANTHOCYANIN, CHLOROPHYLL AND ANTIOXIDANT ACTIVITIES OF YOUNG RADISH SPROUTS

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Abstract

Young radish sprouts (in the family Brassicaceae) are a popular food and known to have benefits to health. This study aims to conduct a quantitative analysis of antioxidant properties and determine the nutritional values of two different colors (colored and white-colored sprouts) and three different ages (young, middle and old for 2-3, 4-5, and 6-7 days) of radish sprouts. Samples were harvested and prepared as a methanolic extract to determine the total phenolic content via Folin Ciocalteu's methods and total anthocyanin content by pH differential method. Total flavonoid content and total chlorophyll content were analyzed with spectrophotometric methods. The contents were calculated in equivalent to quercetin which was used as a standard compound. The antioxidant activity was determined via hydrogen atom transfer mechanism based on DPPH radical scavenging ability assay and single electron transfer mechanism by ferric reducing antioxidant activity (FRAP) assay. Nutritional values were determined by proximate analysis. Results showed the highest percentage yield (5.8 % per fresh weight) in the young age of white-colored radish sprouts. The highest phenolic content (50.8 ± 2.8 mg quercetin equivalent (QE) /g crude extract (CE)) and DPPH value (IC_{50} value of 430.0 ± 40.7 μ g/ml) were detected in colored radish sprout at the middle age ($p < 0.05$). Moreover, lowest age of colored radish sprouts showed the strongest FRAP power (0.4 ± 0.06 mM FeSO₄/g CE). Interestingly, a large amount of flavonoid content (53.1 ± 1.5 mg QE/g CE) was in the middle age of white-colored radish sprouts. Chlorophyll content and anthocyanin content were rich in colored radish sprouts at the lowest age ($p < 0.05$). To be concluded, colored radish sprout exhibited the large number of bioactive compounds, antioxidant activities, and nutrients which can be a benefit to human health as a functional food and further developed as a supplement.

Keywords: Young Radish Sprouts, Antioxidant Activity, Bioactive Compounds, Proximate Analysis.

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ORAL PRESENTATION

EVALUATION OF COMMERCIAL *SYZYGium AROMATICUM* L.
(CLOVE) ESSENTIAL OIL SAMPLES IN ACCORDANCE WITH THE
EUROPEAN PHARMACOPOEIA 10.0 CRITERIA

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Abstract

Clove (*Syzygium aromaticum* L.) oil is widely used in cosmetics, medicine and food industries. Previous studies have revealed many pharmacological effects of clove oil, including anti-infective, insecticidal, antioxidant, anesthetic and anti-inflammatory [1,2]. In this study, clove essential oil samples obtained from different channels in our country have been extensively evaluated in terms of their compliance with European Pharmacopoeia (EP) standards, which is the official guide that includes national and international rules and methods, qualitative and quantitative analysis methods of active substances and excipients used in drug production. Aim of the evaluation is to determine the quality differences between the channels. Quality standards have been determined with the herbal drug monographs in EP [3]. Compliance of plant drugs with pharmacopoeia criteria is crucial for public health.

In this study, 13 clove oil samples obtained from pharmacy and non-pharmacy channels were analyzed according to the criteria specified in the "Clove Oil" monograph in European Pharmacopoeia 10.0. Appearance, solubility, thin layer chromatography, relative density, refractive index, optical rotation, spot control analysis was conducted for all samples. In addition, GC-MS was used to elucidate detailed phytochemical profiles of the samples.

Results revealed that none of the samples from Turkish Market fully met the EP criteria. When the difference between the sources of purchase evaluated, the rate of fulfilling the determined parameters of the products from the pharmacy was calculated as 82.6%, while the rate of the products obtained from the sources other than the pharmacy was found to be 72.5%. These results revealed that there is a significant difference between the sources from which the oils are taken in terms of standards. The deficiency of the clove oils fulfil standards of the EP indicates the requirements of performing the necessary effort and auditing to increase the quality of the products on the market.

Key Words: *Syzygium aromaticum* L. Clove oil, Essential Oil, European Pharmacopoeia, GC-MS

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ORAL PRESENTATION

INVESTIGATION OF PHYSIOCHEMICAL PROPERTIES, POLLEN GERMINATION, VIABILITY AND TUBE LENGTHS OF *Colchicum kurdicum* (BORNM.) STEF. AND *Colchicum szovitsii* FISCH. ET MEY. GROWN IN VAN REGION

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Abstract

Plants called geophytes constitute an important part of the floristic richness in our country. Bulbous plants or flower bulbs are other names for this plant family. The overall number of geophytes with a 35 percent endemism rate in the globe is estimated to be around 4.300. this number in our country is 1.060. Important geophytes are found in the Liliaceae family. *Colchicum* sp. is one of the most important genera in this family. In our country, it is represented by 34 species, three of which are variants. 16 of the species of *Colchicum* are endemic.

In this study, pollen germination, viability and pollen tube lengths and physicochemical properties of *Colchicum szovitsii* Fisch. Et Mey and ve *Colchicum kurdicum* (Bornm.) Stef.species were determined. The samples obtained from the flora study in Van province were compared with each other. In the study, which used of *Colchicum szovitsii* and *Colchicum kurdicum* species, determined pollen height, pollen width, pollen number, pollen viability percentages with viability tests, pollen germination percentage, pollen germination tube length, nitrogen balans index (NBI), chlorophyll, flavonol and anthocyanin amounts. As a result of the research, pollen width, height and number were determined as 25.53µm, 45.98µm and 10 in *Colchicum szovitsii* species, while it was determined as 30.33µm, 59.27µm and 28.0 in *Colchicum kurdicum* species respectively. In the study, determine for the percentage of viability of pollen were applied IKI test (iodized potassium iodide), Safranin test and TTC test (2,3,5 triphenyl tetrazolium chloride). Pollen viability rates in *Colchicum szovitsii* and *Colchicum kurdicum* species were determined as 77.77% and 87.55% in IKI test, 84.90% and 88.72% in Safranin test, and 72.13% and 77.65% in TTC test, respectively. Pollen germination rate and pollen germination tube length were determined as 36.61-88.42µm in *Colchicum szovitsii* species and 64.99%-131.66µm in *Colchicum kurdicum* species. amounts of NBI, chlorophyll, flavonol and anthocyanin were determined as 36.2 dualex index, 62.0 dualex index, 1.89 dualex index and 0.01 dualex index in *Colchicum szovitsii* species, while 39.36 dualex index, 61.3 dualex index, 1.62 dualex index and 0.02 dualex index in *Colchicum kurdicum* species.

Key Words: Chlorophyll, Geophyte, Pollen morphology, Pollen vitality.



ORAL PRESENTATION

**KARYOTYPE ANALYSIS OF *HYACINTHELLA LINEATA* AND
HYACINTHELLA ACUTILOBA (ASPARAGACEAE) IN TURKEY**

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Abstract

The genus *Hyacinthella* Schur is a member of the Asparagaceae family, which includes aromatic and medicinal plants. *Hyacinthella* is represented by 17 species in the world. The genus consists of 12 species, 10 of which are endemic in Turkey. The aim of this study is to determine the chromosome number and morphology of *Hyacinthella lineata* (Steud. ex Schult. & Schult.f.) Chouard and *Hyacinthella acutiloba* K.Perss. & Wendelbo. Chromosome counts were made by germinating bulbs belonging to taxa collected during field studies. The squashing technique was used for chromosome studies and counts were performed at somatic metaphase. The karyotype measurements of the examined taxa were carried out with the KAMERAM program and the taxa were compared in terms of their karyomorphology using asymmetry indices (CV_{CL} , CV_{CI} , AI and M_{CA}). The chromosomal counts confirmed the previous reports of $2n=16$ and $2n=18$ given for *H. lineata* and *H. acutiloba*, respectively. In conclusion, this study is the first report for chromosomal asymmetry indices of analyzed taxa.

Key Words: Chromosome count, endemic, karyomorphology, asymmetry, Turkey.

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ORAL PRESENTATION

NEW BIOACTIVE COMPOUNDS FROM SEMI-SYNTHESIS ON THE
NATURAL PRODUCT (GYPSOGENIN)

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Abstract

Isolation of the natural compound from the plants and the construction of the structure are important studies carried out in plant chemistry for many years. For centuries, plants have been used as a therapeutic and protective agent in human health. Therefore, natural products are important source of new drug discoveries. Saponins are glycosides which have aglycone and sugar moiety. They are found in many plants. It is well known that Gypsophila species contain abundant saponins. These Gypsophila species are known to be used in traditional Chinese medicine. Therefore, these saponins have various biological activities (including antitumor, antimicrobial, antioxidant, antifungal, cytotoxic activity). At the same time, some semi-synthetic derivatives can be originally acquired from many sources (bacterial, fungal, plant and marine). It has been observed that many semi-synthetic derivatives obtained from natural products show many activities. We are working to synthesize new semi-synthetic derivatives on compounds derived from different plants. Some of our work continues in this area. We have synthesized many novel gypsogenin compounds. These compounds display a number of important activities such as anti-tumour activity. In this study, the saponin was isolated from the roots of *Gypsophila arostii* and gypsogenin compound [3-hydroxy-23-oxoolean-12-en-28-oic acid] was obtained from alkaline and then acid hydrolysis of saponin. Column chromatography method was used for purification. Semi-synthesis reactions were carried out by gypsogenin compound that have important biological activities and with various amine derivatives, so it was aimed to obtain new bioactive compounds. The starting material of gypsogenin aglycone was combined with different amine compounds. The purification was carried out using the column chromatographic method. Up to the present, elucidation of the new semi-synthesized compounds has been determined by infrared spectroscopy (IR), nuclear magnetic resonance (¹H NMR and APT) and mass spectrometry (MS) or HRMS analysis.

Key Words: Gypsogenin; Amine; Organic Synthesis.

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ORAL PRESENTATION

PHYTOCHEMICAL PROFILING, *IN VITRO* ANTIOXIDANT, AND
ANTICANCER EFFECTS OF SOME LAMIACEAE SPECIES FROM
TURKIYE

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Abstract

Lamiaceae is one of the largest families, mainly known as ‘mint family’, containing important medicinal plants used in traditional medicine and ethnobotany since ancient times. The species of this family are also considered as an economically valuable source of essential oils (EOs) in the field of food, cosmetics, perfumes, aromatherapy, pharmaceuticals, and medicine. The aim of the present study was to determine chemical composition, anticancer, and antioxidant activities of EOs distilled from some Lamiaceae species, including *Salvia aramiensis* Rech.f., *S. officinalis* L., *S. fruticosa* Miller, *Ocimum basilicum* L., *Lavandula angustifolia* Miller, *Satureja thymbra* L., *Mentha × piperita* L., *Mentha* sp., *Thymbra spicata* L., *Clinopodium serpyllifolium* subsp. *fruticosum* L., and *Rosmarinus officinalis* L. collected in the eastern Mediterranean region of Türkiye. The chemical composition of the EOs was identified using Gas Chromatography/Mass Spectrometry (GC-MS), and antioxidant activity was determined using the DPPH radical scavenging method. In addition, the anticancer potential of the EOs were investigated using thiazolyl blue tetrazolium bromide (MTT) against human cell lines, including hepatocellular carcinoma (HepG2, ATCC®-HB-8065), gastric carcinoma (NCI-N87, ATCC®-CRL-5822), breast adenocarcinoma (MCF-7, ATCC®-HTB-22), human prostate carcinoma (LNCaP clone FGC-Luc2, ATCC®-CRL-1740-LUC2) cancer cells, and non-cancerous human umbilical vein endothelial cells (HUVECs, ATCC®-100010) obtained from the American Type Culture Collection (ATCC). The cells were incubated with various concentrations of EOs from 0 to 1000 µg/mL, and IC₅₀ values were calculated. As consistent with antioxidant activities, the anticancer activities of the EOs of *S. officinalis*, *L. angustifolia*, and *R. officinalis* were determined to be significantly higher than those of the others. However, almost all the EOs were found to inhibit cell viability and induce apoptosis of the cancer cells, while the EOs at different concentrations exhibited the highest anticancer activity against NCI-N87, HepG2, MCF-7, and LNCaP cancer cells, respectively. The major phytochemical components of the EOs from some Lamiaceae species were eucalyptol, carvacrol, camphor, thujone, linalool, methyl cinnamate, linalyl acetate, γ-erpinene, carvone, carvone, linaleic acid, 1-Acetoxy-p-menth-3-one, pulegone, p-Cymene, and α-Pinene at different concentrations in the essential oil samples of each plant species. Overall, the EO samples contained valuable bioactive compounds and accordingly had remarkable biological activities. This study has shown that the EOs obtained from some medicinal plants in the Lamiaceae family could be rich sources of herbal medicines and natural plant products in the future.

Key Words: Lamiaceae, chemical composition, cancer, antioxidant, natural product, herbal medicine



ORAL PRESENTATION

HERBAL AND NATURAL PRODUCTS AS A DOUBLE-EDGED
SWORD IN INTEGRATIVE COMPLEMENTARY MEDICINE FOR
KIDNEY DISEASES

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Abstract

Knowledge of medicinal plants' biological and pharmacological properties and phytochemicals has accumulated over the centuries, providing further evidence to support their continued use in folk medicine as integrative, complementary medicine applications. Due to the increasing interest in documenting traditional knowledge through ethnobotanical studies, conservation of biological resources, and using plants for medicinal purposes in various ethnic communities, the importance of traditional ethnobotanical knowledge is being recognized worldwide. Almost everywhere in the world, up to 5 million people are expected to die from kidney diseases each year, and it is estimated that kidney diseases will be the fifth leading cause of death by 2040. Kidney injury can be triggered by various factors. Moreover, the nephrotoxicity of the used drugs has been specified as one of the critical factors in developing kidney injuries. Due to the several adverse effects of drugs, nephroprotective agents may be used as other alternatives to complement the treatment of these diseases. *Ailanthus excelsa*, *Andrographis paniculata*, *Arbutus pavarii*, *Aristolochia spp.*, *Artocarpus lakoocha*, *Averrhoa carambola*, *Bryophyllum pinnatum*, *Butea monosperma*, *Calotropis procera*, *Celastrus paniculatus*, *Circium vulgare*, *Combretum alatum*, *Commiphora wightii*, *Curcuma longa*, *Cyanthillium cinereum*, *Descurainia sophia*, *Echinacea spp.*, *Ephedra sinica*, *Euphorbia paralias*, *Eurycoma longifolia*, *Eysenhardtia polystachya*, *Ficus benghalensis*, *F. lacor*, *F. vesiculosus*, *Glycyrrhiza glabra*, *Hibiscus sabdariffa*, *Larrea tridentata*, *Mallotus philippensis*, *Mukia maderaspatana*, *Ocimum basilicum*, *Oxalis acetosella*, *Passiflora spp.*, *Pausinystalia yohimbe*, *Pedaliium murex*, *Phyllanthus emblica*, *P. fraternus*, *Pistacia atlantica*, *Pithecolobium lobatum*, *Punica granatum*, *Rhizoma rhei*, *Salix daphnoides*, *Secale cereale*, *Solanum americanum*, *S. anguivi*, *Sonchus wightianus*, *Syzygium cumini*, *S. jambos*, *Terminalia bellirica*, *Theobroma cacao*, *Tinospora cordifolia*, *T. crispa*, *Urtica dioica*, *Vaccinium macrocarpon*, and *Zea mays* are the most common plants used as remedies in the form of powders, decoctions, juices etc., for traditional herbal practices to treat kidney ailments. Considering that people still suffer from kidney diseases and this organ plays a critical role in filtering the blood and eliminating waste products from the body, this review was conducted to gather information about herbal and natural products to reveal scientific evidence in which cases they can cause nephrotoxicity and in which cases they can act as nephroprotective. In this context, the determination of the safe dosage, bioavailability, mechanisms of action of medicinal plants, and the characterization of phytochemicals are the essential issues that play a critical role in deciding whether they are preferred as complementary pharmacological treatment.

Keywords: Medicinal plants, Phytochemicals, Traditional medicine, Nephrology, Kidney ailments.



ORAL PRESENTATION

AN ESTIMATE OF THE *IN VITRO* PLANT EXTRACT TRIGGERED
BLASTOGENIC RESPONSE IN BOVINE TUBERCULOSIS

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Abstract

Objective / Purpose: Tuberculosis, a zoonotic disease, shares pathogenetic processes in both humans and animals. The cell-mediated response, based on T_D lymphocyte reaction is of utmost importance in immunity against the disease. The investigation aimed at establishing the changes of the reactive potential of lymphocytes from infected bovine when stimulated with a mitogen and bovine and avian tuberculin simultaneously with alcoholic plant extracts, implicitly providing information on the immune stimulating potential of certain plants.

Material and Methods: The research was carried out on blood samples from bovine (n=47) on a farm with confirmed tuberculosis (TB), taken before and at the reading of the official skin test, resulting in negative (N), retest (R) and positive (P) groups of animals and subjected to blast transformation technique. For that, the blood was mixed 1:4 with RPMI1640 (Sigma Aldrich, USA), divided in 200µl aliquots in duplicate in 96 well-plates and supplemented with a mitogen (PHA), bovine (TBo) and avian (TAv) tuberculins, alcohol control and alcoholic plant extracts (*Calendula officinalis*, *Arnica montana*, *Symphytum officinale*, *Echinacea angustifolia*, *E. purpurea*), 1.5 µl/well. The plates were incubated at 37°C for 72 h, residual glucose was quantified spectrophotometrically (SUMAL PE2, Karl Zeiss, Jena) and stimulation indices were calculated (SI %). The groups were compared by Student's t test for statistical significance of the results.

Results: All SI were higher for the P and R groups than for the N one, including those to TBo and TAv, with a non significant increase towards the second sampling. The *Arnica montana* extract exerted a significantly increased (p<0.05) stimulating effect in the P, versus R and N, while the *Echinacea purpurea* extract acted inhibiting in all categories.

Conclusion / Discussion: The plant extracts used influenced lymphocyte reactivity based on TB ranking and plant taxonomy/active principles, proving immune stimulating effects comparable or above PHA.

Key Words: bovine tuberculosis, lymphocytes, *in vitro* response, plant extracts



ORAL PRESENTATION

EFFECTS OF *A. ABSITHIUM* SUPPLEMENTED FEED ON THE SPECIFIC CELL-MEDIATED RESPONSE IN PIGS FROM A LOW-INPUT FARM

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Abstract

Objective / Purpose: Parasitic, bacterial and viral diseases cause major losses in swine, thus inducing a high health, welfare and also economic impact. More and more wide-spreading free-range farming depends on the factors targeting environment protection, plant health, animal health, food safety, and consumer health. This research targeted to evaluate exerted by oral administration of *Artemisia absinthium* on the *in vitro* cell-mediated immune responsiveness to other plant extracts in pigs from a low input farm.

Material and Methods: The research was carried out on extensively raised Mangalitza suckling, weaned piglets and sows (n=10 for each group). The feed supplemented with 5% *Artemisia absinthium* L and granulated was administered as daily ratio for 7-10 days/group. Blood was sampled before and after the end of oral treatment period; then it was mixed with RPMI1640 (1:4, Sigma Aldrich, USA), divided in 200µl aliquots in 96 well-plates and supplemented with alcoholic plant extracts (*Calendula officinalis*, *Thymus vulgaris*, *Allium sativum*, *Coriandrum sativum*, *Cucurbita maxima*, 1.5 µl/well). The plates were incubated at 37°C for 48 h, residual glucose was quantified spectrophotometrically (SUMAL PE2, Karl Zeiss, Jena) and stimulation indices (SI %) were calculated. The groups were compared by Student's t test for statistical significance of the results.

Results: The oral treatment with the *A. absinthium* supplemented feed significantly (p<0.05-0.001) decreased all SI%, the least in suckling piglets. None of the extracts acted stimulating, the lowest indices being recorded for *C. sativum*, within the negative range in weaners and sows. Only the extract of *C.maxima* acted stimulating in suckling piglets (53.37±7.13%).

Conclusion / Discussion: The results indicated negative effects of *A. absinthium* on the specific immune response when administered orally in pigs, suggesting the eventual reconsideration of its administration dosage/protocole.

Acknowledgements

This research was funded by PPILOW project of the European Union's Horizon 2020 programme, grant agreement 816172.

Key words: *A. Absinthium*, swine, oral administration, immune response, low-input farm



ORAL PRESENTATION

THE VALORISATION OF ESSENTIAL OILS AS BIOSTIMULANTS ON PLANTS APPLIED BY SEED COATING ON WHEAT AND ROOTFEEDING ON TOMATO IN TUNISIA

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Due to climate change in the mediterranean area, emerging problems more severe foliar and soil diseases are causing losses on strategic crops such as wheat and tomato. Therefore, coating seeds and application by rootfeeding with plant extracts as biostimulants appears to be a promising approach to maintain the productivity of plants under stressed conditions. The effect of essential oils extracts in inducing defense response of the plant to fungal diseases was evaluated. In this study, we tested thyme (*Thymus capitatus*) and lemongrass (*Cymbopogon citrates*) essential oil, for their ability to control fungal disease both under controlled conditions and in field. These biostimulants were tested on seedlings driven in a hydroponic medium in an oxygenated nutrient solution. Daily sampling of the leaves carried out according to a kinetics of 7 days made it possible to carry out on the one hand biochemical analysis (peroxidase, phenolic compounds and H₂O₂ levels). On the other hand, infections by pathogenic fungus (*Botrytis cinerea* for tomato and *Septoria tritici* for wheat) can reveal the induction of defense reactions according to the evolution of the necrotic spots on leaves. Later on, inoculations are made by a soil borne pathogen through the injection of a suspension of Fusarium spores into the nutrient solution for tomato as well as wheat with the appropriate pathogen species. Biochemical analysis (phenolic compounds; catalase, chitinase and glucanase activities) were also performed according to kinetics in an infectious context. These data were also confirmed through molecular analysis performed at the leaf level which revealed the presence of acquired systemic resistance response through the overexpression of genes involved in defense response. Alltogether these results allowed highlighting the presence of both an inducing and priming effect of the biostimulants tested and showed an effect that can lead to the protection of the target plant.

Key Words: Disease, thyme, lemongrass, essential oil, defense response, inducing.



ORAL PRESENTATION

CONSERVATION AND MICROPROPAGATION STUDIES IN
OPERCULINA TERPETHUM (L.) SILVA MANSO: AN ENDANGERED
MEDICINAL PLANT OF WESTERN GHATS OF INDIA

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Abstract

One of the environmentally richest areas on earth is India's Western Ghats, which boasts over 4000 species of higher plants, 500 of which are medicinally important. Over time, medicinal plants that were once commonly distributed in the Western Ghats have made it into the IUCN Red List. The preservation of medicinal plants that are close to going extinct is urgently needed. *Operculina terpehthum* L. stands out among the many plant species for its potent therapeutic properties. It is a highly useful plant with a variety of medicinal uses and pharmacological effects. Fever, cough, asthma, anaemia, ascites, anorexia, constipation, gout and rheumatism are among conditions that can be treated using roots that contain the active ingredients and turpentine. One of the primary causes of *Operculina terpehthum* declining population has been overharvesting for its established medical applications. Efforts have been made for Ex-situ conservation and micro-propagation of this species. Micropropagation was conceded out to make a multiplication technique using matured nodal segments as explants cultured on MS medium supplemented with various hormone combinations. Along with other combinations, excellent results were obtained in terms of shoot length, number of shoots and number of leaves by using a combination of BAP (2.0 mg/l) + NAA (0.80 mg/l). In-vitro preservation was achieved by lowering the development and other growth-related aspects and maintaining a temperature of 100 °C. This made it possible to preserve the material for six months. To determine the genetic stability of micro-propagated plants, genetic fidelity evaluation of in-vitro produced *Operculina terpehthum* plants was carried out. The genetic stability was assessed using 15 RAPD markers. There was no genetic variation in in-vitro produced plants since all banding profiles from micro propagated plants were monomorphic and mirrored those of the mother plant. The results of this work suggest that nodal segment explants and in-vitro shoot multiplication have the ability to provide rapid clonal proliferation and conservation with low risk of soma clonal variation.

Key words: Western Ghats, Medicinal plants, conservation, propagation, genetic fidelity and multiplication



ORAL PRESENTATION

**EFFECT OF TRIPTOLIDE-LOADED EXOSOMES ON HUMAN MCF-7
BREAST CANCER CELL PROLIFERATION AND APOPTOSIS**

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Abstract

Triptolide is a bioactive diterpene triepoxide isolated from traditional Chinese plant, *Tripterygium wilfordii*, and proven to be as a potent immunosuppressant and anti-inflammatory agent. Although, it possesses remarkable anticancer and anti-inflammatory activities, its application in the clinical oncology is very poor, due to its poor water solubility and possible toxicities. In this research, a triptolide-loaded exosomes delivery system (TP-Exos) was used and MCF-7-exosomes were collected by ultracentrifugation and ultrafiltration centrifugation. MCF-Exos and TP-Exos were characterized by transmission electron microscopy, western blotting, nanoparticle tracking analysis, and high-performance liquid chromatography. Cellular uptake of exosomes, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, bromodeoxyuridine (BrdU) cell proliferation assay, and cell apoptosis assay were used to analyze the efficiency of TP-Exos on breast cancer. The cytotoxicity assay of various concentration of free TP and TP-Exos towards MCF-7 cells were revealed that encapsulation into exosomes may reduce or postpone the cytotoxicity of TP against MCF-7 cells. The results demonstrated that TP-Exos can induce the cytotoxicity and apoptosis-inducing capabilities of TP against MCF-7 cells but increase the inhibitory activities of TP on cell proliferation. In addition, TP-Exos had less cytotoxic and apoptotic effects on MCF-7 cells than free TP, but higher tumor cell proliferation suppression and tumor growth reduction than free TP. The findings showed that TP-Exos has both the general characteristics of exosomes and high drug encapsulation efficiency. As the main result, the TP-Exos may be a promising approach for breast cancer, particularly, MCF-7 breast cancer cells, but they need to be further optimized to reduce toxicity in liver, kidney or other organs.

Keywords: Exosomes, phytochemicals, drug delivery, breast cancer, apoptosis.



ORAL PRESENTATION

**PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF
PISTACIA LENTISCUS FRUIT EXTRACTS DURING RIPENING:
POTENTIAL USES IN THE FOOD INDUSTRY**

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Abstract

Medicinal plants are believed to be an important source for the discovery of potentially-important molecules for human health, due to their various biological activities. The phenolic composition of *Pistacia lentiscus* fruits extracts at different maturation stages were investigated for their phenolic composition, antioxidant capacity and enzyme-inhibitory potential against α -glucosidase and acetylcholine esterase activity. Optimization of the extraction of phenolic compounds from the last stage of ripening has also been undertaken.

This study revealed the presence of thirty molecules, including nine anthocyanins, two phenolic acids, one newly-identified stilbene, seven flavanols, seven flavonols, two flavanones, one flavanonol and one dihydrochalcone. The early stages of ripening were the richest in flavonoids and phenolic acids, while anthocyanins accumulated towards the end of fruit development. The extracts of *Pistacia lentiscus* fruits showed good antioxidant and α -glucosidase-inhibitory potential as well as a moderate inhibitory action against acetylcholine esterase activity, with variations depending on the stage of maturation, although the early stages of fruit development presented the greatest potential. Optimization of the extraction allowed the implementation of an eco-responsible anthocyanin extraction model, thereby saving time, solvent and plant material.

The results obtained in the present study indicate that ripe *Pistacia lentiscus* fruits are an important source of anthocyanin-based nutraceuticals and for food, while unripe fruits would be an interesting source of other flavonoids to produce natural extracts enriched in flavonols and flavanols.

Key Words: *Pistacia lentiscus*, Maturation stages, anthocyanins, Stilbene, Biological activity.

Acknowledgements:

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ORAL PRESENTATION

**SPECIES-SPECIFIC *IN VITRO* IMMUNE RESPONSES IN
COHABITING ANIMALS ON A LOW-INPUT FARM**

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Abstract

Objective / Purpose: The cost-effectiveness of raising animals depends primarily on their health and welfare, while losses from infectious diseases could be relatively high when the technology is disregarded. Low-input farms, where cohabitation of various species is frequent, provide outdoor free-roaming and also offer the opportunity for extensive locomotion and sunlight, considered key factors for health and welfare. The study aimed at investigating the immune potential of cohabitants (pigs, bovine and sheep) from a low input farm by testing their *in vitro* reactivity to stimulation.

Material and Methods: The research was carried out on blood samples from bovine (n=5), pigs (n=7) and sheep (n=15) cohabiting on the same low-input farm. To monitor the *in vitro* blast transformation capacity of lymphocytes, the blood was mixed 1:4 with RPMI1640 (Sigma Aldrich, USA), divided in 200µl aliquots in duplicate in 96 well-plates and supplemented with a mitogen (PHA), alcohol control and alcoholic extract of *Symphytum officinale*, 1.5 µl/well. The plates were incubated at 37°C (72 h-ruminants, 48 h-pigs), residual glucose was quantified spectrophotometrically (SUMAL PE2, Karl Zeiss, Jena) and stimulation indices were calculated (SI %). The groups were compared by Student's t test for statistical significance of the results.

Results: The spontaneous SI was higher in cows (55.7± 10.3%) while PHA induced SI was higher in swine (64.61±7.88%, p<0.05) than in bovine (53.2±9.21%) and sheep (37.81±5.08%). The *Symphytum officinale* extract exerted a significantly increased (p<0.05) stimulating effect in pigs (59.88 versus 41.33%, p<0.05), but not in bovine or sheep.

Conclusion / Discussion: Considering the similar influential factors acting on cohabiting animals on a low-input farm, there was a species-specific response of the immune system to stimulation, and presumably disease, which the *Symphytum officinale* extract could influenced.

Acknowledgements

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Key Words: bovine, pigs, sheep, *in vitro* cellular response, *Symphytum officinale*



ORAL PRESENTATION

GREEN SYNTHESIS AND BIOLOGICAL ASSESSMENT OF NANOPARTICLES USING *TAGETES ERECTA* EXTRACTS

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Abstract

Nanotechnology presents various applications in many fields, such as medicine, agriculture, pharmaceutical and food industries, as well as environmental pollution control. This is due to the unique characteristics of nanoparticles, especially of silver ones (AgNPs), out of which we mention their remarkable electrical, optical, and antimicrobial properties. Since biological synthesis methods are environmentally friendly and accessible, we have directed our attention towards the plant kingdom for the synthesis of silver nanoparticles with potential biological activities.

The present study reports an eco-friendly synthesis method of AgNPs using different *Tagetes erecta* extracts. When exposed to different active constituents of the plant, silver ions are reduced, consequently resulting in the formation of nanoparticles. After performing the optimization of the synthesis by studying the influence of several parameters, the AgNPs were characterized using various analytical techniques. The UV–Vis spectrum of the obtained nanoparticles showed a maximum peak in the 400-450 nm range. TEM analysis revealed that the particles were spherical with a size < 20 nm. The elemental composition of the synthesized AgNPs analyzed using EDX revealed the signal peak at 3 keV corresponding to Ag, but also signals corresponding to other elements (e.g., C, O and N). The average diameter values obtained through DLS analysis were higher than those acquired through TEM, given that DLS measures the hydrodynamic size of AgNPs. The negative Zeta potentials indicated the stability of the colloidal solutions. Moreover, the biological assessment was performed through different antioxidant and antimicrobial assays, that showed that the synthesized AgNPs presented enhanced antioxidant and antifungal activities compared to the corresponding extracts. In conclusion, given the facile obtaining of such nanoparticles and their improved biological properties, further studies are justified for the elucidation of their *in vitro* and *in vivo* activities.

Keywords: eco-friendly synthesis, silver nanoparticles, marigold, antioxidant, antimicrobial activity.



ORAL PRESENTATION

COMPARATIVE BIOLOGICAL AND CHEMICAL PROFILE OF
THREE ROMANIAN TEUCRIUM SPECIES

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Abstract

The aerial parts of each species (*T. chamaedrys*, *T. montanum* and *T. polium*) were collected in June 2019 from the northeastern region of Romania from the wild flora around the cities of Horlești and Bicaz. The corresponding methanolic extracts were obtained for each plant material. The chemical composition was assessed qualitatively and quantitatively (UPLC-PDA, spectrophotometry). The antioxidant, antimicrobial and cytotoxic activity was evaluated by specific means. The phytochemical analysis indicated that *T. polium* contains the highest amounts of total polyphenols and total flavonoids, which is supported by the spectrum of components determined and quantified chromatographically. Quantitatively, *T. polium* is followed by *T. chamaedrys* and then by *T. montanum*. The antioxidant potential of the investigated extracts is correlated with the chemical composition. The best scavenger of DPPH radicals was the methanolic extract of *T. polium*, followed by the samples of *T. chamaedrys* and *T. montanum*. Also, all samples were active against lipoxygenase. The antimicrobial potential revealed that the most valuable species remains *T. polium*, followed by *T. chamaedrys* and *T. montanum*, although among the three species investigated, *T. chamaedrys* stands out with the best activity against strains of gram-negative pathogens. The methanolic extract of *Teucrium montanum* has selective cytotoxic effects, the activity being expressed only on the melanoma line. The methanolic extract of *Teucrium chamaedrys* was shown to be active on the melanoma cell line at the concentration of 50 µg/mL, but showed similar effects on the healthy cell line. Corroborating all the results of biological tests we note the special potential of the three species of *Teucrium* included in the study, which have a variable spectrum of activity from one species to another, while depending on the type of solvent used to obtain extracts.

Key Words: *T. chamaedrys*, *T. montanum*, *T. polium*, antioxidant, antimicrobial, cytotoxicity

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ORAL PRESENTATION

BIOGENIC SILVER NANOPARTICLES FOR PHOTOCATALYTIC DEGRADATION OF ORGANIC DYES: A GREEN APPROACH

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Abstract

The synthesis and use of nanoparticles, especially silver ones (AgNPs), represent a developing field with various applications. The synthesis of AgNPs can be achieved by physical or chemical methods. However, these methods are expensive and require complex equipment or involve the use of polluting substances. Such disadvantages led to the development of biological methods that use bacteria, fungi, algae or plants, the last ones being accessible and offering a good cost-efficiency ratio. Moreover, since synthetic dyes are some of the most produced water pollutants, being resistant to degradation, finding materials for wastewater treatment is a topic of interest.

Thus, the aim of the present study was to investigate the photocatalytic action of some AgNPs synthesized from different aqueous plant extracts. Initially, the AgNPs synthesis conditions were established, after which the AgNPs formation was confirmed by different methods. The change in the color of the extract:silver salt mixture from yellow to brown and the presence of SPR in the 400-500 nm range by UV-Vis spectroscopy showed the formation of colloidal AgNPs. FTIR analysis revealed the presence of bioactive phytochemicals that participate in the reducing and capping/stabilizing processes. The AgNPs size and morphology were investigated by DLS and TEM, revealing a well dispersed, spherical uniform morphology and dimensions <100 nm. The EDX analysis demonstrated the presence of metallic silver and of some extract biomolecules, while the negative zeta potential values confirmed that AgNPs are stable.

The catalytic capacity of the synthesized AgNPs to remove toxic organic dyes was evaluated following the degradation of malachite green. The degradation was visually evaluated by the decreasing of color intensity from dark blue to light blue, and by UV-Vis spectroscopy. The results showed that the synthesized AgNPs can act as efficient photocatalysts for the degradation of malachite green.

In conclusion, the study demonstrates that plants represent an important source of biomolecules that can participate in the eco-friendly synthesis of AgNPs which can be used as useful materials for environmental pollution control.

Key Words: silver nanoparticles, green synthesis, photocatalytic activity, environmental pollution control.

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ORAL PRESENTATION

FOLIAR TREATMENT WITH CALCIUM AND NITROGEN IMPROVE
PRODUCTION PARAMETERS IN OREGANO

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Abstract

Oregano (*Origanum vulgare* L.) is a perennial herbaceous plant belonging to *Lamiaceae*. It is widely spread throughout Europe and North Africa both as a wild and cultivated plant. In addition to the fresh and/or dried uses of flowers and leaves, oregano provides benefits for human health thanks to its antioxidant, antimicrobial and antifungal properties. Oregano shows variation in biomass yield and essential oil (EO) content and quality because of the influence of abiotic and biotic factors in crop production. Mineral nutrition represents a fundamental aspect for cultivation of medicinal and aromatic plants due to positive effects on biomass yield and EO content. Particularly, foliar fertilisation could be a valid technique to reduce the negative impact of poor soil water availability, improving the production and qualitative parameters of open field crops. The aim of this study was to assess the effects of different foliar applications based on calcium (Ca) and nitrogen (N) on fresh and dry biomass yield, relative water content (RWC), chlorophyll content, EO content and yield in oregano. Tests were carried out in Sicily (Italy) in 2020-2021. Each year, eight foliar applications based on Ca and N were applied. Only flowers and leaves were used for the extraction of the EO. For all parameters in the study, except for the RWC, the highest values were found in the treatments applying different doses of Ca and N with respect to the control. The foliar applications with Ca and N allowed to obtain the highest production values in terms of biomass and EO. The results highlight that foliar treatments with Ca and N permit to increase crop production in environments with poor water availability.

Key Words: foliar application, essential oil, yield, mineral nutrition, oregano.

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ORAL PRESENTATION

THE NORMOGLYCEMIC AND ANTIOXIDANT EFFECTS OF *MORUS*
LEAVES EXTRACTS

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Abstract

Morus leaves water extracts are used for a long time in traditional medicine for their antidiabetic, anti-inflammatory effects and to treat cardiovascular diseases. The aim of our study was to evaluate the hypoglycemic and antioxidants effects of *Morus* leaves extracts obtained by using different solvents. The leaves collected from *Morus alba* and *Morus nigra* species were extracted with ethanol-water solution, with methanol and hot water. The dried extracts were dissolved in dimethylsulfoxide and used for biochemical tests. For *Morus alba* leaf extracts, the polyphenol content varied between 42.4 mg gallic acid equivalents/g methanolic extract and 62.5 mg gallic acid equivalents/g ethanol-water extract. For the extracts made from *Morus nigra* leaves, the content in polyphenols varied between 69 mg gallic acid equivalents/g methanolic extract and 95.2 mg/g water extract. All extracts have the ability to control alpha-amylase and alpha-glucosidase and thus can control the level of post-prandial hyperglycemia. The most intense effect on alpha-amylase was presented by the methanolic extract from *Morus nigra* leaves (EC₅₀ 69.72±1.17 µg/mL), and on alpha-glucosidase by the ethanol-water extract from *Morus nigra* leaves (EC₅₀ 69.86±0.03 µg/mL). From the point of view of antioxidant effects, the extracts inhibit lipoxygenase, with the maximum effect in the case of the ethanol-water extract from *Morus alba* leaves (EC₅₀ 23.75±1.77 µg/mL). The ethanol-water extracts have a higher iron chelating capacity compared to those in methanol or water, the most active being the ethanol-water extract from the leaves of *Morus nigra* (EC₅₀ 213.32±0.26 µg/mL). The conducted study highlights the ability of the analyzed extracts to control the enzymes involved in the digestion of carbohydrates and to reduce oxidative stress.

Key Words: *Morus alba*, *Morus nigra*, alpha-amylase, alpha-glucosidase, antioxidant

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ORAL PRESENTATION

PHYTOCHEMICAL INVESTIGATION AND BIOLOGICAL
ACTIVITIES OF *CENTAUREA* SP. COLLECTED IN TURKEY

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Abstract

Centaurea genus, belonging to the Asteraceae family, is represented by approximately five hundred species distributed through the World. Previous phytochemical studies have revealed the presence of acetylenic compounds, flavonoids and sesquiterpene lactones [1]. Associated to this exuberant chemodiversity, a several of pharmacological actions has been reported for total extracts and isolated compounds, the most significant being anticancer, antimicrobial, anti-inflammatory and antidiabetic activities. In the frame of our contribution to the characterization of biological and chemical profiles of Turkish plants [2] and considering the lack of detailed phytochemical studies, we carried out a comprehensive characterization of two different species of *Centaurea* collected in Turkey. The apolar organic extract obtained from aerial parts of *Centaurea drabifolia* Sibth. & Sm. subsp. *detonsa* (Bornm.) Wagenitz, growing wild in Turkey, was investigated for the first time for its secondary metabolite composition. Seven sesquiterpene lactones belonging to the guaiane class, including anew compound, along with a fatty acid lactone derivative, were isolated. The phytochemical investigation of the aerial parts obtained from the Turkish plant *Centaurea kotschyi* var. *persica* led to the isolation of nine sesquiterpene lactones belonging to the guaiane class, including the undescribed kotschyols A and B, a monoterpene lactone (daphnauranin E), four known lignans (matairesinol, matairesinoside, arctiin and arctigenin) and an undescribed dihydrobenzofuran neolignan (4-O-glucosylcrataegifin A). The structures of these compounds were defined by spectroscopic analysis, including ECD and 1D/2D NMR, and chemical conversion

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ORAL PRESENTATION

MS-BASED METABOLOMIC APPROACH FOR CHEMICAL PROFILE
ANALYSIS OF *CENTELLA ASIATICA* LEAVES

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Abstract

Centella asiatica (L.) Urban is a perennial herbaceous plant native to the Indian subcontinent and diffused in the entire southeast Asia and in the wetland zones of Southeastern America. The aerial parts are widely known since pre-historical time as medicinal plant mainly renowned for its cognitive enhancing effects, anti-inflammatory properties, and its ability to regenerate wound healing. The health benefits of *C. asiatica* extracts have been exploited for the production of many natural supplements and phytotherapies with several applications, such as psychotropic, veinotonic and anti-ulcer. Among its secondary metabolites, the main bioactive constituents of *C. asiatica* leaves are pentacyclic triterpenes that are responsible for the main therapeutic properties ascribed to the plant.^[1,2] The aim of our study was to provide a detailed phytochemical profile of the plant extract used in the phytotherapeutic formulations. To this aim, the preliminary LC-HRMS fingerprint and Molecular Networking visualization completed by isolation and NMR-based characterization of constituents can empower the exploration of chemical composition, focusing on the minor constituents. In this field, we capitalized on a protocol developed in our studies for expeditious untargeted dereplication, extraction and fractionation using MPLC and HPLC methods equipped with different types of detectors and on an extensive spectroscopic analysis based on MS and NMR for the structural elucidation of isolated metabolites. The investigation led to the isolation and characterization of about 24 secondary metabolites including 10 polyphenols and 14 ursane- or oleanane-type triterpenoids in the saponin or saponin form, including two previously undescribed saponins, named isomadecassoside and isoterminolloside. The anti-inflammatory potency of the isolated compounds was tested for their ability to reduce nitrite levels in LPS-stimulated macrophages.^[3]

Key Words: *Centella asiatica*, triterpenoid saponins, metabolomics, phytochemicals

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ORAL PRESENTATION

GREEN EXTRACTION OF PHENOLIC COMPOUNDS FROM OLIVE LEAF BY MEANS OF DEEP EUTECTIC SOLVENTS: PRINCIPAL COMPONENT ANALYSIS AND OPTIMIZATION

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Abstract

Olive leaf extract has been recommended as natural additive for drug and food industries because of its rich phytochemical content [1]. Therefore, it is very important to evaluate this valuable component with green methods. From this point of view, deep eutectic solvents (DESs) have been utilized as alternative green solvent. This third type solvents can easily synthesis by mixing at least two components (hydrogen bond donor and hydrogen bond acceptor) [2].

The aim of present research is to suggest a DES-based microwave assisted extraction of phenolic substances from olive leaves by application of chemometric study. 15 individual citric acid-ethylene glycol DESs were synthesised with three molar ratios (1:2, 1:3, 1:4). Additionally, effect of different water addition level in DES (10, 30, 50, 70 and 90%) was investigated, and evaluated by principal component analysis (PCA). It was found that the best DES combination was obtained with 1:2 molar ratio and 70% water addition. Once the best combination was chosen, response surface method (RSM) was applied as statistical experimental design approach through Box-Behnken design. The effect of independent parameters was selected as microwave power, extraction time and solid/solvent ratio with three levels (300-500 W, 40-60 s and 0.2g/20 mL-0.2/40mL). The greatest phenolic (TPC), antioxidant activity and oleuropein yields of olive leaf extract were calculated with optimization study. Furthermore, analysis of variance (ANOVA) test was used to define the impacts of process parameters, their interactions and second powers by means of Design-Expert version 12 (Stat-Ease, Minneapolis, MN, USA) software trial. ANOVA also provided analysis of the model fitting indicators

Key Words: Phenolic compounds, oleuropein, microwave assisted extraction, PCA, Box-Behnken-RSM.

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ORAL PRESENTATION

HPLC DETERMINATION AND METHOD VALIDATION OF
CHLOROGENIC ACID FOUND IN *CHAEROPHYLLUM BYZANTINUM*
BOISS. EXTRACTS

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Abstract

The genus *Chaerophyllum* is represented by 15 species and 4 of them are endemic in Turkey. (Davis 1988). In this study, crude extracts of aerial parts and fruits of *Chaerophyllum byzantinum* (CBH, CBF) and sub-extracts (CBH1-CBH5 and CBF1-CBF5) were tested for total phenolic content. Four extracts with the highest total phenolic content were selected for quantitative analysis. A novel and sensitive HPLC method has been developed and validated for chlorogenic acid quantitation, in which the amounts of chlorogenic acid in extracts were detected as 20.5 µg/mL for CBH2, 15.8 µg/mL for CBH4, 7.64 µg/mL for CBF2 and 3.7 µg/mL for CBF4. Our findings showed that the linearization regression coefficient value (R²) was 0.9997 in 7-point calibration. Linearity of the method was tested in the range of 2.5-400 µg/mL chlorogenic acid standard solution. The method represented good linearity (R²=0.9997), precision (RSD %: <2%), LOD (0.1186 µg/mL), LOQ (0.3593 µg/mL), accuracy, selectivity and stability, in terms of establishing the suitability of the method. To the best of our knowledge, chlorogenic acid quantification was performed for the first time in *C. byzantinum* extracts.

Key Words: *Chaerophyllum byzantinum*, Chlorogenic acid, Total phenolic content, HPLC, Validation

Acknowledgements

The authors are grateful to Dr. Emine Akalın (İstanbul University, İstanbul, Turkey) for identification of plant material.

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ORAL PRESENTATION

HPLC QUANTIFICATION OF CAFFEINE FOUND IN COFFEE BEANS
OF DIFFERENT ORIGINS SOLD IN TURKEY

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Abstract

Caffeine, which belongs to the alkaloid group, is found in many prescription and over-the-counter drugs as a central nervous system stimulant, analgesic and respiratory distress reliever, and has wide range of effects within the body [1]. It is recommended not to consume more than 400 mg per day for those who are sensitive to caffeine, and 300 mg for pregnant women in order not to be affected from its negative outcomes. This amount is 4-5 cups for adults, maximum of 3-4 cups for pregnant women [2]. Albeit, the amount of caffeine in a coffee beverage varies according to the region where the bean is grown, in addition to the methods of roasting, grinding and brewing. Therefore, it is critical to determine the caffeine quantity found in coffee. In this study, the amount of caffeine in ground coffee produced from coffee beans sold in a local coffee shop chain in Turkey and obtained from different regions (Guatemala, Kenya, Costa Rica, Brazil, Colombia) was determined by HPLC in order to estimate the amount to be consumed without exceeding the daily limit. Linear dynamic range was 1.125–80 $\mu\text{g.mL}^{-1}$, and coefficient of determination (R^2) was 0.9995. The limit of detection and the limit of quantification were found to be 0.46 and 0.50 $\mu\text{g.mL}^{-1}$, respectively. As a result, the amount of caffeine in a cup (240 mL) of coffee brewed from coffee beans originated from Guatemala, Kenya, Costa Rica, Brazil, and Colombia was found 117.77, 145.85, 140.38, 163.33 and 106.44 mg, respectively. These findings indicate that coffee from Brazil should be consumed less, which is followed by Kenya and Costa Rica.

Key Words: Coffee, Daily intake, HPLC, Caffeine, Determination

Acknowledgements

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ORAL PRESENTATION

FOLK MEDICINES IN GERZE (SINOP, TURKEY)

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Abstract

The aim of our research to make an inventory of folk remedies used in Gerze District (Sinop). For this purpose, scientific trips were organized to the study area in May-August 2009. In the visited locations ethnobotanical information was collected by face-to-face interviews and open and semi-structured questionnaire. After interviews, the plants that are being used as folk medicine were collected in situ under guidance of informants. Scientific names of the plants were determined and plant specimens were deposited in Gazi University Faculty of Pharmacy Herbarium (GUEF). To assess the reliability of results, ethnobotanical data was analysed through three quantitative indices: “informant consensus factor (FIC)”, “use value (UV)” and “relative frequency of citation (RFC)”.

As a result of these interviews conducted in 18 villages with 92 people (29 women; 63 men), 65 plant species from 42 families have been determined to be used as folk medicine in Gerze District. Rosaceae family is the most used family in the preparation of folk remedies with 7 species. Most cited plant species was *Olea europaea* L. (27 citation) and followed by *Sambucus ebulus* L. (24 citation), *Brassica oleracea* L. (21 citation) and *Urtica dioica* L. (20 citation). The highest FIC value was obtained in dermatological disorders (0.752); respiratory and musculoskeletal system disorders are other pharmacological categories which has high FIC value (0.6860 and 0.6825, respectively). *Olea europaea* L. (UV: 0.293, RFC: 0.184) and *Sambucus ebulus* L. (UV: 0.260, RFC: 0.239) were the species that have highest UV and RFC values.

As a result of this study, once again it was revealed that the folk medicine knowledge is rapidly disappearing in Gerze District as in other regions of Turkey and the necessity of conducting ethnobotanical inventory studies in the whole country.

Keywords: Folk medicine, Gerze, Ethnobotany, Sinop, Medicinal plant.



ORAL PRESENTATION

FORMULATION OF HERBAL TOOTHPASTE TABLET FOR
ANTICARIOGENIC ACTIVITY AGAINST *STREPTOCOCCUS MUTANS*
AND EVALUATE THE SATISFACTION OF THE VOLUNTEERS

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Abstract

Herbal toothpaste, in the form of powders, has caused many problems such as difficulty in the amount of use, difficulty in carrying when traveling, and complications in moisture, which affected the quality. Toothpaste tablets are known for being an eco-friendly alternative to toothpaste. These products also offer benefits in terms of portability and resistance to temperature changes. **Objective:** The objective of this research aims to develop toothpaste tablets from Thai herbs, determine the anticariogenic activity of herbal toothpaste tablets against oral bacteria (*Streptococcus mutans*), study the stability of the herbal toothpaste tablets, and assess the satisfaction of using the products. **Methods:** A total of five formulations were developed. Each herbal toothpaste tablet was formulated by incorporating the eight types of herbs such as cuttlebone, toothbrush tree, clove, borneol, camphor, menthol, alum, and salts and consist of seven types of excipients such as disintegrants, flavoring agents, binders, surfactants, lubricants, and diluents. The methods consisted of wet granulating and tableting. Using the disk diffusion method, the antibacterial sensitivity of the tablets was evaluated. The survey method and self-administered questionnaire were used for satisfaction data collection. **Results:** The results showed that the fourth and fifth formulations could be compressed as tablets that were brown circled-shaped, 250 mg of weight, 30-50 N of hardness, and less than 1% of friability according to USP39/NF35 criteria. The tablets were tested for their antimicrobial susceptibility test and the largest zone of inhibition observed was 12.2 +/- 0.54 mm. For assessing the satisfaction as a pilot test, the best formulation was the fourth and fifth formulation, which has microcrystalline cellulose PH101 as diluent and 2% clove oil, and 3% peppermint oil as a flavoring agent. **Conclusion:** The herbal toothpaste powder could be compressed as a tablet with the high stability of the formulation. The formulated toothpaste capable of tooth and oral hygiene shows antimicrobial activity against the pathogen. Overall, the product is interesting and accepted among participants that could be used for further commercial development.

Key Words: Herbal formulation, Toothpaste tablet, Anticariogenic activity, *Streptococcus mutans*, Satisfaction

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ORAL PRESENTATION

FRAGRANCES OF *PUNICA GRANATUM* YELLOW LEAVES

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Abstract

The supercritical carbon dioxide extraction of pomegranate, *Punica granatum*, yellow leaves was performed under different pressure, temperature and carbon dioxide flow rate. A three-level Box-Behnken design was used for the determination of the optimal process conditions. The optimum extraction conditions are at 11.82 MPa and 40°C with the carbon dioxide flow rate of 3 kg/h. Under these conditions the optimum yield is 5.75%. The optimum extraction conditions for the maltol extraction are at 17.68 MPa at 40°C with the carbon dioxide flow rate of 2.5 kg/h. The supercritical carbon dioxide is a reliable technique for obtaining the fragrance compound maltol from *P. granatum* yellow leaves.

Key Words: *Punica granatum*, yellow leaves, supercritical carbon dioxide extraction, maltol.



ORAL PRESENTATION

THE CONTRIBUTION OF ADRENERGIC RECEPTORS IN THE
ANALGESIC EFFECT OF RUTIN

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Abstract

Rutin has been known that is a flavonoid that has analgesic and anti-inflammatory activity and is found in many medicinal plants. In this study, it was aimed to evaluate the analgesic efficacy of rutin with hot plate and tail immersion tests in mice and to investigate the role of adrenergic receptors in the analgesic effect of rutin. Rutin was administered intraperitoneally at doses of 25, 50, 100, and 200 mg/kg in mice, and 300 mg/kg (i.p.) dipiron were administered as positive control. Prazosin (α -1 adrenoceptor antagonist), and yohimbine (α -2 adrenoceptor antagonist) (both; 1 mg/kg, i.p.) were used to examine the involvement of the adrenergic system in the analgesic effect provided by rutin. Rutin significantly reduced pain induced by hot plate and immersion tests at all doses. 100 mg/kg rutin showed analgesic effect similar to that of 300 mg/kg dipyrone in both hot plate and tail immersion test and was chosen to investigate the mechanism of analgesic effect. Prazosin significantly reversed the analgesic effect of rutin, while yohimbine reduced the analgesic effect relatively and rutin still had a significant effect compared to the control group in the hot plate test. In the tail immersion test, both prazosin and yohimbine reduced the analgesic effect relatively and rutin still had a significant effect compared to the control group. According to the data, it has been determined that the adrenergic system partially plays a role in the analgesic effect of rutin. The elucidation of the effect and mechanisms of actions of rutin will contribute to new therapeutic approaches and provide guidance for new analgesic drug development studies.

Keywords: Rutin, analgesic effect, adrenergic system, hot plate, tail immersion

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ORAL PRESENTATION

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF
DRACOCEPHALUM MOLDAVICA L. EXTRACTS

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Abstract

Dracocephalum moldavica L. (Moldavian dragonhead) is one of species belonging to Lamiaceae family, which has received a lot of attention in the last years, due to its significant pharmacological potential and its cultivation adaptability [1,2]. **Aim.** The present study aimed to evaluate the chemical profile and the antioxidant, antimicrobial and antiproliferative activities of a hydroalcoholic extract (70% V/V ethanol in water) obtained from some *D. moldavica* cultivars. **Materials and methods.** The chemical composition of the tested samples was investigated using spectrophotometrical and LC/MS methods [3]. The protective effect of the ethanolic extract against free radicals was assessed *in vitro* using the DPPH, FRAP and CUPRAC methods. The antimicrobial potential was screened using agar-well diffusion and broth microdilution assays, while the cytotoxicity and antiproliferative potential were tested on human fibroblast (BJ) and colorectal adenocarcinoma (DLD-1) cell lines using MTT assay. **Results.** The chemical analysis demonstrated the presence of flavonoids (hyperosid, luteolin-7-O-glucoside, rutin) and of phenolic acids (caffeic acid, chlorogenic acid) in significant amounts. Tested samples displayed important *in vitro* antimicrobial efficacy against all reference strains, in particular in the case of *Staphylococcus aureus* and MRSA. Furthermore, MTT test pointed out no toxicity against the normal cell line and cytotoxic potential expressed towards the tumoral line. **Conclusion.** The study highlighted relevant antioxidant, antimicrobial and antiproliferative activity for the tested extracts, emphasizing a significant medicinal potential of the species.

Keywords: *Dracocephalum moldavica* L., ethanolic extracts, chemical composition, antioxidant, antiproliferative, antimicrobial

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ORAL PRESENTATION

EFFECT OF SALICYLIC ACID FOLIAR APPLICATION ON PROLINE, CHLOROPHYLL AND CARETENOID CONTENT IN *GALANTHUS ELWESII* HOOK UNDER ZINC STRESS

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Abstract

Zinc (Zn), which is a nutrient element, has a toxic effect on plant tissues in high concentrations, but at higher concentrations, it is toxic. On the contrary, salicylic acid (SA) is a regulator of molecules responsible for growth and development, which reduces environmental stress tolerance in plants. Therefore, spray application of SA can protect against various types of stress, such as Zn toxicity. The objective of this study was to investigate the effect of salicylic acid concentrations on the proline and chlorophyll content in snowdrop under zinc stress. The experiments were conducted as a factorial using a randomized pilot design with five replications, in a greenhouse. The factors included SA (0, 0.5, 1 and 2 mM) and Zn (0, 40, 80 and 120 mM) concentrations. Proline, chlorophyll and caretenoid contents were determined by spectrophotometric methods. Lower SA concentrations, especially a concentration of 0.5 mM, were generally more effective in enhancing proline, chlorophyll and caretenoid content. Proline increased with treatment by 2.86 times (0.5 mM). Chlorophyll and caretenoid increased in the salicylic acid treatment by 2.99 and 3.29 times, respectively. On the other hand, a statistically significant negative correlation was registered between the content of chlorophyll and caretenoid and the concentration of Zn in the leaf ($p < 0.05$). The results of the study revealed that salicylic acid application against Zn stress had a positive effect on proline, chlorophyll and caretenoid content.

Key Words: Amaryllidaceae, Bulbous plants, Medicinal plants, Micro element

Acknowledgement: This study is a part of Yasemin Kirgeç's master's thesis.



ORAL PRESENTATION

**EXAMINATION OF CHEMICAL COMPOSITION OF ESSENTIAL OIL
AND BIOLOGICAL ACTIVITY OF ETHANOLIC PLANT EXTRACT
DISSOLVED IN DIFFERENT SOLVENTS OF *CIRSIMUM
PSEUDOPersonATA* BOISS. & BAL. SUBSP. *PSEUDOPersonATA***

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Abstract

Cirsium pseudopersonata Boiss. & Bal. subsp. *pseudopersonata* is one of the endemic species of *Cirsium* genus in Turkey. The genus is derived from the Greek word “khirsos,” meaning “swollen vein.” As a result of the literature review, no study has been found on the chemical content and antimicrobial activity of this endemic species, known as the Turkish “huge Kangal”. This study is aimed to analyze the chemical composition of essential oil of *C. pseudopersonata* Boiss. & Bal. subsp. *pseudopersonata* collected from the Ayder plateau, Turkey, in 2020-2021. Furthermore, the antimicrobial and antioxidant activities of the parts of the plant extracted with ethanol, prepared in different solvents with different polarities, were demonstrated.

The chemical composition of the plant's essential oil was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The Kirby- Bauer agar diffusion method was performed for the extracts' antimicrobial activity on eleven pathogens. Total phenolic and flavonoid contents were calculated according to Folin-Ciocalteu and AlCl₃ methods, respectively. The solvent-induced effect on the antioxidant activity was evaluated by examining each extract solution's 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging efficiency.

Forty-two compounds representing 99.9% of the essential oil were identified. Santalol (α (30.22%), Benzene, methyl- (CAS) (20.36%), Lauryl alcohol (15.42%), Germacrene D (7.22%), and Formate propyl (5.38%) were found to be the major compounds in the oil. *Bacillus subtilis* and *Salmonella enterica* subsp. *enterica* serovar *typhimurium* were resistant to all extracts. The inhibition zone diameter in the extracts with antimicrobial activity ranged between 8.75-27.65 mm. The antimicrobial activity results of the cyclohexane extract were promising. While the total phenolic content was determined for the extract soluble in tetrahydrofuran to be the highest, the methanol solution was in second place. Correspondingly, the DPPH radical scavenging activity is most effective in the case of the preparation dissolved in methanol. While the flavonoid content can only be calculated in the case of extracts dissolved in ethyl acetate, tetrahydrofuran, methanol, and diethyl ether due to solvent interference, the results of all these tests are correlated with each other.

Key Words: Endemic plant, antimicrobial, antioxidant, solvent effect, GC-MS, essential oil



ORAL PRESENTATION

**THE EVALUATION OF THE ANTIOXIDANT CAPACITY OF APPLE
FRUIT EXPOSED TO TEBUCONAZOLE**

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Abstract

The aim of our study was to evaluate the effect of pesticide on the characteristics of apple fruits and their antioxidant capacity. The study was carried out on two apple varieties, Golden delicious and Jonathan, which were treated with tebuconazole alone or in combination with other pesticides, in a normal or double dose. After harvesting, the peel and pulp of the apple fruits were subjected to methanol extraction by the microwave-assisted extraction method. Methanol extracts were used for pesticides analysis by GC-MS, for the determination of polyphenols and flavonoids content, respectively for the evaluation of antioxidant properties of these. Tebuconazole was determined only in the peel of fruits from the Golden delicious variety that were treated with the double dose of pesticide. The content of polyphenols is lower in the fruits treated with tebuconazole compared to the untreated ones, for example for the Jonathan variety it is 133.49 µg GAE/mL methanolic extract from the skin of the untreated fruits and 49.80 µg GAE/ml extract from the skin of the fruits treated with the double dose. Treating the trees with tebuconazole alone or in combination induces a reduction in the chelating capacity of the ferrous ion with an increase in the EC50 value from 0.132 ± 0.007 mL equivalent extract (untreated Jonathan) to 0.165 ± 0.017 mL equivalent extract (Jonathan treated with pesticides combination). The lipoxygenase inhibition capacity for Jonathan peel extract decreases from 47.14% (untreated fruits) to 26.32% (fruits treated with double dose pesticides). For the Golden delicious variety, the lipoxygenase inhibition capacity of the fruit peel extracts decreases from 29.54% (untreated fruits) to 18.02% (fruits treated with a double dose of tebuconazole). The study highlights the importance of respecting the dose of pesticide used and the time from the last pesticide application to fruit harvesting.

Key Words: apple, antioxidant, lipoxygenase, iron

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ORAL PRESENTATION

THE ANTIBACTERIAL AND ANTIMYCOTIC ACTIVITY OF SOME
PELARGONIUM SPECIES

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Abstract

The aim of the study was to evaluate the antimicrobial activity of *Pelargonium hispidum*, *Pelargonium grandiflorum*, *Pelargonium radens*, *Pelargonium peltatum* and *Pelargonium zonale*, methanolic and ethanolic extracts. The activity was tested in Mueller-Hinton agar for disk diffusion, according to the international standards, with recommended quality control organisms, medium pH, agar depth and uniformity, and characteristics of the zones of inhibition. The evaluation was made against *Staphylococcus aureus* ATCC 25923, *Sarcina lutea* ATCC 9341, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028, *Candida glabrata* ATCC MYA 2950, *Candida parapsilosis* ATCC 22019. These were carried out by taking the organic extracts at a concentration of 10 mg/ml and their activities were recorded by estimating zones of inhibition as produced by disc-diffusion method on Mueller-Hinton agar media. For positive control were used tablets of Ciprofloxacin (5 µg/tablet), Ampicillin (25 µg/tablet), Fluconazole (25 µg/tablet), Voriconazole (1 µg/tablet), Nystatin (100 µg/tablet). The plates were incubated for 24 hours at 37°C for bacteria and 48 hours at 24°C for yeasts. The results confirmed the data from the literature, so the methanolic extracts were much more active on gram negative strains than the ethanolic extracts and both types of extracts were active against *Staphylococcus aureus*. The ethanolic extracts were much more active against gram positive strains. The differences between these two types of extracts is correlated with the differences in the active compounds. The high concentration of the polyphenolic compounds in the methanolic extracts can prove their antimycotic activity.

Key Words: methanolic extracts, ethanolic extracts, antimicrobial activity, *Pelargonium species*.

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ORAL PRESENTATION

EVALUATION OF *PEGANUM HARMALA* L. AND ALKALOIDS AS
POTENTIAL MATRIX METALLOPROTEINASE INHIBITORS:
AN EXPERIMENTAL AND *IN SILICO* STUDY

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Abstract

Matrix metalloproteinases (MMPs) playing an important role in collagen metabolism are suggested to contribute to hemorrhoidal disease due to the degrading properties of the extracellular matrix proteins, especially elastic fibers. These effects were particularly associated with the overexpression of MMP-2 and MMP-9 enzymes (1, 2). In this research, methanolic extract and a non-alkaloid fraction of the seeds of *Peganum harmala* L., a folk medicine utilized against hemorrhoids, as well as harmala alkaloids (harman, harmine, harmol, harmaline, harmalol, vasicine, vasicinone) were evaluated for their gelatinase inhibitory activities both *in silico* and *in vitro*. Docking studies were performed on MMP-2 and MMP-9 crystal structures, and the *in vitro* experiments were carried out spectrophotometrically. Enzyme inhibition assay results were expressed as remaining activity (%) of the enzyme and inhibitory activities (%) of the samples on the 10th minute. All the tested samples were found to be more effective on MMP-2 rather than MMP-9. The extract inhibited MMP-2 by 59.2%, whereas the non-alkaloid fraction inhibited only 9.6% at 100 µg/ml, indicating that the alkaloids were responsible for the enzyme inhibition. Among the tested alkaloids, harman, harmol, and harmaline demonstrated potent MMP-2 inhibitory activity in a range of 79.6-89.8% at 50 µM concentration. On the other hand, the best two remaining activities were seen on harmine (41.5%) and harmaline (48.4%) at 200 µM concentrations for MMP-2. As to MMP-9, harmalol exhibited the highest inhibitory activity at 63.6% and the best remaining activity at 52.1% at 100 µM. *In silico* studies revealed that the β-carboline alkaloids showed superior protein binding capabilities, similar to a known gelatinase inhibitor NNGH, compared to the quinazoline alkaloids for both MMPs. In conclusion, *in silico* and *in vitro* studies both suggested that β-carboline alkaloids may act as potential MMP-2 inhibitors and anti-hemorrhoidal agents due to enhancing properties on the connective tissue.

Key Words: Hemorrhoids, MMP-2, MMP-9, molecular docking, enzyme inhibitor, harmala alkaloids

Acknowledgments

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ORAL PRESENTATION

CONTENT OF ESSENTIAL AND FATTY OIL OF *Foeniculum vulgare*
L.(FENNEL) GROWN UNDER INORGANIC AND ORGANIC
CONDITIONS

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Abstract

In recent decades, the over-use of chemical fertilizers has imposed many environmental challenges worldwide. Nowadays, the use of vermicompost manure (VM) and sheep manure (SM) of great interest to environmental security and are effective as a good nitrogen source for sustainable crop production. Therefore, determining the effective doses of VM and SM that will be an alternative to chemical fertilizers, are also important to improve soil fertility and produce healthy products. This study aimed to determine the effects of VM, SM and ammonium sulfate (AS) fertilizers on the fatty oil and essential oil content of fennel (*Foeniculum vulgare* L.).

Three levels of VM applications (1000, 1500, 2000 kg/da), SM (1000, 1500, 2000 kg/da) and AN (4, 8, 12 kg/da) with a control (no fertilizer or manure) were placed in the experimental farm. Essential oil yield showed differences depending on the dose of VM, SM and AS fertilizer applications. The essential oil content range was found to be 2.40 to 3.35% in fennel fruits, and the highest essential oil content was obtained from the treatment of 2000 kg/da VM. In addition, the fatty oil content range was found to be 17.39 to 33.61% in fennel fruits, and the highest fatty oil content was obtained from the treatment of 2000 kg/da VM. VM applications of 2000 kg/da increased the essential oil and fatty oil yield of fennel, which may be related to the presence of more nutrients or effects of organic manure on soil structure.

The findings of the study suggest that the application of VM has promising effects on fennel fruit and can be considered as a suitable substitute for chemical fertilizers when growing fennel, a plant with increasing importance and demand.

Key Words: Fennel, Essential oil, Fatty oil, Organic manures

Acknowledgements

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ORAL PRESENTATION

INFLUENCE OF DIFFERENT ORGANIC MANURES ON THE COLOR CHARACTERISTICS OF *Salvia officinalis* L. (SAGE) UNDER FIELD CONDITIONS

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Abstract

Salvia officinalis L.(sage) is a medicinal plant extensively used as a raw material in the medicine, pharmaceutical, food, and perfume industry. Due to the economic values of sage, it has attracted the interest of producers. Organic farming is becoming more integrated in the agro-food chain and the global market, while environmental, food safety regulations contribute to making agriculture more sustainable and producing food that is safe for the consumer. Therefore, it is necessary to grow sage in accordance with the standards and of high quality for sustainable agriculture worldwide.

The present study aimed to evaluate the influence of doses of organic manures as sheep (500, 750, 1000, 1250 kg/da), chicken (500, 750, 1000, 1250 kg/da) and vermicompost (50, 100, 150, 200 kg/da) with a control (no manure) and conventional fertilizer on the color parameters of sage under field conditions. Furthermore, our study was undertaken to determine relationships between color measurements and pigment concentrations. According to statistical analysis results, there were significant differences in terms of L*, a*, b*, C*, and H*. In the case of sage leaves showed L* values in the range 51,93 and 66,66, a* values among 2,79 and 17,35, b* values among 12,89 and 17,35, C* values among 15,12 and 24,09 and H* values among 30,60 and 77.80. Among the organic manure applicaitons, The highest L* and C* were found from 500 kg/da chicken manure dose and the highest a* and b walues were noted as 17,35 under different organic manure doses. The lowest b* and h° values were observed from 500 kg/da chicken manure application. The lowest L*, a* and c* values were found from different organic manure applications. In conclusion, chicken and sheep manure applications had positive effects on leaf color parameters sage under field conditions, and 500 kg/da chicken manure applicaiton had the best result in field conditions.

Key Words: Sage, Leaf color parameters, Organic manures, *Salvia officinalis* L.

Acknowledgements

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ORAL PRESENTATION

THE FOOD USED IN MATERNAL AND NEWBORN HEALTHCARE IN BISKRA (SOUTHERN EAST OF ALGERIA): THE CASE OF HERBAL SWEET

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Abstract

Background: Food is considered to be the first means to health care, especially when is prepared with special ingredients, so there are dishes associated to special celebration in different civilisation. The birthday of a new born is one of the important celebrations in Algerian's families that are marked with special food one of them is the herbal sweet of Biskra, served to mothers after the first day of delivery till 40th day, to know its specificity we laid an investigation about its composition.

Methods: The survey was carried out on a simple of 60 females informants from Biskra region (Southern East of Algeria), during the two academic years 2020/2021 and 2021/2022. The main purpose of this study is to determine the level of women knowledge from Ziban region in maternal and newborn healthcare, to enumerate what are the herbs, spices and medicinal plants used for the preparation of special dishes using a questionnaire prepared in Arabic language. In this case, we study the herbal sweet (R'fiss l'hechahech)

Result: Our investigation in sweet herbal allowed us to inventory about 25 species of medicinal aromatic and herbal plants belonging to 11 botanic families. The oldest informants (grand-mothers and mothers) give us more information than the youngest informants (single and students). The most sited species are *Rosmarinus* and *Thymus* with 100 % of responses. The leaves are the most used part with 56 %, the most medicinal uses of the listed species are: promote lactation, calm abdominal pain and intestinal gas, and help the postpartum recovery.

Conclusion: There remain, however many dishes that deserve further study, and it is very important to document them because the transfer of knowledge from the old generation to the new generation is diminishing.

Key words: maternal healthcare, investigation, herbs, spices,



ORAL PRESENTATION

INVESTIGATION OF BIOACTIVE PROPERTIES OF *TEUCRIUM CHAMAEDRYS* LEAVES: PROCESS OPTIMIZATION USING CENTRAL COMPOSITE DESIGN

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Abstract

Teucrium chamaedrys leaves have been reported as alternative herbal medicine rich in phytochemicals such as phenolics and flavonoids [1]. Therefore, the selected biomass has been investigated in order to develop a green extraction method for recovery of its bioactive substances. Homogenizer-assisted extraction (HAE) method was used by means of a GRAS (generally recognized as safe) solvent (aqueous ethanol). Central composite design of Response Surface Method (RSM) was used to optimize the HAE system through 20 experimental runs. Ethanol concentration (30-90%, v/v), extraction time (30-90 sec) and homogenizer speed (4000-10000 rpm) were independent process parameters. Responses (dependent variables) of the system were total phenolic content (TPC), total flavonoid content (TFC), individual phenolic compound (4-hydroxycinnamic acid) and antioxidant activity of the extracts. DPPH (2,2-diphenyl-1-picrylhydrazil) assay was used to measure the antioxidant activity. In order to identify the significance of the process parameters, their interactions and second powers, analysis of variance test (ANOVA) was employed via RSM. Model statistics such as p and F values, lack of fit, coefficient of variation (C.V.), coefficient of determination (R^2) and adjusted R^2 findings have demonstrated the reliability of the second order models produced for the present system.

Key Words: Green chemistry, teucrium chamaedrys, antioxidant, multivariate optimization methods.

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ORAL PRESENTATION

IN VITRO ANTIFUNGAL AND ANTIBIOFILM ACTIVITY OF
ARTEMISININ AGAINST *CANDIDA* SPECIES

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Abstract

Artemisinin is a sesquiterpene lactone derivative of *Artemisia annua* L. Previous research has shown that artemisinin and its derivatives have important pharmacological properties. *Candida* species are opportunistic infections with high mortality and morbidity rates worldwide. There is growing evidence that natural agents have the ability to treat *Candida* infections. The demand for new drugs has increased in recent years due to treatment costs, toxicity and resistance to conventional antifungal drugs. The aim of this study was to investigate the antifungal and antibiofilm activity of artemisinin. The antifungal activity of artemisinin against *Candida* strains was performed in 96-well microtiter plates using the broth microdilution protocol of Clinical and Laboratory Standards Institute document M27-A3. The inhibitory effect of artemisinin on *Candida* biofilms compared to fluconazole was measured by MTT assay. Total RNA was isolated from control and treated biofilm cells using the YeaStar RNA Kit. Synthesis of cDNA from total RNA was performed using the Transcriptor High Fidelity cDNA Synthesis Kit. Expression analysis of biofilm-related genes was investigated using qRT-PCR. Statistical analysis was performed using a one-way test ANOVA with Bonferroni post hoc. Artemisinin showed significant antifungal activity against fluconazole-susceptible and -resistant strains of *Candida*, as well as an inhibitory effect on *Candida* biofilms. In RT-qPCR experiments, changes in the expression of biofilm-related key genes were observed after artemisinin. The current results suggest that artemisinin has the potential to treat biofilm-associated *Candida* infections. Further translational research is needed to investigate whether the anti-biofilm activity of artemisinin is useful in a therapeutic context.

Key Words: Artemisinin, *Candida*, Antifungal, Antibiofilm.



ORAL PRESENTATION

***H. PERFORATUM* L. AFFECTS LOCOMOTOR ACTIVITY, BODY WEIGHT AND BLOOD GLUCOSE**

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Abstract

Hypericum perforatum is a perennial herb that is widely distributed in all Mediterranean and mainly contains hyperforin and hypericin. It is commonly used as an antidepressant. In rats fed a high-fat diet, *H. perforatum* was found to prevent weight gain. It has been shown to cause the total glucose level to remain elevated for six weeks. We investigated how different doses of *H. perforatum* extract affected Syrian hamster locomotor activity, body weight, food consumption, and blood glucose levels. The aerial parts of *H. perforatum* were subjected to maceration in ethanol using a Soxhlet apparatus. Syrian hamsters received injections of plant extract at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg. The animals' daily body weights and food intake were calculated, along with their locomotor activity for the course of the 10-day experiment. Blood glucose levels were measured twice, before and after the extracts were injected. Results showed that as doses increased, animals' locomotor activity, body weight, and food intake decreased. After being administered into hamsters, an extract at a dose of 300 mg/kg considerably raised blood glucose levels. High doses of *H. perforatum* extract can therefore be thought of as both an antidepressant and an anti-obesity medication. The decrease in daily activity rhythms, in particular, may be an indication of *H. perforatum's* sedative effect.

Key Words: *H. Perforatum*, locomotor activity, body weight, food intake, blood glucose



ORAL PRESENTATION

CHEMICAL COMPOSITION AND PHYTOTOXIC ASSESSTMENT OF SEVEN EUCALYPTUS ESSENTIAL OILS FROM TUNISIA

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Abstract

The identification of bioactive allelochemicals in several *Eucalyptus* species essential oils have developed research into potential natural herbicidal compounds for weed management [1]. Nowadays, only 8% of conventional herbicides are derived from natural compounds even if natural product-based herbicides are considered to be safer than conventional chemical herbicides [2]. The present study was carried out to characterize chemical composition of seven *Eucalyptus* species EOs *E. griffithsii* Maiden, *E. hemiphloia* Benth., *E. lesouefii* Maiden, *E. longicornis* Maiden, *E. pyriformis* Turcz., *E. viminalis* Labill. and *E. wandoo* Blakely, as well as their phytotoxic activity. The EOs were analyzed using GC/MS and the potential *in vitro* phytotoxicity was evaluated against germination and radical elongation of *Raphanus sativus* L., *Lolium multiflorum* Lam., and *Sinapis arvensis* L. seeds. Moreover, this study will associate the evaluation of phytotoxicity with the activity of α -amylase that has an active role in the hydrolysis of the starch during seed germination and is involved in the growth regulation [3]. Several differences were found in the phytochemical profile of the *Eucalyptus* EOs investigated. 1,8 cineole was most abundant compound in all essential oil studied (ranging from 40.8% for *E. lesouefii* EO to 73.6% for *E. wandoo*) except for that of *E. pyriformis* where is present but at 15.1%. *E. pyriformis* and *E. lesouefii* EOs are the most active against both germination and radical elongation. These EOs showed phytotoxic activity against *S. arvensis* seeds: at lower concentration (125 μ g/ml), germinated only 3.4% of seeds treated with *E. pyriformis* EO and 6.9 % of seeds treated with *E. lesouefii* EO; and the radical elongation decreased at 5.2% with *E. pyriformis* EO and at 9.4% *E. lesouefii* EO at the same concentration. Evaluation of results of anti- α -amylase activity are ongoing.

Key Words: Eucalyptus, essential oil, phytotoxic activity, anti α - amylase activity

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ORAL PRESENTATION

GERMACRANOLIDE-TYPE SESQUITERPENOIDS WITH
PLASMODIUM TRANSMISSION BLOCKING ACTIVITY

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Abstract

Malaria is an infectious disease that represents one of the most concerning human health problems, particularly in sub-Saharan Africa, where the infection is spreading and leads to severe effects in terms of morbidity and mortality. Currently, almost 100 countries are endemic for malaria and the urgent need to develop a valuable vaccine, drug resistance, combined with the occurrence of insecticide-resistant vectors, could deteriorate the already dramatic situation.¹ Nowadays, the main drugs used in the treatment of malaria are represented by a few classes of compounds, such as artemisinin analogues, quinolones, and antifolates, and thus new highly efficacious antimalarial remedies are urgently required. In this regard, the screening of secondary metabolites from natural sources has already shown to be an effective approach.²

The genus *Daucus*, belonging to the Apiaceae family, includes almost 90 accepted species, widespread mainly in Europe, West Asia and North Africa. Among the 90 species of *Daucus* genus, eleven species and seven subspecies are distributed in Tunisia, and they have been used for centuries in traditional Tunisian medicine to treat cutaneous infections and for their diuretic properties.³ Phytochemical investigation of different *Daucus* species led to the isolation of several new germacranolide-type sesquiterpenoids, whose stereostructure has been determined by using MS, NMR spectroscopy, and chemical derivatization. These metabolites were investigated *in vitro* for inhibition of *Plasmodium* Early Sporogonic Stages (ESS) development, the non-pathogenic *Plasmodium* stages responsible for the transmission of malaria. Due to a lack of compounds active against this stage of the parasite's life cycle, the screening of natural products represents a useful tool to find new antimalarials with transmission-blocking activity.

Key Words: Antimalarial activity, *Daucus* species, Germacranolide-type sesquiterpenoids, Transmission-blocking.

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ORAL PRESENTATION

REGULATIONS ON MEDICINAL AND AROMATIC PLANTS: FROM
RAW MATERIAL TO FINAL PRODUCTS

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Abstract

The interest and demand for the use of Medicinal and Aromatic Plants (MAPs) and the natural products obtained from them has increased rapidly in recent years. The number of MAPs used in many industries such as food, health, pharmacy, cosmetics, textile etc. has gradually increased nowadays. Although, most of these plants that are used as raw materials in related industries, are collected from nature, some of them are insufficient in terms of quality and demanded in quantity. Most of the MAPs grown in the world and in our country are used as spice and herbal tea, as well as wild fruits and vegetables are valuable food sources and they are among the MAPs grown in agricultural lands in recent years. Good agricultural practices are the basic rule of MAPs cultivation, while monitoring, protection-use-development strategies are followed in wild harvests in terms of protection of plant biological diversity and supply of quality raw materials. In this direction, “Good Collection Practices (GCP)” and “Good Agricultural Practices (GAP)” published by the World Health Organization (WHO) are applied especially by developed countries and other countries are expected to adopt these standards. While the yield and quality of secondary metabolites is the main point for MAPs collected from nature, scientific studies have shown that post-harvest processing plays a very important role in the production of medicinal and aromatic plants. In recent years, remarkable scientific studies have been carried out on post-harvest processing, storage, processing, packaging and marketing. The quality criteria and standards of MAPs in almost all the industries are constantly regulated by well-known published institutions. When these issues are regulated and monitored by the FDA (Food and Drug Administration) in the USA, the responsible authorities in the European Union countries are EMA (European Medicines Agency), EFSA (European Food Safety Authority) and THIE (Tea & Drug Administration Herbal Infusions Europe). As for in Turkey, the Ministry of Agriculture and Forestry is responsible for the wild collection, agricultural production, processing and marketing of MAPs. Both the Ministry of Agriculture-Forestry and Ministry of Health manage regulations and public health of these plants. Moreover, studies on the licensing and inspection of herbal medicines and cosmetic products are carried out by the units of affiliated to the Ministry of Health in Türkiye.

Keywords: MAPs, law and legal issues, GCP, GAP, natural products, herbal medicine



ORAL PRESENTATION

THE PERFORMANCE AND SAFETY OF BIOAPIFIT® ANTI HEMORRHOIDAL OINTMENT IN PREMARKET/POSTMARKET STUDY IN THE TREATMENT OF HEMORRHOIDAL DISEASE

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Abstract

Objective / Purpose: Hemorrhoids are a very common anorectal condition defined as the symptomatic enlargement and distal displacement of the normal anal cushions. The objective of this study was assessment of the efficacy and safety of Bioapifit® anti hemorrhoidal ointment consisted of honey, *Cera flava*, glycerin, the oil macerates of *Achillea millefolium* L., *Plantago major* L., *Quercus robur* L., *Salvia officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Calendula officinalis* L., *Matricaria chamomilla* L., essential oils of *Melaleuca alternifolia*, *Thymus vulgaris* ct. Thymol and *Origanum vulgare* for the treatment of hemorrhoids of grade 1 to 3.

Materials and methods: 40 participants for premarket and 35 per year for post market investigation were recruited. Bioapifit® ointment was 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: The external and rectal application of Bioapifit® ointment resulted in significant decrease of all the symptoms of haemorrhoidal disease both in premarket and post market studies at third day of the treatment ranging from 58% in premarket application to 61% in post market study. In the end of the treatment overall subjective discomfort decreased for 96% in premarket and up to 96.4% in post market investigation. There was no significant difference in the product efficacy between premarket and post market investigation. Clinical cure rate was observed in 85% of the patients both in premarket and post market investigation. None of the patients experienced any discomfort or adverse effect including allergic reaction, worsening of the existing or the occurrence of new symptoms during the treatment.

Conclusion / Discussion: Physical parameters like low pH, high osmolarity/low water activity, high viscosity, greasiness, coating and lubricating effect resulted in significant decrease of the symptoms of hamorrhoidal disease such as bleeding, itching, irritation, and pain as well as wound infection.

Keywords: hemorrhoidal disease, honeybee's products, herbal macerate



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POSTER PRESENTATIONS



POSTER PRESENTATION

POTENTIAL OF THAI HERBAL FORMULA, NCP, ON DEPRESSION
TREATMENT INDUCED BY UCMS MICE MODEL AND POSSIBLE
MECHANISMS

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Abstract

Depression development as a result of prolonged stress near to urban culture is a current health issue. Many researches demonstrate that herbal medications and herbal formulae have neuroenhancement benefits. The NCP formula is made up of three different therapeutic herbs: *Nelumbo nucifera*, *Centella asiatica*, and *Piper nigrum*. The current work aims to assess the antidepressant-like action of the NCP formula and examine probable causes using a mouse model of depression generated by chronic mild stress (CMS). NCP formula administration to CMS mice greatly improved all, anhedonia, and hopeless behavior. NCP treatment effectively restored hypothalamic-pituitary-adrenal (HPA) axis hyperactivity in CMS mice by lowering blood corticosterone (CORT) levels, downregulating serum- and glucocorticoid-inducible kinase 1 (SGK1), and upregulating glucocorticoid receptor (GR) mRNA expression in the frontal cortex and hippocampus of CMS mice. Furthermore, the NCP formula increased neurogenesis in both brain areas by raising cyclic AMP-responsive element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) mRNA expression. The screening of monoamine oxidase (MAO) activity revealed that extracts of each of the NCP herbal components reduced the activity of both MAO-A and MAO-B. Piperine, ferulic acid, two terpenoids (madecassoside and asiaticoside), and five flavonoids were the most abundant bioactive components found in NCP formula extract (quercetin, kaempferol, kaempferol-3-O-glucoside, rutin, and luteolin-7-O-glucoside). **Keywords:** NCP formula; Chronic mild stress; Depression; *Nelumbo nucifera*, *Centella asiatica*, *Piper nigrum*.

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POSTER PRESENTATION

ANTIOXIDANT ACTIVITY OF *ARBUTUS UNEDO* FRUIT

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Abstract

Arbutus unedo is an evergreen shrub that belongs to the Ericaceae family, lives in temperate climates. It is cultivated in the garden for its ornamental qualities, and for its edible fruits. It is a medicinal plant used in Algerian traditional medicine to treat various diseases such as diabetes and kidney problems. The objective of our work is focused on the evaluation of the antioxidant activity of the fruits of the *Arbutus unedo* by Ferric reducing antioxidant power or FRAP assay. The fruits were subjected to extraction by maceration in water-acetone (30/70) followed by liquid-liquid fractionation by n-butanol. The results obtained by the FRAP method show that the organic fraction has better activity compared to the crude extract and the aqueous fraction with EC₅₀ of 0.7206 mg / mL, 1.614 mg / mL, and 2.1755 mg / mL, respectively. In conclusion, the extracts of the fruits of the *Arbutus unedo* exert a significant antioxidant effect.

Key words: *Arbutus unedo*, antioxidant activity, FRAP, maceration.



POSTER PRESENTATION

ANTIFUNGAL ACTIVITY OF STRAWBERRY TREE
(*ARBUTUS UNEDO*) FRUIT

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Abstract

The *Arbutus unedo* is a shrub that grows in regions with a mild climate. It is cultivated in the garden for its ornamental qualities and for its edible fruits, also it is used in traditional medicine to treat several diseases such as urinary infections. The objective of this study is the evaluation of the antifungal activity of the extracts: water-acetone, n-butanol and aqueous obtained from the fruits of *Arbutus unedo*, belongs to the Ericaceae family; a medicinal plant of the traditional pharmacopoeia of Algeria.

During this study, we undertook several steps to investigate the fruits of "Lindj" from Algeria. After harvesting, the fruits were macerated in a water-acetone (30/70), followed by a liquid-liquid extraction with n-butanol. The antifungal activity was tested by the diffusion method (disc method and well method) which results in a zone of inhibition more or less important depending on the sensitivity of the yeasts studied: *Candida albicans* ATCC 10231 and *Candida albicans* IPP 444.

The results obtained showed that the *Candida albicans* ATCC 10231 strain has a variable sensitivity towards the two water-acetone and n-butanol extracts of *Arbutus unedo*, with inhibition zones of 12 and 16 mm, respectively, while, the aqueous fraction has no effect on the two fungal strains.

In conclusion, the results obtained in this study clearly show that this plant has an interesting antifungal activity.

Keywords: *Arbutus unedo*, lindj, antifungal activity, water-acetone, n-butanol.



POSTER PRESENTATION

THE EFFECT OF DIFFERENT NITROGEN DOSE APPLICATIONS ON
PRODUCTION AND QUALITY IN AŞOTU VARIETIES (*Coriandrum
sativum* var. *microcarpum* AND *Coriandrum sativum* var. *vulgare*) IN AMİK
PLAIN CONDITIONS

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This study was conducted on the research area of Telkaliş, Hatay Mustafa Kemal University, Agricultural Faculty (36°15'14.6"N; 36°30'13.1"E), in 2020 winter growing season to investigate the effect of five different nitrogen (0, 3, 6, 9 and 12 kg da) applications on the yield and yield components with essential oil rates and yields of coriander (*Coriandrum sativum* var. *vulgare* L).

At the end of the study, it was found that the nitrogen doses did not have a significant effect on all the features examined. The highest wet herba yield (827.74 kg / da) was obtained from N0, and the highest volatile oil yield (0.438) was obtained from N6 application.

There is *Coriandrum sativum* in research. Since plants cannot be obtained from the seeds of *microcarpum*, there is only *Coriandrum sativum. vulgare* variety were evaluated.

Key Words: Coriander, *Coriandrum sativum* L, Nitrogen, Essential oil, Yield

References

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POSTER PRESENTATION

***HELICHRYSUM SANGUINEUM* AND *H. PLICATUM* ESSENTIAL OIL
COMPOSITION**

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Abstract

Helichrysum Mill. genus from Asteracea family contains approximately 600 species that distributed Africa, Madagascar island, Mediterranean basin, middle Asia and India. The genus presented with 24 species in the flora of Türkiye. In the present study, *Helichrysum sanguineum* (L.) Kostel and *H. plicatum* DC. essential oils obtained by hydro distillation were analyzed with GC/MS. Total of 36 components were determined from *H. sanguineum* essential oil. The main componenets were caryophyllene (18.341%), neryl-acetate (12.754%), lobeline (10.219%), pyrazole-3-carboxylic acid (6.711%), 2-naphthalenemethanol (4.712%) and butanoic acid (4.362%). *H. plicatum* subsp. *plicatum* essential oil were resulted with 42 components. The main components of *H. plicatum* were neryl-acetate (21.278%), caryophyllene oxide (14.124%), α -pinene (11.890%) and β -selinene (6.277%). This study is the first report for *H. sanguineum* essential oil analysis. The results found very promising with desireable components. Further studies should be continuing for to investigate biological activities of the essential oils.

Key Words: neryl-acetate, gas chromatography, essential oil, caryophyllene

Acknowledgements

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POSTER PRESENTATION

OBTAINING THE SUM OF PROANTACYANIDES FROM GRAPE SEEDS AFTER FLUID EXTRACTION

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Abstract

In Uzbekistan, different varieties of red grapes are grown for the production of wine and juice. The waste from these industries (pomace) is disposed of unproductively, thus presenting a problem for producers and an environmental hazard. At the same time, pomace is a rich source for obtaining medicines and nutritional supplements.

Today, grape seed oil and extracts are in high demand on the world market and are used in the food, cosmetic and pharmaceutical industries. Previously, we proposed a method for obtaining oil from grape seeds by fluid extraction.

The purpose of this study was to obtain the amount of proanthocyanidins from grape seed meal after fluid extraction, as well as to create a technology for the complex processing of grape seeds to obtain oil and the amount of proanthocyanidins. As an object of study, we used the seeds of technical varieties of grapes, harvest 2021 at JSC "Parkent winery" of the Republic of Uzbekistan.

It is known from literary sources that proanthocyanidins are well extracted with 40% ethyl alcohol. With this in mind, the meal was extracted with 40% ethanol, then the extract was concentrated and the optimal mode for drying the extract obtained from the grape seed meal was determined.

The process of drying the extract from the meal was studied on such installations as the ShSV-45K vacuum-drying cabinet (Russia) and the Anhydro No. 2 nozzle-type spray dryer (Denmark).

The meal was extracted five times at room temperature with 40% ethanol with a hydromodulus of 1:12. The combined extracts were concentrated; the concentrate was divided into two equal parts. The first batch of the concentrate was thickened on a rotary evaporator to a thick mass, which was then dried in a vacuum oven for 10 hours at a temperature of 70–90°C and a vacuum of 0.6–0.8 kgf/cm². A dry extract with a moisture content of 4.2% was obtained.

The second portion of the test solution was dried in a spray dryer at a coolant temperature of 170°C at the inlet and 80°C at the outlet, and the air pressure for spraying the solution was 0.2 MPa. In this case, the time spent was 50 minutes. A dry extract with a moisture content of 3.8% was obtained. The extract dried in a vacuum-drying cabinet is a resinous mass, which was difficult to separate from the surface of the dryer and, when crushed, stuck to the mill knife. The extract dried in the spray dryer had a powdery appearance. Time consumption in a spray dryer is 10-12 times less than that in a drying cabinet. Therefore, a spray dryer was chosen for drying the extract of proanthocyanidins from grape seeds. As a result of studying the parameters that affect the drying process, the following mode was selected to ensure optimal operation of the dryer with a high yield of the finished product: the heat carrier temperature at the inlet is 160–170°C, the outlet is 75–80°C, the solution feed rate is 2.0 l/h · m³, dry residue of the dried solution 10–15%. The amount of proanthocyanidins in the extract was at least 65%.



POSTER PRESENTATION

PHYTOCHEMICAL INVESTIGATION OF *ARTEMISIA ALBIDA* WILLD.

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Abstract

Artemisia albida Willd is not only a medicinal, coloring, food, vitamin, essential oil plant, but also used in pharmacology, anatomy, pathology, immunology and disease prevention. *A. albida* Willd is potential sources of unique and new natural products and new chemical structures exhibiting diverse biological activities and leading to the development of safe and effective phytopreparations against common diseases in Kazakhstan and the Central Asian region [0, [1], [2]]. The latest work of our research group is intended for *A. albida* Willd. Qualitative and quantitative phytochemical content was also investigated, including the determination of 20 amino acids and organic acids in the medicinal plant, and nine macro- and microelements were identified [[3]].

The MeOH extract of *A. albida* Willd showed a high potential to inhibit α -glucosidase (55.8%), PTP1B (85.5%) and BNA (95.5%), the presence of these bioactive components may indicate that the plant has substances, antiviral nutraceuticals to fight diabetes, obesity and bacterial infections [[5]]. Phytochemical and pharmacological investigation of *Artemisia albida* Willd. that collected from the Tarbagatai Mountains of Kazakhstan are going continued. The main goal is to separate and identify biologically active compounds targeting diabetes and influenza.

Keywords: *Artemisia albida*, biological activity, phytochemistry, PTP1B, BNA, Kazakhstan.

Acknowledgment

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POSTER PRESENTATION

STUDYING THE CHEMICAL COMPOSITION AND BIOLOGICAL
ACTIVE CONSTITUENTS OF THE FERULA FOETIDA

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Abstract

The nature of Kazakhstan differentiates with its unique and endemic plants that have useful medicinal properties. One of the plants with valuable properties is *Ferula foetida*. It has several pharmacological activities like anti-flatulent, antibacterial, antiviral, antifungal, anti-ulcerogenic, antidiabetic, anti-hepatotoxic properties [1]. Indian researchers investigating anti-helminthic activity led to the result that extract of *Ferula foetida* with the concentration 100 mg/mL showed the highest activity and significantly higher than standard medicines [2].

The research work shows the results of quantitative analysis of aerial and underground parts of *Ferula foetida* that include the compositional substituents and phytoconstituents analysis of the main organic groups. From aerial part of *Ferula foetida* extractive substances – 10,35%, organic acids – 0,155%, polysaccharides – 1,7%, alkaloids – 1,56%, coumarins – 1,96%, saponins – 2,15% were identified. The underground part of *Ferula foetida* showed content of extractive substances – 22,69%, flavonoids – 0,237%, polysaccharides – 2,8%, alkaloids – 1,34% and tannins – 8,5%. Eleven macro and micro elements from the ash of plant were identified by atomic absorption spectrometry method. It showed that underground part ash contains more microelements like Fe (11,826 µg/ml), Mn (2,4748 µg/ml), Cu (0,9230 µg/ml) and macroelements as K (1401,45 µg/ml), Ca (560,640 µg/ml), Na (158,08 µg/ml). Comparatively, ash of the aerial part showed higher concentration of macroelements that includes K (371,350 µg/ml), Ca (590,410 µg/ml), Na (250,675 µg/ml), Mg (209,625 µg/ml) and also microelements such as Fe (3,0438 µg/ml) and Cu (0,7856 µg/ml). The quantitative analysis was conducted according to the methodology of the State Pharmacopoeia of the Kazakhstan Republic. This study of *Ferula foetida* plant are going continued in order to do in-depth research of the chemical composition and biological active substances of both parts of the *Ferula foetida*.

Keywords: *Ferula foetida*, phytoconstituents, biological active compounds, quantitative analysis, macro-micro elements.

Acknowledgment

The Ministry of Education and Science of the Republic of Kazakhstan (AP09259567) supported this work.

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POSTER PRESENTATION

PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF
CERATONIA SILIQUA L. PULP EXTRACTS

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Abstract

Our study focused on a plant native to the mediterranean region called *Ceratonia siliqua* L. (locally known as Kharoub) belonging to the *Fabaceae* family. It is both a fruit and forest tree widely used in traditional Algerian medicine for its several biological properties which are mainly due to its richness in secondary metabolites. The aim of this study is to explore the chemical composition and to evaluate the antioxidant activity of the acetone and ethanol extracts obtained from the carob pulp of *C. siliqua* L. The extracts were obtained by maceration with respective yields of 9.337% and 1.53% (w/w). Phytochemical screening revealed the presence of polyphenols, flavonoids, condensed tannins, quinones and terpenoids in both extracts. The colorimetric determination of total polyphenols by the Folin-Ciocalteu method showed levels of 73.55 ± 0.36 and $20.62 \pm 0.76 \mu\text{g GAE/mg}$ of ethanolic and acetone dry extract respectively, while the dosage of flavonoids on one hand and flavones and flavanols on another hand by the method of aluminum trichloride in the same extracts revealed respective contents 1.884 ± 0.266 and $4.75 \pm 0.063 \mu\text{g QE/mg DE}$ and of 0.486 ± 0.012 and $1.205 \pm 0.247 \mu\text{g QE/mg DE}$. The condensed tannins determined in the same extracts by the vanillin method showed contents of 134.38 ± 0.537 and $106.685 \pm 0.544 \mu\text{g CE/mg DE}$ respectively. Evaluation of the antiradical power by the DPPH test and that of the reduction of iron FRAP *in vitro* showed activities expressed in CI_{50} of $54.14 \mu\text{g/mL}$ and $\text{CE}_{50} = 612.75 \mu\text{g/mL}$ for the acetone extract and $193.38 \mu\text{g/mL}$ and $2495.55 \mu\text{g/mL}$ for the ethanolic extract. The phosphomolybdate test showed a total antioxidant capacity expressed in quercetin and ascorbic acid equivalent 14.44% and 64.66% for the acetone extract; whereas, the ethanolic extract showed activities of 7.20% and 32.22% respectively. These results lead us to conclude that the acetone extract from carob pulp is richer in active compounds with antioxidant potential and consequently confirms the use of this plant for its therapeutic virtues as a food additive (or complement).

Key words: *Ceratonia siliqua* L, maceration, pulp, ethanol extract, acetone extract, phenolic compounds, antioxidant activity.



POSTER PRESENTATION

**BIOLOGICAL ACTIVITY OF *CHAMEROPS HUMILIS* FRUIT FROM
TESSALA REGION**

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Abstract

Chamaerops humilis.L., commonly called Doum Palm is a species whose different components are widely used for the manufacture of different objects handicrafts (ropes, mats, cuffins) which are of social and economic use. In traditional Algerian medicine the fruit named “ha bel gaz” are used as an anti-inflammatory, antidiabetic, antispasmodic and as a treatment against gastrointestinal disorders and prostatic hypertrophy.

Qualitative evaluation of the secondary metabolites presents in the different extracts of saw palmetto fruits. obtained by maceration, showed the presence of polyphenols including flavonoids, anthocyanins and tins, cardiac glycosides, terpenoids, sterols and saponosides, while alkaloids, coumarins and anthraquinones were absent.

Quantitatively, the dosage of total polyphenols by adopting the method of Folin ciocalteu reveals the presence of a moderately large quantity of polyphenols and which was of the order of 15.47 mg Eq of gallic acid / g of dry matter.

The antioxidant power of the hydroalcoholic extract of this species was determined by the DPPH free radical scavenging method. The 50% effective concentration (EC50) obtained (1.09 mg/mL) was lower than that of ascorbic acid used as reference antioxidant (1.13 mg/mL).

The alcoholic extract of the integuments of the fruit of *Chamaerops humilis* showed a high content of polyphenols, which results in very interesting pharmacological activities, including mainly an antioxidant and anti-inflammatory activity to enhance traditional recipes in the management of pathologies. prostate by analyzing the chemical composition and biological properties of the fruits of *Chamaerops humilis* L.

The pathologies of the prostate complicate the life of the patient on many levels, which makes daily life difficult to live with, benign prostatic hypertrophy, dysuria or urinary urgency, all well-known pathologies. At the end of this study, we can say that the traditional use of the fruits of *Chamaerops humilis* is justified.



POSTER PRESENTATION

**LICHENS AS BIO-INDICATORS OF AIR POLLUTION IN THE CITY
OF SIDI BEL ABBÉS IN ALGERIA**

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Abstract

Lichens have a large ecological amplitude and a remarkable sensitivity to pollutants which makes them suitable for biomonitoring studies as they provide information on local, site-specific levels of air quality. In this work we evaluate the air quality by applying lichen index based on German environmental engineers in 4 zones (forest, rural, industrial and urban) spread across Sidi Bel Abbes city. Lichen diversity for the study sites, comprised twelve common species and the overall estimated air quality Index for the stations is 45.99 which places the town of Sidi Bel Abbes in “low” class pollution. The effect of chemical pollutants may be observed on the distribution and diversity of lichens, elevated levels of nitrogen are indicated by the presence of *Xanthoria parietina* and *Xanthoria polycarpa*, a widely distributed and nitrogen tolerant species while Sulfur deposition is indicated by the absence of *Parmelia sulcata* however, *Anaptychia ciliaris* is rare and only found in places where air quality scores fall in the “best” category. The present study demonstrates that biomonitoring using lichens can be a reliable and cost-effective method.

Key words: atmospheric pollution, lichens, Air Quality Index, Algeria



POSTER PRESENTATION

BIOLOGICAL AND PHYTOCHEMICAL EVALUATION OF
METHANOLIC EXTRACTS FROM PENNYROYAL AND LAVENDER
SPECIES OF THE PORTUGUESE FLORA

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Medicinal and aromatic plants represent a healthier alternative to synthetic phenolic compounds, with a positive impact on therapeutic, aromatic and dietetic or gastronomic purposes. The *in vitro* biological activities of the phenolic compounds are an important parameter to select the most promising extracts for use in industrial applications. Thus, this study aimed to evaluate the *in vitro* antioxidant, antibacterial and cytotoxicity properties of 17 pennyroyal (*Mentha pulegium* L.) and 18 lavender (*Lavandula pedunculata* L.) accessions from the Portuguese flora, conserved in the Portuguese Genebank, in order to select the accessions with the highest yield and high content of active ingredients for use in the different industrial sectors. The antioxidant activity was determined spectrophotometrically using the Folin-Ciocalteu, Ferric Reducing Antioxidant Power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging methods. To evaluate the antimicrobial activity of the extracts, a screening was made using a colorimetric assay in different fungi and Gram positive and negative bacterial strains. Moreover, the phenolic profile was assessed by HPLC-DAD-ESI/MS. The lavender extracts showed the highest total phenolic content (TPC), ranging from 79.45 to 242.89 mg GAE.100g⁻¹ (gallic acid equivalents/100g) and the highest antioxidant activity, ranging from 2.40 to 6.55 mmol.100 g⁻¹ (µM Fe⁺²/100g), obtained by the FRAP method, and for DPPH scavenging activity, the minimum inhibitory concentration (EC₅₀) ranged from 0.51 to 1.61 mg.mL⁻¹. In general, all the samples revealed antimicrobial activity in some of the tested strains, which can be related to their phenolic composition. The lavender accessions revealed a prevalence of rosmarinic acid, while pennyroyal accessions were wealthier in quercetin-3-*O*-rutinoside. The results showed that the methanolic extracts of *L. pedunculata* L, from the Portuguese flora, can be considered as good sources of natural antioxidants and the extracts of lavender and pennyroyal as sources of antimicrobial compounds that can be used for therapeutic, cosmetic or food purposes.

Keywords: Total phenolic compounds, antioxidant capacity, FRAP, DPPH, antibacterial activity, methanolic extracts.

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POSTER PRESENTATION

APPLICATION OF BOX-BEHNKEN DESIGN FOR OPTIMIZATION OF EXTRACTION YIELD AND THE CONTENT OF PHENOLICS AND FLAVONOIDS FROM THAI TRADITIONAL HERBAL FORMULA, TRI-KAYSORN-MAS

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Objective / Purpose: Tri-Kaysorn-Mas (TKM) is a Thai traditional herbal formula available in Thailand's National List of Essential Medicines (herbal medicines) for health tonic. The Box-Behnken design was intended for use in experiment design (DOE). It is a selective technique for investigating the significance of the various factors influencing a specific process, their relationship, and their impact on the results of this process [1-2]. The aim of this study is to apply the Box-Behnken design (BBD) with the response surface methodology (RSM) for optimization of extraction yield and the content of phenolic and flavonoids from Tri-Kaysorn-Mas formula.

Material and Methods: The Tri-Kaysorn-Mas formula contains three herbs in a 1:1:1 ratio: *Jatropha multifida* L bark, *Nelumbo nucifera* Gaertn. petal, and *Aegle marmelos* (L.) Corr. fruit. Seventeen laboratory experiments at three levels (-1, 0, +1) and three factors of BBD with RSM were used to evaluate the optimal conditions of the extraction process. The experimental design of this study was performed using Design-Expert software (Version 12, Stat-Ease Inc., Minneapolis, USA). Factor of this study were type of solvent, number of extraction time, and material-to-solvent ratio (g/m). For extraction process, seventeen experimental sets of TKM (5 g in 100 mL) were extracted along with each set of variable factors. After that, the extract solutions were filtered, evaporated (40°C) and freeze-dried for 24 hr. Percentage yield of crude extract was calculated for each set of experiment. For phytochemical analysis, total phenolics content in TKM formula was determined using the Folin-Ciocalteu method. Its result was expressed as mg GAE/g extract. Total flavonoids content was determined using the aluminum chloride colorimetric method and its result was expressed as mg QE/g extract.

Results: The optimization for the highest extraction yield was obtained using ethanol: water (60:40) for three times with material-to-solvent ratio (g/m) 1:6. The optimization for the highest of phenolics content was obtained using ethanol: water (70:30) for two times with material-to-solvent ratio (g/m) 1:6. The optimization for the highest of flavonoids content was obtained using 95% ethanol for one time with material-to-solvent ratio (g/m) 1:6.

Conclusion: BBD was used to investigate and optimize process parameters (type of solvent, number of extraction times, and material-to-solvent ratio). The findings revealed that all of the process variables had a significant impact on the responses. As a result, the extraction process should be concerned with various extraction conditions in order to obtain the highest content of the active compound.

Keywords: Tri-Kaysorn-Mas, Box-Behnken design, Extraction, Phenolics, Flavonoids

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POSTER PRESENTATION

PHYTOCHEMICAL STUDY AND *in vitro* ANTICHOLINESTERASIC ACTIVITY OF EXTRACTS OF *DIOCLEA virgata* (RICH.) AMSHOFF

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Abstract

Dioclea genre has about 50 plant species distributed in tropical areas, most of them found in Central and South America, especially in the Amazon¹. Species of this genus are used in popular medicine to treat kidney and prostate diseases. Bioactive substances have been isolated from species of this genus, presenting biological activities such as: antibacterial and anticholinesterase², analgesic³ and *in vitro* antioxidant⁴. This work describes the phytochemical study of the aerial parts of *Dioclea Virgata* (Rich.) Amshoff and the evaluation of *in vitro* anticholinesterase activity of its extracts. *D. Virgata*'s leaves and stems were collected and its botanical identification performed by the botanist Dr. Luciano Paganucci de Queiroz. The plant material was dried, crushed and the extraction of metabolites was performed by cold maceration with methanol. After partitioning the crude extracts with solvents of different polarities (hexan, chloroform and ethyl acetate), the extracts obtained were applied to a column chromatography using silica gel 60H as stationary phase and organic solvent in polarity gradient (hexane, chloroform, ethyl acetate and/or methanol) as moving phase. *In vitro* anticholinestean activity tests were performed with extracts, following the methodology described by Ellman⁵ (1961), with adaptations⁶. The results showed that the ethyl acetate extract of both leaves and stem presented the ability to inhibit the ACHE enzyme, with 68.4 and 65.8% inhibition, respectively, in the initial concentration of 1 mg/mL. These data suggest that there are substances present in *D. Virgata* extracts with bioactive potential, which makes it a promising source of drug candidates. Using classic chromatographic techniques it was possible to isolate and identify from the stem acetate extract the 3,4-dihydroxybenzoic and salicylic acids, as well as the flavonoids 3,7-dihydroxy-6-metoxiflavanone and 7,3',4'-trihydroxyflavone.

Key Words: *Dioclea virgata*, Phytochemistry, Anticholinesterase activity, Phenolic compounds.

Acknowledgements

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POSTER PRESENTATION

CHEMODIVERSITY OF THE ESSENTIAL OILS FROM *PIPER* SPECIES (PIPERACEAE): A CASE OF STUDY ON AN ISLAND IN THE BRAZILIAN COAST

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Abstract

This study aimed to investigate for the first time the chemodiversity pattern from the chemical composition of the essential oils (EOs) from leaves (dry and rainy seasons) of ten species and two varieties of *Piper* (*P. amalago* L.; *P. amplum* Kunth; *P. anisum* (Spreng.) Angely; *P. arboreum* Aubl. var. *arboreum*; *P. arboreum* var. *hirtelum* Yunck.; *P. diospyrifolium* Kunth; *P. divaricatum* G. Mey.; *P. gaudichaudianum* Kunth; *P. lepturum* (Kunth) C.DC. var. *lepturum*; *P. lepturum* var. *angustifolium* (C.DC.) Yunck.; *P. mollicomum* Kunth and *P. vicosanum* Yunck.) collected in Marambaia Island, Rio de Janeiro (Brazil). EOs were obtained by hydrodistillation of fresh leaves in a modified Clevenger-type apparatus. The analyzes were performed by Gas Chromatography (GC) coupled with Mass Spectrometry (MS) and by GC coupled with a Flame Ionization Detector (FID). Multivariate analyzes were performed to determine clusters according to the identified compounds and in accordance to taxonomy. Twenty monoterpenes and 96 sesquiterpenes were identified, demonstrating an important chemical diversity. The main identified compounds were Linalool, Guaiol, 1-Butyl-3,4-methylenedioxybenzene, iso-Leptospermonene, α -Eudesmol, *E*-Nerolidol, Germacrene D, β -Elemene, 7-*epi*- α -Selinene, 1,8-Cineol, α -Eudesmol, α -Copaene, *E*- γ -Bisabolene, Bicyclogermacrene and β -Bisabolol. *E*-Caryophyllene was registered as a possible chemical marker, since it was identified in all samples. The occurrence of arylpropanoids in the EO of *P. mollicomum* leaves is described for the first time. All previous records of the chemical composition from leaves of this species showed mono- and sesquiterpenes (Ramos et al., 2021; 2022). Multivariate analyzes showed the formation of two main clusters: one with *P. anisum* that biosynthesize metabolites of the shikimate pathway and other with those from acetate-mevalonate pathway (other species and varieties). This study presents, for the first time, the volatile chemical composition of congener species of Piperaceae on Marambaia Island, a fragment of Brazilian Atlantic Forest with unique sandy soil characteristics.

Key Words: terpenes, *E*-Caryophyllene, volatile composition, Atlantic Forest, Marambaia Island.

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POSTER PRESENTATION

ETHNOBOTANICAL SURVEY ON THE USES OF *CAPPARIS SPINOSA*
IN SOUTHEASTERN OF ALGERIA (BISKRA)

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Abstract

This document constitutes the first ethnobotanical study of the medicinal plant *Capparis spinosa* conducted in the Biskra region, the gateway to the Algerian desert, located in the southeastern part of Algeria. *Capparis spinosa* is an evergreen plant with large white flowers with a large number of stamens in a light purple color that adorns the mountains of the region and is widely used by the locals.

Alternative medicine or traditional medicine is still in demand today by people who cannot afford modern medicine, and trust traditional healers who administer remedies extracted from medicinal plants. This botanical ethnographic study aims to highlight the different uses of *Capparis spinosa* in the city of Biskra. During the full six months in 2021 (200 informants), a questionnaire was used with local residents, herbalists and medicinal herbs in the study area. This study helped in providing important information about the medicinal plant and collecting information regarding its various uses by the local people, and the information required in the informant questionnaire includes age, gender, origin, marital status, educational level.

The results of this study showed the most frequently used part Its :leaves 80% , fruits 40% , seeds 35%, and *Capparis spinosa* is mainly used for its therapeutic properties (80%), and most of the diseases that are specifically treated in this field include treatment of rough knees and analgesia of rheumatic joint pain (75%) against digestive diseases (30%). The majority of treatments were prepared by decoction (60%) and were generally taken orally as tisane (65%) and 30% as sprays. The cure rate was about 80% , capers are used for edible leaves (30%) as flavorings and fruits as pickles (40%). Research information about the plant used indicated its therapeutic benefits, and the phytochemical examination, which clarified that the plant is rich in Secondary metabolites such as alkaloids, tannins, sterols and saponins. The plant can serve as a research vector and source of a biologically active product for the formulation of new drugs

Keywords: *Capparis Spinosa*, Biskra, Ethnobotanical study, Traditional uses, therapeutic uses, phytochemical examination



POSTER PRESENTATION

EVALUATION OF DIACETYLCURCUMIN MANGANESE COMPLEX FOR THE RADICAL SCAVENGING ABILITY AND NEUROPROTECTIVE ACTIVITY ON ROTENONE INDUCED NEUROTOXICITY IN SH-SY5Y NEUROBLASTOMA CELLS

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Abstract

Introduction: Oxidative stress is the main toxic leading to neurodegenerative disorder, Parkinson's disease by damaging dopaminergic neuron. Superoxide dismutase is a first line of defense against oxidative stress. Diacetylcurcumin manganese complex (DAC-MnCpx) was synthesized to mimic superoxide dismutase in order to improve the efficacy for scavenging various free radicals. **Objectives:** This study aimed to investigate the neuroprotective effect and mechanism of diacetylcurcumin manganese complex against rotenone induced neurotoxicity. **Methods:** Superoxide radical, hydrogen peroxide, and hydroxyl radical scavenging activity of DAC-MnCpx were detected by PMS/NADH-NBT system, the reaction of 1,10-phenanthroline and ferrous ammonium sulphate, and deoxyribose degradation assay, respectively. The neuroprotective effect was investigated in SH-SY5Y neuroblastoma cells by pretreatment with diacetylcurcumin manganese complex for 4h at the optimal concentration following by 150 nM rotenone for 48h for induction of neuronal toxicity. Cell viability was assessed using MTT assay. **Results:** Diacetylcurcumin manganese complex effectively reduced the generation of superoxide radical in concentration dependent manner. It showed greater superoxide radical, hydrogen peroxide, and hydroxyl radical scavenging than diacetylcurcumin, its parent compound. Moreover, rotenone induced cell death in SH-SY5Y cells was significantly reduced by pretreatment with DAC-MnCpx, indicating the neuroprotective effects of DAC-MnCpx. **Conclusion:** DAC-MnCpx had the potent scavenging activities resulting in the neuroprotective effects against rotenone-induced neuronal cell death via oxidative stress pathway.

Key Words: Diacetylcurcumin manganese complex, Rotenone, Oxidative stress, Parkinson's disease, Neurodegenerative disorder

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POSTER PRESENTATION

SEPARATION OF GREEN PROPOLIS BIOMARKERS BY
COUNTERCURRENT CHROMATOGRAPHY

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Abstract

Propolis is a natural resinous mixture produced by honeybees with numerous biological activities, such as: antibacterial, antifungal, antioxidant, antiparasitic, among others¹. In the isolation of compounds from complex matrices it is essential to choose a process that is not expensive, time consuming, unreproducible and with sample losses during each step. Countercurrent chromatography (CCC) is a liquid-liquid partition technique that allows full recovery of the analyte due to stationary phase retention without the use of a solid support². For these reasons, this work proposes the isolation of biomarkers from green propolis from Rio de Janeiro by using the CCC technique. The selection of the appropriate solvent system for the fractionation of the hexane extract of green propolis (HEGP) was made by the shake-flask experiment where the sample (2mg of HEGP) was dissolved in hexane-ethanol-water solvent system in several different ratios. Based on visual estimation of the partition coefficient (*K*) of target compounds by thin layer chromatography (upon spotting equal volumes of upper and lower phases of the biphasic liquid system), hexane-ethanol-water in the ratios 4:3:1 (v/v) was chosen for the fractionation of 450mg of HEGP. The sample was dissolved in 5mL of both upper and lower phases of the liquid system and injected in a P.C. Inc equipment equipped with a 70mL column, mobile phase at a flow rate of 2mL/min, rotation speed at 890rpm. Two isocratic fractionations were performed, one in normal elution mode and the other in reversed phase elution mode. Collected fractions were analyzed by HPLC/DAD and GC/MS. The CCC fractionation of HEGP using the normal elution mode afforded the biomarkers baccharin (26.2mg), artepelin C (73.6mg) and chromene (5.7mg) with purities (by HPLC/DAD) of 89%, 72% and 84%, respectively. The fractionation in the reversed phase elution mode, instead, resulted in a better purification of the chromene, whereas baccharin and artepelin C eluted in overlapped peaks. This work highlights the importance of choosing both the adequate solvent system and elution mode in a CCC separation. By using this technique, semi-purified green propolis biomarkers could be obtained at a low operating cost with excellent reproducibility.

Key Words: green propolis biomarkers, countercurrent chromatography, biphasic solvent systems.

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POSTER PRESENTATION

BENEFICAL EFFECTS OF *ALLIUM ROSEUM* L. ON SOME METABOLIC PARAMETERS STATUS IN NORMAL AND STREPTOZOTOCIN-DIABETIC RATS AND POSSIBLE REGENERATION OF THE LIVER AND KIDNEY DAMAGES

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Abstract

Diabetes affects a large segment of the population worldwide, and the prevalence of this disease is rapidly increasing. Despite the availability of medication for diabetes, traditional remedies are desirable and are currently being investigated. The objective of this study is to investigate the beneficial effects of *A. roseum* (fresh and dried) in normal and streptozotocin diabetic rats. The parameters followed were the body weight gains or loss, daily food intake, organs weight, plasma glucose, some metabolic parameters (urinary protein, creatinine, total cholesterol, HDL cholesterol and triglyceride) and kidney and liver morphology, anatomy and architecture. Diabetes was induced by a single dose of STZ (65 mg/kg, b.w.) given intraperitoneally. Ninety (90) albino rats were divided into seven groups normal: control and treated (2.5%, 5%, and 10% *A. roseum*: fresh and dried, respectively) and seven groups diabetic: control and treated (2.5%, 5%, and 10% *A. roseum*: fresh and dried, respectively) and a groupe received drug for diabetes (Glibenclamide). After 4 weeks, the low doses of *A. roseum* increased significantly the daily food intake of the normal groups; however, they significantly decreased the weight gains mainly for the highest doses. The slimming and appetizing effect of *A. roseum* was confirmed by the significant negative correlation between weight loss and the daily food intake, which is -0.27 in normal rats and -0.69 in diabetic rats. Compared to control diabetic rats, *A. roseum*-treated rats had less serum glucose, lower serum cholesterol levels and lower urinary protein. In addition, triglyceride levels in *A. roseum*-treated diabetic animals were more than 50% lower compared to the diabetic controls. A significant regeneration of liver and kidney damages was recorded in treated diabetic rats compared to controls. Therefore *A. roseum* might be of value in diabetes treatment.

Key Words: *A. roseum*, STZ-diabetes, diabetes, hypolipidaemic activity, Body weights, kidney; Liver



POSTER PRESENTATION

**THE NEUROTHERAPY EFFECT OF HONEY-SIDR FROM THE
ALGERIAN SAHARA ON THE NEUROPATHOLOGY OF
ALZHEIMER'S DISEASE STUDY IN MICE**

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Abstract

The objective of our studies is based on the determination of the effect of honey-sidr from the Algerian Sahara on the neuropathology of Alzheimer's disease which is characterized by the progressive accumulation of senile plaques and neurofibrillar degeneration.

Experimental animal models make it possible to demonstrate the neuropathology effects of honey on this disease, female mice were given by the oral route of Alcl3 at 100mg/kg/d, and D-galactose intraperitoneally at 120mg / kg / day for 45 days, after this period we proceeded to treatment with three doses of honey (100mg/kg/d, 200mg/kg/d and 300mg/kg/d) what were administrated by oral route for 45 days.

Histological results show changes in the hippocampus of mice, cortical atrophy, cytoplasmic vacuolation in neurons, thus the presence of damaged neurons and a decrease in cell density, these alterations are caused by the strong absorption of Aluminum and which characterize Alzheimer's disease.

The marked alterations in mice treated with honey-Sidr are less important and almost absent compared to mice caused by this disease, this affirms the anti-Alzheimer effect of Honey-Sidr from the Algerian Sahara on the brain.

Keywords : Alzheimer, Honey, Neurotherapy, Mice.



POSTER PRESENTATION

ANTIOXIDANT, ANTIDIABETIC AND ANTICHOLINESTERASE
ACTIVITIES OF HEXANE EXTRACT OF *GENISTA CARINALIS*

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Abstract

Diabetes mellitus has become one of the main threats to human health and an increasing public health issue [1]. As a result of this situation, effective anti-diabetic agents with negligible side effects are necessary. In this context, studies on the search for effective and safe inhibitors to replace synthetic inhibitors of natural products, especially plant-based secondary metabolites, have gained momentum [2]. *Genista* species are used to treat diseases. Hyperglycemic [3] and vasoconstrictor [4] effects of extracts of *Genista* plants were determined.

Genista carinalis was collected in the Trakya region of Turkey. The collected and dried plants were divided into small portions and extracted in methanol. After the methanol was evaporated in the evaporator, a small quantity of water was added to the crude extract obtained and extracted with n-hexane, chloroform, ethyl acetate and n-butanol in order of polarity. Then the solvents were evaporated in the evaporator, and crude extracts were obtained. Anticholinesterase activity [5], antioxidant activity [6] and α -glucosidase/ α -amylase inhibitory activities [7] of chloroform extract of *Genista carinalis* were determined.

This study is the first in the literature to report the anticholinesterase, antioxidant and antidiabetic activities of hexane *G. carinalis* extract.

Key Words: *Genista*, *G. carinalis*, anticholinesterase activity, antioxidant activity, α -amylase, α -glucosidase.

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POSTER PRESENTATION

ANTICHOLINESTERASE, ANTIDIABETIC AND ANTIOXIDANT
ACTIVITIES OF CHLOROFORM EXTRACT OF *GENISTA CARINALIS*

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Abstract

Alzheimer's disease, a global health problem, is a chronic disease associated with the loss of consciousness and is considered the most common type of dementia. According to the World Health Organization report in September 2020, 50 million people are struggling with this disease and 10 million new people are added to this number every year. It should be 82 million by 2030 and 152 million by 2050 [1]. *Genista* is an important genus of the Fabaceae (Leguminosae), with about 100 species distributed mainly in the Mediterranean and West Asia [2]. Many *Genista* species show interesting biological properties such as antioxidant, hypoglycemia [3], anti-inflammatory, antiulcer [4] and cytotoxic activity against different human cancer cell lines [5]. *Genista carinalis* was collected in the Trakya region of Turkey. The collected and dried plants were divided into small portions and extracted in methanol. After the methanol was evaporated in the evaporator, a small quantity of water was added to the crude extract obtained and extracted with n-hexane, chloroform, ethyl acetate and n-butanol in order of polarity. Then the solvents were evaporated in the evaporator, and crude extracts were obtained. Anticholinesterase activity [6], antioxidant activity [7] and inhibition activities of α -glucosidase/ α -amylase [8] were determined in the chloroform extract obtained from *Genista carinalis*. This study has reported anticholinesterase, antioxidant and antidiabetic activities of *G. carinalis* chloroform extract for the first time.

Key Words: *Genista*, *G. carinalis*, anticholinesterase activity, antioxidant activity, α -amylase, α -glucosidase.

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POSTER PRESENTATION

STUDY OF THE GEOGRAPHICAL DISTRIBUTION OF *ZIZYPHUS LOTUS* L IN THE REGION OF GHARDAÏA (SOUTH OF ALGERIA)

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Abstract

Our work is devoted to the study of the geographical distribution of *Zizyphus lotus* L in the different regions of the wilaya of Ghardaïa (case of Zelfana and case of the commune Metlili). There were 35 subjects of *zizyphus lotus* L in our study area (17 subjects of *zizyphus lotus* in Metlili and 18 subjects of *Zizyphus lotus* in Zelfana). The number of species inventoried is 15 species, divided into 13 botanical families, the most representative of which is that of Asteraceae. The biological spectrum of these species reveals the dominance of Hemicryptophyts (40%) and therophyts (26.66%) and chamephyts (20%), and on the physiognomic level, the existing formations are bushy with a dominance of the herbaceous layer, biogeographically, the Saharan element is predominant. Data processing by calculating the Similarity index (Is) equal to 0.4 and the disturbance index equal to 46.66%.

Key words: *Zizyphus lotus* L, Metlili, Zelfana, Hemicryptophyts, Similarity index, disturbance index.



POSTER PRESENTATION

EVALUATION OF THE POLLEN TYPES PRESENTS IN
GEOPROPOLIS PRODUCED BY *Melipona scutellaris* COLLECTED IN
COSTA DO SAUIPE-BA (BRAZIL)

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Abstract

Palinology is one of the tools used in the botanical identification of species responsible for the availability of plant resins that make up geopropolis (propolis produced by *Melipona scutellaris* bee results of mixture of vegetable resins, wax and soil), and botanical identification is one of the steps that assists in the standardization of propolis and geopropolis extracts¹. The chemical profile of the samples is dependent of the plant species that are close to the hives, and seasonality has a direct influence on the chemical composition of the resins collected by bees². In this sense, the objective of this work was to identify the polynic types in geopropolis samples collected in the Meliponário Costa do Sauípe, located in the city Mata de São João-BA (Brazil). Geopropolis was collected in the interval of 7 months to include dry and rainy season (January to July). The samples were processed using the method described by De Erdtman (1960)³. The microscope slides obtained were compared with polynic atlases for botanical identification of the polynic types. As results, 33 polynic types were identified, which are distributed in 21 botanical families. At the family level, Fabaceae was highlighted by the presence of five pollen types, among which *Mimosa caesalpiniiifolia* was very frequent in all samples, demonstrating the visitation pattern of the *Melipona scutellaris* bee in the aforementioned plant species. In addition, the study shows the diversity of plant species visited by *M. Scutellaris*, suggesting the importance of meliponiculture in the conservation of the Atlantic Forest, through the pollination of plant species in this biome.

Key Words: Pollen, Atlantic Forest, Conservation, Standardization.

Acknowledgements

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POSTER PRESENTATION

EVALUATION OF THE PRESENCE OF POTENTIALLY TOXIC
ELEMENTS IN GEOPROPOLIS COLLECTED AT BAHIA'S
RECONCAVO, BRAZIL

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Abstract

The propolis produced by *Melipona scutellaris* is called geopropolis, as it is the result of mixture of vegetable resins, wax and soil¹. Its main aspects are granules of heterogeneous consistency and odorless, dark brown color and bitter taste². The objective of this work was to evaluate the presence of potentially toxic elements in extracts of geopropolis produced by *M. scutellaris* at Bahia's Reconcavo, Brazil. The elements aluminum (Al), boron (B), barium (Ba), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), silicon (Si), strontium (Sr), vanadium (V) and zinc (Zn) were quantified by microwave-induced plasma optical emission spectrometry (MIP-OES). Approximately 0.25 g of the extract was subjected to assisted digestion directly in a closed microwave container, applying 30% H₂O₂ + 7.0 mL of HNO₃ to the system. The geopropolis samples showed no potentially toxic elements. This result is important because contamination by heavy metals, such as cadmium and lead, makes propolis and geopropolis samples unsuitable for human consumption, when at concentrations above those recommended. The results obtained for the *M. scutellaris* geopropolis collected in Cruz das Almas-BA showed it had better quality due to the absence of contaminants such as Al, Ba, Cd, Co, Cr, Cu, Fe, K, Ni, Pb, Sr, V and Zn. Therefore, the absence of potentially toxic elements may be indicative of a good quality of the material. Brazilian propolis is sought worldwide for its chemical and therapeutic characteristics in addition to its quality, being a product of economic importance for the country.

Key Words: *Melipona scutellaris*; lead metal; Contaminants; Therapeutic safety

Acknowledgements

We are grateful for the financial support from the Fundação de Amparo à Pesquisa da Bahia (FASPEB), Higher Education Personnel Improvement Coordination (CAPES) and National Council for Scientific and Technological Development (CNPq).

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POSTER PRESENTATION

THE IMPACT OF *INULA HELENIUM RADIX* AQUEOUS EXTRACT ON WISTAR RATS' DIGESTIVE SYSTEM: A PILOT STUDY

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Abstract

Maintaining the normal functioning of mucin-secreting cells is essential for the health of gastrointestinal tract mucosa, reduction in the number or secretions causing alteration of mucus barrier, followed by morphological lesions of digestive tract mucosa [1]. One of the medicinal plants known for its beneficial effects on digestive system is *Inula helenium* L., a plant widely used in the traditional herbal medicine throughout Europe and Asia. In this context, the aim of our study was to evaluate the possible modifications of the digestive system structure and functions induced by long-term oral administration of aqueous extract of *I. helenium* roots. During 8-week study, groups of 8 rats (n = 4/sex) received normal saline solution (control), 300 and 600 mg/kg of aqueous extract of *I. helenium*. Body weight and feed consumption were recorded daily. At day 29 an interim sacrifice was performed (n = 4/group). The remaining animals continued receiving treatment until the day 57, when the final sacrifice was made. Blood analysis, coproculture, necropsy examination, relative organ weight, histology (Tricrom Goldner coloration) and histochemical (PAS - alcian blue coloration) analysis of digestive system organs were performed on both interim and final sacrifice, for all the animals included in the study. Resulting histological and histochemical findings were consistent and confirmed that the direct contact between the extract and mucosa of the digestive tract did not cause any irritation, inflammation or functional alteration.

Key Words: *Inula helenium* L., aqueous extract; Wistar rats; digestive system, mucin-producing cells, toxicity study.

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POSTER PRESENTATION

STANDARDIZATION OF THAI TRADITIONAL KRATOM PRODUCT

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Abstract

Introduction: The Ministry of Public Health of Thailand, Department of Thai Traditional and Complementary Medicine considered and announced the recipes containing kratom for treating diarrhea, pain, and opium substitute. **Objective:** This study selected one recipe, namely, YA-GAE-BID-NA-RON (YGBNR), and performed the multiherbal recipe standardization according to the Thai Herbal Pharmacopoeia (THP) [1]. **Methodology:** The recipe of YGBNR, composed of kratom (*Mitragyna speciosa*), ginger (*Zingiber officinale*), Java long pepper (*Piper retrofractum*), and chili (*Capsicum annum*), was compounded for 12 independent recipes. Each herb's qualities and formula were determined for loss on drying (or water content), total ash, acid-insoluble ash, water-soluble extractive value, and ethanol-soluble extractive value. The content of mitragynine was determined using HPLC. Total phenolic content, total tannin content, and total flavonoid content were determined using colorimetric methods. Antioxidative power was estimated by DPPH and FRAP assays. The TLC and HPLC fingerprints were established. **Results:** The YGBNR is designated to use fresh herbs. To simplify the formulation as a dry powder, we mimic the ratio based on the drying indices of each herb. According to the THP monograph, the quality of each herb (ginger, long pepper, chili) met the THP requirements. Although kratom has not yet been included in the THP so far, the values of kratom were acceptable. It can be noted that the quality of YGBNR was dependent upon the quality of kratom. The formula's antioxidant power is related to the amounts of total phenolic and tannin contents. Establishing TLC and HPLC could distinguish the fingerprints and discriminate the adulteration. **Conclusion:** This is the first report on compounding the YGBNR and standardization. The simple recipe was formulated easily. The YGBNR can be used as an alternative for antidiarrhea and flatulence.

Key Words: kratom; Thai traditional medicine; anti-diarrhea; YA-GAE-BID-NA-RON

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POSTER PRESENTATION

PHYTOCHEMICAL STUDY AND BIOLOGICAL ACTIVITIES OF ORGANIC EXTRACTS FLOWER HEADS, LEAVES, AND ROOTS OF RHAPONTICUM ACAULE L. (DC) FROM NORTHWESTERN ALGERIA

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Abstract

Rhaponticum acaule L. (DC) called “Tafgha” is a wild medicinal plant belonging to the flora of the region of Tlemcen. The chemical composition of its capitula is interesting, given the presence of polyunsaturated fatty acids with values between 20.2% linoleic acid and 52.7% linolenic acid. Proteins have an average percentage in capitula (11.37%) with a remarkable composition of essential amino acids. The highest content of total polyphenols (135.31mg EAG/g) was reported in leaf extracts and flavonoids (0.24mg CEQ/g) in capitulas extracts... The effect of capitula methanolic extract on cell proliferation of the two lines 4T1 and AMB-231 showed a reduction in breast cancer cell proliferation in a dose-dependent manner, with an IC50 ranging from 13.33µg/ml to 11.61µg/ml.

Keywords: Cell proliferation, polyphenols, polyunsaturated fatty acids, Rhaponticum acaule.



POSTER PRESENTATION

INHIBITORY EFFECT OF PRUNUS PERSICA L. EXTRACT ON
ALPHA-AMYLASE ACTIVITY

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Abstract

Today, medicinal plants are a crucial source for the development of novel therapeutic compounds that are highly effective against a wide range of illnesses. Especially for the treatment of diabetes, a condition that is widespread throughout the world. Contrary to natural herbs, which have minimal to no effects, it is treated with insulin and oral antidiabetics, both of which can have major side effects.

This research is a component of the evaluation of the alpha amylase activity-inhibiting properties of phenolic extracts from peach leaves, scientifically known as *Prunus persica L.* of the Rosaceae family. A tree with several medicinal benefits, it is commonly utilized in traditional medicine in Algeria. *Prunus persica L.* leaves were extracted under reflux in hexane and then in a methanol/acetone combination. The yield of phenolic extract is 29%.

The results of the phytochemical screening revealed the absence of saponins and coumarins as well as the presence of alkaloids, tannins, flavonoids, and terpenoids in varied concentrations. The Folin-Ciocalteu reagent was used to calculate the total polyphenol content, which was found to be 134.66 mg GAE/g DM. The flavonoids' concentration is 50.63 mg CE/g DM and they were assessed using the aluminum trichloride method (AlCl₃). The tannins' content, which was determined using the vanillin method, is 53.01 mg CE/g DM. Then studies based on the inhibition of alpha amylase using acarbose as a reference molecule were conducted. The findings indicate that leaf extracts have an inhibitory effect on alpha amylase, with IC₅₀ values of 1.71 mg/ml and 0.056 mg/ml for our extract and acarbose, respectively. This demonstrates the extracts of *Prunus persica L.* have an inhibitory impact on alpha-amylase.

Key Words: Alpha amylase, Phenolic compounds, *Prunus persica L.*, Under reflux.



POSTER PRESENTATION

BIOACTIVE CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF
SOME SELECTED CAMBODIAN EDIBLE FLOWERS

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Abstract

Cambodian edible flowers have been used for treatment and prevention of diseases for centuries and few studies have been conducted so far to prove its bioactive compounds and antioxidant properties. This study was conducted with an aim to quantify the bioactive constituents and screen the antioxidant activities of flower of *Clitoria ternatea* L., *Nymphaea nouchali* Burm.f., *Sesbania grandiflora* (L.) Pers. and *Eichhornia crassipes* (Mart.) Solms were collected and prepared as methanolic extracts. Total phenolic content was screened via Folin Ciocalteu's method and total anthocyanin content was determined by the pH differential method. Total alkaloid content and total flavonoid content were quantified using spectrophotometric method. Antioxidant activities were accessed via ferric reducing antioxidant power (FRAP) assay, ABTS, and DPPH radical scavenging assay. As a result, the highest percentage yield and alkaloid content was found in *Clitoria ternatea* L. (35.4 ± 0.9 mg atropine equivalent /g of crude extract (CE)). *Nymphaea nouchali* Burm.f. showed the highest phenolic and flavonoid content, strongest radical scavenging activities in DPPH assay (IC_{50} at 25.1 ± 5.8 μ g/ml) and in ABTS assay (153.1 ± 1.0 mM trolox equivalent/g CE), and the highest in FRAP value (0.04 ± 0.005 mM $FeSO_4$ /g CE) than other extracts. Noticeably, anthocyanin content was highest in *Eichhornia crassipes* (Mart.) Solms as 8.1 ± 1.4 mg cyanidin-3-glycoside/g CE. Our study found different contents of bioactive compounds as well as *in vitro* antioxidant effects in each sample. The flower of *Nymphaea nouchali* Burm.f. seems to have the highest bioactive capability among all flowers. Our study suggested that those species could serve as potential sources of bioactive substances with antioxidant properties. The results of this study could be a useful source for further development of food additives and/or supplements.

Keywords: Edible Flower, Antioxidant, DPPH Radical Scavenging Assay, ABTS Radical Scavenging Assay

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POSTER PRESENTATION

KRATOM ORAL SYRUP ATTENUATES OF ULCERATIVE COLITIS SYMPTOMS IN MICE

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Abstract

Introduction: *Mitragyna speciosa* (Korth.) Havil. [Rubiaceae] or kratom [Thai] is a unique source of indole alkaloids and oxindole alkaloids [1]. Thai traditional use of kratom extract was for relieving diarrhea. The prominent mitragynine in kratom possesses opioid agonist activity and reduces gastrointestinal tract movement [2]. **Aim of the study:** The present study aimed to formulate kratom-containing oral syrup and evaluate its effect on acetic acid-induced colitis mice. **Methodology:** Kratom leaves were extracted with ethanol by maceration. The extract was evaporated and dried with lyophilization to obtain the kratom dry powder. The mitragynine content was determined using HPLC. The formulation of oral syrup began with an investigation of co-solvents, which assist the solubility of kratom dry powder. Then, kratom syrup at 2 mg mitragynine equivalent per mL was composed of co-solvent, sweetener, preservative, and kratom extract. Mice induced with 5% v/v acetic acid were administered orally with kratom oral syrup as a single dose for five days. Each group (n = 6) was treated at the amount of mitragynine equivalent to 5, 10, and 20 mg/kg. Loperamide was used as a positive control drug. **Results and discussion:** The kratom oral syrup was formulated and appeared as a clear, viscous yellow liquid with sweet-bitter and sour. A dose of 10 and 20 mg/kg of mitragynine could reduce the DAI score, increase the colon length, and prolong the upper gut transit time. These results indicated that the 10 mg/kg and 20 mg/kg kratom syrup relieved colitis severity. In addition, kratom syrup has been shown to recover the impairment of paracellular permeability function.

Key Words: kratom (*Mitragyna speciosa*), co-solvent, oral syrup, ulcerative colitis mice, mitragynine

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POSTER PRESENTATION

VASELINE BASED ON THE OIL OF "Urticadioica L."

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Abstract

The product is a natural petroleum jelly made from nettle oil to fight eczema, as it is made up of a group of natural oils that are helpful in moisturizing the skin and fighting eczema, but the main ingredient is nettle oil due to its great benefits and components that effectively help fight eczema, the raw material is composed of: nettle oil, coconut oil, Olive Oil, Stoches Lavender Oil, Melted Beeswax, Oats and Vitamin E.

In the realization of our therapeutically and economically very interesting product "vaseline from nettle" and after overcoming difficulties during the preparation of the recipe and the determination of the raw materials that can be used, and also the determination necessary quantities, and avoid the presence of crumbs. We can offer recommendations represented in the following: Try to find and choose the useful plant in the treatment of specific diseases. First try to find the appropriate recipe for the manufacture of natural products and specify the necessary quantities, which must be well studied. Finally, Be careful when using essential oils, because when they exceed the required limits, they can be toxic or cause allergies or burns, and their effect will be counterproductive, one must be careful and serious in the use of these oils.

Key Words: Urticadioica L., Vaseline, Eczema, Skin application, vegetable oils.

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POSTER PRESENTATION

QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL COMPOSITIONS
AND ANTIOXIDANT ACTIVITY OF DIFFERENT PARTS OF
SOLANUM TORVUM SWARTZ

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Abstract

Solanum torvum Swartz (in the family of Solanaceae) has a long history in Khmer traditional remedies in the treatment of pain, cough, stomachache, and menopause. So far, there has not been any study conducted on this plant in Cambodia. Thus, this study aims to conduct quantitative analysis and screen antioxidant properties of different parts of *Solanum torvum* Swartz. Three different parts (leaves, flowers, and fruits) were collected and prepared as the methanolic extract to determine total phenolic content via Folin Ciocalteu's method and total anthocyanin content via the pH differential method. Flavonoid and alkaloid contents were analyzed via spectrophotometry. Antioxidant activities were screened with DPPH radical scavenging activities and ferric reducing antioxidant power (FRAP) assays. Results showed that the leaf extract exhibited the highest percentage yield (8.9 % per fresh weight), phenolic content (66.8 ± 2.2 mg gallic acid equivalent /g of crude extract (CE)), alkaloid content (0.3 ± 0.0 mg atropine equivalent / g CE), and FRAP value (0.1 ± 0.0 mM FeSO₄ / g CE). Interestingly, the flower extract showed the highest content of flavonoid (48.4 ± 3.0 mg quercetin equivalent (QE)/ g CE) as well as the strongest radical scavenging activities via DPPH assay (IC₅₀ value at 162.4 ± 8.6 µg/ml). The highest content of anthocyanin showed in the fruit part (8.0 ± 1.2 mg cyanidin-3-glycoside/g CE). It is indicated that each part of this plant comprised different types of compounds which attributed to different antioxidant activity. Our study found this plant possesses nutraceutical importance and could be studied for the development of functional foods or pharmaceuticals.

Keywords: *Solanum torvum* Swartz, Antioxidant Activities, Total anthocyanin content, Total alkaloid content, DPPH radical scavenging activities.

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POSTER PRESENTATION

**BIOACTIVE, ANTIOXIDANT, AND ANTIBACTERIAL PROPERTIES
OF *PSIDIUM GUAJAVA* L. LEAVES**

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Abstract

Psidium guajava L. has a long history in Khmer traditional medicine to treat various infectious diseases, especially diarrhea, fever, and inflammation. So far, there has not been any scientific evidence to prove its therapeutic effects yet in Cambodia. Thus, this study aims to quantify the phytochemical compounds, antioxidant, and antibacterial activities of these leaves. The leaves were harvested and prepared as methanolic extracts. Phenolic content was determined via Folin-Ciocalteu's methods. Total chlorophyll content, total flavonoid content, and total alkaloid content were determined using UV-spectrophotometry. The content was calculated in equivalent to the standard compounds. Antioxidant activities were screened via DPPH and ABTS radical scavenging activities assays and ferric reducing antioxidant power (FRAP) assay. Two different grams of bacteria were treated with the extract and incubated for 24 hours. As a result, phenolic content was found to be 90.3 ± 4.01 mg gallic acid equivalent /g of crude extract (CE). Flavonoid content was 5.0 ± 0.4 mg quercetin equivalent /g CE. Chlorophyll content was 0.02 ± 0.001 mg/g CE and alkaloid content was 0.7 ± 0.004 mg atropine equivalent /g CE. Radical scavenging activities via DPPH was showed the IC₅₀ value of 103.3 ± 13.0 µg/mL, while ABTS was 2.13 ± 0.24 mM trolox equivalent /g CE. Moreover, FRAP values was 0.07 ± 0.2 mM FeSO₄ /g CE. The diameter of the inhibition zone was higher in gram-positive *Staphylococcus aureus* (8.0 ± 0.8 mm) than in gram negative at 24 hours. Our study found the potential of antioxidant activities, and antibacterial activity of leaves extract from *Psidium guajava* L. The evidence would be useful for the potential development of new antibiotics.

Keywords: *Psidium guajava* L. leaf, Antioxidant Activities, Bioactive Properties.

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POSTER PRESENTATION

PROXIMATE ANALYSIS, PHYTOCHEMICAL CONSTITUENTS AND
ANTIOXIDANT ACTIVITY OF GREEN PEA SPROUTS

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Abstract

Many studies have been conducted on the bioactive compounds of green pea seeds, however, there is some limited information on the nutritional status and its pharmacological properties of its sprout. Hence, in this study, green pea (*Pisum Sativum* L.) sprout was cultivated between the age 4-5 days from a microgreen farm to screen its phytochemical compositions, antioxidant activities and nutrients. Young green pea sprouts were collected and prepared as methanolic extracts (1% HCl in methanol). Total phenolic content was determined via Folin-Ciocalteu's method. Total chlorophyll content and total flavonoid content were determined via UV-spectrophotometry. Radical scavenging activities were screened via DPPH and ABTS assay and ferric reducing antioxidant power was screened via FRAP assay. The fresh weight of 100 g was assessed for nutritional content via proximate analysis. Results showed that chlorophyll content was 0.003 ± 0.0 mg/g of crude extract (CE), phenolic content showed 60.1 ± 3.9 mg gallic acid equivalent/g CE, and flavonoid content was 11.1 ± 0.5 mg quercetin equivalent/g CE. Interestingly, radical scavenging antioxidant activities via DPPH showed inhibitory concentration (IC_{50}) at 715.3 ± 31.8 μ g/ml while ABTS value showed 0.05 ± 0.0 TE mM/g CE. Moreover, ferric reducing antioxidant power via FRAP assay showed 0.002 ± 0.0 mM/g CE. The fresh green pea sprouts consisted a total fat, carbohydrate and energy (496.8 ± 12.8 Kcal) but not protein. Our study showed the presences of bioactive compounds and its nutritional status indicated the potential of the green pea sprouts as a functional food. This information may be useful for further development of a natural health products.

Keywords: Green Pea Sprouts, Antioxidant, Chlorophyll, Proximate Analysis

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POSTER PRESENTATION

SUBCRITICAL CO₂ EXTRACTION OF *CAPPARIS SPINOSA* SEEDS

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Abstract

The plant *Capparis spinosa* is widespread in Turkey, Iran, Spain, Central Asia, including Uzbekistan. This plant can be used as a raw material for the production of biologically active substances. Dozens of tons of above-ground parts can be harvested annually without damage to the reserve habitats. Currently, buds and fruits of *C. spinosa* are exported from Uzbekistan to foreign countries as food products.

The diet of men should include capers, because the compounds in these fruits have a beneficial effect on muscle tissue, the musculoskeletal system and, most importantly, support the health of the cardiovascular system. And the seeds of *Capparis spinosa* are used for headaches.

Caper fruits contain about 18% proteins and 36% fats, in seeds - up to 30% oils. The fruits contain thioglycosides, steroidal saponins, rutin (up to 0.3%), ascorbic acid (up to 136 mg/%), dyes; in the roots - the glycoside capparidine and the alkaloid stachidrine. The fruits contain a fairly large amount of iodine (up to 27 mg /% in terms of dry weight).

The purpose of this work is to develop a technology for extracting oils from *C. spinosa* seeds using subcritical CO₂ extraction to isolate oils. Based on the goal, the main task is to study the influence on the extraction process of such factors as the degree of grinding of seeds, the choice of the state of the extractant, the pressure in the extraction process, the multiplicity and duration of extraction, which play a major role in obtaining a high oil yield.

The seeds of *C. spinosa* collected in September 2021 in the Tashkent region of the Republic of Uzbekistan were used as the object of study. The seeds of *C. spinosa* were extracted from the fruits, then they were dried in drying at a temperature of 40±2°C. Oil samples were obtained by subcritical CO₂ extraction using a Deyang Strong Tech laboratory extraction system. Ltd (China manufacturer).

The obtained research results show that under subcritical conditions, the extraction of *C. spinosa* seeds, crushed to a size of 0.1-0.2 mm, is advisable to carry out at a pressure of 65 bar for 80 minutes. In this case, the oil yield is 18.50% by weight of the seeds. The resulting oils contain mainly oleic, linoleic and linolenic which make up 25-50%, the remaining oils are palmitic and stearic. Oils are widely used in the food, cosmetic and pharmaceutical industries.



POSTER PRESENTATION

STUDY THE SYNTHESIS AND CHARACTERIZATION OF N-SUBSTITUTED QUINONE ANALOGUES

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Abstract

Quinones, including heterocyclic analogs, natural and synthetic compounds. The quinonic compounds have great importance due to their dye and pharmacological properties. Especially, it is displaying a wide range of biological activities, such as antitumor, antibacterial, antimalarial and antifungal activities. Since quinones are fast redox cycling molecules, they have the potential to bind to amine, thiol, and hydroxyl groups. They play an important role in electron transport processes and oxidative phosphorylation processes. Because of these features, they have always been the focus. Especially, the main focus of these compounds is based on their potential to produce tumor-selective toxicity. At the same time, 1,4-Naphthoquinones are many clinically important antitumor drugs. In this study, some N-substituted-1,4-naphthoquinone compounds were synthesized by the reactions of 2,3-dichloro-1,4-naphthoquinone with various amine nucleophiles. The purification of reaction mixture has been carried out by using chromatographic methods (with column chromatography). Elucidation of the new synthesized compounds has been determined by infrared spectroscopy (IR), nuclear magnetic resonance (¹H NMR, ¹³C NMR), and mass spectrometry (MS) analysis.

Key Words: Quinone; Bioorganic Compound; Amine; Spectroscopy; Organic Synthesis

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POSTER PRESENTATION

EVALUATION OF THE EFFICIENCY OF THYME ESSENTIAL OIL
ON IN VITRO ROOTING AND ACCLIMATIZATION OF
STRAWBERRY PLANTS: PRELIMINARY ASSAY

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Abstract

To improve the micropropagation protocol and the regeneration rate of strawberry (*Fragria* × *ananassa* Dutch) Camarosa viraty plants, the use of natural biostimulants was investigated. For this purpose, the effect of Thyme essential oil (EO) as well as microalgae mixture (Ma), used as reference, were studied. These treatments were tested at 10 and 100 ppm during *in vitro* rooting phase using nodal segments of the plant inoculated on MS medium. *In vitro* root development assessed after four weeks of incubation in the growth chamber exhibited a significant difference between treated and control plants. Results showed that supplementation of the medium with Thyme EO accelerated initiation of rhizogenesis. Indeed, while treated roots appear within 7 days, and completed formation at 4 weeks by giving rise to the highest number of roots (average of 20/explant) at both concentrations of EO, they only show up after 20 days in control media with only 10 roots/explant. For the aerial part of the plant, we noticed a significant effect on the height as well as on the number of formed shoots after application of Thyme EO and Ma at the highest concentration (100 ppm). Later, the impact of both treatments on the growth of *ex-vitro* strawberry seedlings was studied during the acclimatization phase. It revealed that at 10 ppm thyme EO significantly increased the chlorophyll content and photosystem II of seedlings. Besides, biochemical analysis confirmed these results as they revealed that plants treated with thyme EO at both concentrations reduced accumulation of phenolic compounds by almost 48%. and moderately the peroxidase activity compared to control ones. These results could be explained by the protective role of thyme EO against plant stress. More investigations on the effect and the mode of action of the EO as a biostimulant on strawberry micropropagation are currently being investigated.

Keywords: micropropagation, strawberry, Thyme Essential oil (EO), phenolic compounds, chlorophyll content, acclimatization.



POSTER PRESENTATION

TRI-KESORN-MAS REMEDY PALLIATE COGNITIVE BEHAVIOR THROUGH OXIDATIVE STRESS ON OVARIECTOMY MODEL

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Abstract

Generally, the predominance of patients suffering from Alzheimer's disease (AD) are women, and women have a higher risk of cognitive impairment than men. It may be related to the longer life span of women, and the sharp decline in estrogen during menopause may also contribute to this situation. Hormone replacement therapy has been demonstrated to protect menopausal women against cognitive impairment, which leads us to consider the relationship between estrogen and memory. Herbal medicines have become an interesting target remedy since there is evidence showing the multiple mechanisms of action. Thai remedy, Tri-Kesorn-Mas (TKM), compose of *Nelumbo nucifera* stamen, *Jatropha multifida* bark and *Aegle marmelos* fruit. Thai remedy, Tri-Kesorn-Mas (TKM), compose of *Nelumbo nucifera* stamen, *Jatropha multifida* bark and *Aegle marmelos* fruit. This remedy exhibited anti-acetylcholinesterase activity and anti-oxidation, but no evident data revealed the effect of TKM on estrogen deprivation conditions. To verify the palliative cognitive behavior of TKM extract on estrogen deprivation, an ovariectomy mouse model was used. Y-maze test, novel object recognition test (ORT), and Morris water maze test (MWM) were determined for cognitive behavior. Mice were divided into seven groups: sham, OVX, estradiol (E2), vitamin E, and TKM (50, 100, 400 mg/kg). The result showed that ovariectomy significantly impaired learning and memory compared to sham mice, while E2 and vitamin E enhanced memory deficits. TKM 100 and 400 mg/kg showed significantly improved learning and memory. Moreover, ovariectomized mice significantly increased lipid peroxidation compared to sham, while TKM 100 and 400 mg/kg, including positive control E2 and vitamin E significantly decreased lipid peroxidation in the hippocampus and frontal cortex. This finding revealed that ovariectomy caused cognitive impairment. Tri-Kesorn-Mas remedy can palliate cognitive behavior and may indicate the mechanism via oxidative stress. Thus, the TKM remedy is interesting and possible drug development for the amelioration of suffering from dementia in menopausal women. To confirm more clearly scientific evidence, further biochemical experiments and related gene expression are ongoing investigations.

Key Words: Tri-Kesorn-Mas, *Nelumbo nucifera*, *Jatropha multifida*, *Aegle marmelos*, Cognition, Ovariectomy

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POSTER PRESENTATION

ANTIBACTERIAL ACTIVITY, PHYTOCHEMICAL EVALUATION
AND ANTIOXIDANT ACTIVITIES OF *PHYLLANTHUS AMARUS*
ORIGINATED IN CAMBODIA

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Abstract

Phyllanthus amarus (in a family of Euphorbiaceae) has a long history in Khmer traditional medicine for hepatoprotective, gastroprotective, treating ulcers, urinary tract stone, antimicrobial, antimalarial, and hepatitis. But so far, there is no scientific evidence on antibacterial activities, phytochemical constituents, and antioxidant property of this plant in Cambodia yet. This study aims to investigate the phytochemical compounds, antioxidant activities, and antibacterial activities of this plant originated in Cambodia. The leaf part of this plant was collected and prepared as methanolic extract. Total phenolic content was determined via Folin-Ciocalteu's method. Total chlorophyll content, total flavonoid content, and total alkaloid content were determined by spectrophotometric method. The content was calculated in equivalent to the standard compound. Radical scavenging activities were screened via DPPH and ABTS assays. Reducing antioxidant power was screened via ferric reducing antioxidant power (FRAP) assay. Two different grams of bacteria were treated with the extract and incubated for 24 hours. As the results, phenolic content was 768.4 ± 38.8 mg gallic acid equivalent/g of crude extract (CE), flavonoid content was 73.7 ± 7.0 mg quercetin equivalent /g CE, chlorophyll content was 0.02 ± 0.002 mg/g CE, and alkaloid content was 7.9 ± 0.09 mg atropine equivalent/g CE. Radical Scavenging activities via DPPH was showed the IC₅₀ value of 67.4 ± 2.7 µg/ml, while ABTS showed 1.1 ± 0.02 mM trolox equivalent/g CE. Moreover, FRAP values showed 2.1 ± 0.1 mM/g CE. The diameter of the inhibition zone was shown to be higher in gram-positive *Staphylococcus aureus* (11.0 ± 0.0 mm) than in gram-negative bacteria in 24 hours. Our study found the amounts of phytochemical constituents, antioxidant activities, and antibacterial activity of the methanolic extract of *Phyllanthus amarus*. The evidence is promising for further isolation, characterization, and synergistic evaluation of the phytochemical constituents of this plant. However, many studies are required to discover a new class of antimicrobial agents that can be used to prevent antimicrobial resistance.

Keywords: *Phyllanthus amarus*, Antioxidant Activities, Antibacterial activity

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POSTER PRESENTATION

CHEMICAL PROFILE AND BIOLOGICAL ACTIVITIES OF
FRANKENIA HIRSUTA L.

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Abstract

Frankenia L. genus, which belongs to Frankeniaceae family, consists of salt tolerant aromatic plants. *F. hirsuta* L., which is one of the three *Frankenia* species, is widely distributed in Turkey. However, research on this halophyte plant is very limited. In this study, hydrodistilled essential oil obtained from the aerial parts of the plant was analysed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). Phenolic compounds of methanolic extract prepared from aerial parts of the plant was qualified and quantified by a LC-MS/MS analysis. Total phenolic compound, in vitro antioxidant and antimicrobial activities of methanol extract and its hexane, dichloromethane, ethyl acetate and water fractions were evaluated.

Nineteen compounds were identified representing 89.2% of the total oil, hexadecanoic acid (48.5%) was characterized as the main constituent with heneicosane (7.0%), hexahydrofarnesyl acetone (5.6%), tetradecanoic acid (4.9%) and (E)-nerolidol (4.1%). LC-MS/MS analysis indicated that tannic acid was the most abundant phenolic acid (7596.32±387.41 µg/g extract), whereas hyperoside was the most abundant flavonoid (2628.94±128.94 µg/g extract). The highest total phenolic content was determined 652.55 mg GAE/g extract in ethyl acetate fraction. The antioxidant capacity was evaluated by DPPH and total antioxidant capacity (TAC) assays and stronger antioxidant activity in ethyl acetate fraction was highlighted (DPPH: IC₅₀=12.748 µg/mL; 0.698±0.014 mM UAE, 1527.36±30.49 µM CRE). Methanol extract and fractions are more active against Gram positive bacteria than Gram negatives and showed considerable growth inhibitions against tested fungi.

In conclusion, this study indicated that *F. hirsuta* is a promising plants that can be evaluated in pharmaceutical, cosmetics and food industry.

Key Words: *Frankenia hirsuta*, Frankeniaceae, essential oil, phenolics, GC-MS, LC-MS/MS, antioxidant, antimicrobial.



POSTER PRESENTATION

CHEMICAL FINGERPRINTING AND EVALUATION OF BIOACTIVE PROPERTIES OF *LIMONIUM CASPIUM* (WILLD.) GAMS

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Abstract

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, 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 cancer to skin disorders.

The genus *Limonium* Mill. (Plumbaginaceae), is represented by 27 halophytic taxa in Turkey. Taking in consideration of possible high levels of polyphenols and high antioxidant capacity of halophytes, in this study, phenolic compounds of *L. caspium* (Willd.) Gams were qualified and quantified by a LC-MS/MS analysis, total phenolic content, in-vitro antioxidant, antimicrobial and enzyme inhibitory activities of the methanol extract from aerial parts and its hexane, dichloromethane, ethyl acetate and water fractions were determined. Tannic acid (6316.55±322.14 g/g) and hyperoside (633.15±31.02g/g) were found to be the most abundant phenolic acid and flavonoid, respectively. The highest total phenolic content (678.82±7.52 mg GAE/g extract), DPPH radical scavenging activity (IC₅₀=16.01±0.32 g/mL) and total antioxidant capacity (0.353±0.001 mM UAE, 771.733±2.565 mM CRE) determined in ethly acetate fraction. Methanol extracts and fractions showed moderate growth inhibitions against tested bacteria and fungi. While hexane fraction showed weak pancreatic lipase inhibition, the highest tyrosinase inhibitory activities were observed with hexane and ethyl acetate fractions (IC₅₀=171.04±2.35 and 171.19± 3.52 g/mL, respectively).

Results of the present study indicated that *L. caspium* is a promising plant that can be evaluated in pharmaceutical, cosmetics and food industry.

Key Words: *Limonium caspium*, Plumbaginaceae, phenolics, LC-MS/MS, antioxidant, antimicrobial, pancreatic lipase, antityrosinase.



POSTER PRESENTATION

PHARMACOBOTANICAL ANALYSIS OF *PAEONIA BROTERI*

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Abstract

Paeoniaceae is a monogeneric family where the genus *Paeonia* includes 35 species [1]. Some are used in traditional Chinese medicine for their anti-inflammatory and antioxidant properties, among others [2; 3; 4]. Despite this, the information available on the species mentions only a few characters that are not sufficient for their control and the distinction between species has raised some doubts, which can risk their safety and efficacy [5; 6]. We completed microscopic and histochemical analyses to establish botanical authentication for roots, leaves and powdered materials of *P. broteri*, endemic in Iberia [7].

Plant material was collected from native populations in SW Portugal, identified and vouchers deposited at LISU. Light and scanning electron microscopy and histochemical tests followed standard methods [8; 9]. *P. broteri* tuberous roots have a secondary growth in which some phelloderm cells are filled with simple and compound starch grains. The first are circular/polyhedral, with a cleft hilum and no striae; the second have 2-3-4 aggregate elements. Some vacuoles of phelloderm cells have druse calcium oxalate crystals. Throughout the root phelloderm, stone cells, positive for lignin are seen. The analysis of the light yellow-brown root powder confirmed these characters. Both leaves epidermal faces show irregular cells with sinuous cell walls. Elliptical and circular anomocytic stomata appear on the leaves lower epidermis. Among the mesophyll cells, druse calcium oxalate crystals are also found, accompanying both the secondary and the main vessels. The powdered leaves acquire an olive-green color and its microscopic observation confirms the previous characters. Histochemical tests on *P. broteri* roots and leaves allowed the differentiation of the main chemical groups, except alkaloids.

This is the first pharmacobotanical study of *P. broteri* revealing some of the main identifying characters of this species which as diagnostic tools can contribute to the botanical authentication promoting its pharmaceutical and economic importance.

Key Words: *Paeonia broteri*, microscopy, histochemistry; roots and leaves, powdered analysis.

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POSTER PRESENTATION

THE CONTRIBUTION OF SEROTONERGIC RECEPTORS IN THE
ANALGESIC EFFECT OF KAEMPFEROL

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Abstract

Kaempferol is a flavonoid-derived compound known to have various pharmacological activities such as antioxidant, anti-inflammatory, anticancer, neuroprotective, and cardioprotective effects. But, its effect on pain and mechanisms of this effect has not been studied in detail before. In this study, it was aimed to evaluate the analgesic efficacy of kaempferol and to investigate the role of serotonergic receptors in the analgesic effect of kaempferol. Hot plate and tail immersion tests were used to evaluate the central analgesic effect, and the acetic acid-induced writhing test was used to evaluate the peripheral analgesic effect. Kaempferol was administered intraperitoneally at doses of 25, 50, 100, and 200 mg/kg in mice. WAY-100635 (5-HT_{1A} receptor antagonist), ketanserin (5-HT_{2A/2C} receptor antagonist), and ondansetron (5-HT₃ receptor antagonist) (all; 1 mg/kg, i.p.) were used to examine the involvement of the serotonergic system in the analgesic effects of kaempferol. Kaempferol showed both central and peripheral analgesic effects at all doses administered. 50 mg/kg kaempferol was chosen to investigate the mechanism of analgesic effect. It was observed that WAY-100635, ketanserin, and ondansetron inhibited both the peripheral and central analgesic effects of kaempferol. It was determined that the serotonergic system significantly contributed to the analgesic effect of kaempferol. The results obtained provide data in the literature that kaempferol can be used in the treatment of pain.

Keywords: Kaempferol, analgesic effect, serotonergic system.

Acknowledgements

This study was supported by a grant of Anadolu University (BAP- 1806S241).



POSTER PRESENTATION

TOTAL PHENOLIC CONTENT, ANTIOXIDANT AND TYROSINASE
INHIBITORY ACTIVITIES OF SELECTED *SIDERITIS* L. SPECIES

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Abstract

Numerous species of medicinal and aromatic plants have been consumed as tea and herbal medicine in Turkey for many years. The species of *Sideritis* L. (Lamiaceae), which are widely known as Ada çayı (Island tea), Dağ çayı (Mountain tea) and Yayla çayı (Plateau tea) in Anatolia, have an important place among the plants used as herbal tea and household remedy since the time of Dioscorides.

The genus *Sideritis*, which comprises more than 150 species worldwide, is represented by 53 annual and perennial taxa including 39 species, 12 subspecies and 2 varieties in our country. Of which 39 taxa are endemic and endemism ratio being 74%. Many of *Sideritis* species collected from nature and used, some are sold in the market. When bioactivity studies were examined, researches on anti-inflammatory, antispasmodic and anti-ulcerogenic etc. activities which are related to usages of the species were highlighted. Phytochemical studies show that *Sideritis* species are rich in volatile oils, terpenic and phenolic substances.

Literature survey have proven that biological activity studies on the *Sideritis* species growing in our country is still limited. In this study, anti-tyrosinase activity of methanolic extracts obtained from three endemic *Sideritis* species, *S. tmolea* P.H. Davis, *S. rubriflora* Hub.-Mor. and *S. hololeuca* Boiss. & Heldr. apud Bentham were investigated for the first time. The results were evaluated together with total phenolic contents (TPC) and antioxidant properties. The highest TPC and DPPH radical scavenging activity were found in *S. tmolea* (161.57 ± 0.95 mg GAE/g extract and IC₅₀= 155.5 ± 2.09 µg/mL). Only *S. hololeuca* showed significant tyrosinase inhibition (IC₅₀=1565.25 ± 17.59 µg/mL) among tested samples.

Key Words: *Sideritis*, total phenolic content, antioxidant, tyrosinase inhibition.

Acknowledgements

This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 2111S196.



POSTER PRESENTATION

**TOTAL BOTANICAL INVESTIGATION ON A HERBAL TEA:
SIDERITIS TMOLEA P.H. DAVIS**

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Abstract

The genus *Sideritis* L. (Lamiaceae) is distributed in an area stretching from the Mediterranean region to Europe, Bahama's, Western China and Morocco. In the Flora of Turkey and the East Eagean Islands, 38 *Sideritis* species were reported by Huber-Morath in 1982. Since then, 6 species and 2 new records have been described in the flora of Turkey and the number of *Sideritis* species reached to 46. The genus *Sideritis* is divided into two sections in Turkey. Section *Hesiodia* Benthham is known with reliable taxonomic characters. Section *Empedoclia* (Rafin) Benthham which shows a high level of endemism was reported with a few clear-cut species.

The species of *Sideritis* L. (Lamiaceae), which are widely known as Ada çayı (Island tea), Dağ çayı (Mountain tea) and Yayla çayı (Plateau tea) in Anatolia, have an important place among the plants used as herbal tea and household remedy since the time of Dioscorides.

As a part of our ongoing studies on *Sideritis* species growing in Turkey, in this study we have explained morphological and anatomical characteristics of an endemic *Sideritis* species, *S. tmolea* P.H. Davis, which belong to *Empedoclia* and used as herbal tea.

Key Words: *Sideritis tmolea*, Labiatae, morphology, anatomy.

Acknowledgements

This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 2111S196.



POSTER PRESENTATION

GIVEN ARABIC NAMES RELATED TO PLANT'S NAMES

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Abstract

Background The meaning of the given names to the newborn (male or female) in Arabic culture in general and in the Algerian culture specially has a great importance. The belief of the blessful of plants and the auspicious meaning of the plant's name it can be seen in Arabic culture in general, when we use the name of plant as given name of people, for the two gender as well. The aim of this paper is to collect the names of plants (medicinal, aromatic and, ornamental plant) that their vulnaculaire Arabic names is used as Arabic given names to people.

Methods In this paper we examined the given names of Algerian people to both genders related with the Arabic plant's names. The information was collected from books, encyclopaedia scientific articles and internet sites, written in Arabic French and English languages. The Arabic given names could be common in Arabic countries and at the same time rare in Algeria (the opposite is true). To evaluate the frequency of given names we exanimate more than 1000 names from students lists (primary school and university).

Result This study allowed us to list about 44 plants: medicinal, aromatic ornamental plants or fruit tree that are used as given names in Algerian culture 10 plants names are used for male gender and 43 plants names for the female gender. From the lists of students names the common use of plant as given name is given to the female gender than the male one, the more used first name from the same lists is Zahra or Fatima Zahra (*Citrus*), Lyna (*Phoenix dactylefera*) and Nesrine (*Rosa canina*).

Conclusion According to algerian beliefs, given names must have auspicious, bless full, lucky names, there are more than 40 given names related to aromatic, medicinal, ornamental plants and fruit tree.

Kye words: female names, Algerian belief, bless full, aromatic plant.



POSTER PRESENTATION

CHARACTERIZATION OF VOLATILE COMPOUNDS OF
CENTAUREA BINGOELENSIS BEHÇET & İLÇİM

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Abstract

The genus *Centaurea* L., a member of the Asteraceae family, has a high endemism rate in Turkey (Uysal, 2012). *Centaurea bingoeleensis* Behçet & İlçim is an endemic species that spreads in a narrow area in Eastern Anatolia (Behçet et al., 2017). In the present study, volatile components of *C. bingoeleensis* were investigated using hydrodistillation and IM-SPME techniques, and analyzed by GC-GC/MS. The main components of essential oil were characterized as phytol (21.3%), hexadecanoic acid (12.5%), spathulenol (11.4%), (E)-nerolidol (9.7%), hexahydrofarnesyl acetone (8.6%), caryophyllene oxide (6.5%). In addition, main components of hydrolate over 5% were characterized linalool (24.5%), spathulenol (16.7%), α -terpineol (7.5%) and phenyl acetaldehyde (5.9%). As a result, the volatile components of *C. bingoeleensis* species were investigated for the first time.

Key Words: *Centaurea bingoeleensis*, essential oil, IM-SPME, GC-GC/MS.

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POSTER PRESENTATION

INVESTIGATIONS ON
HYPERICUM TURCICUM ÖZBEK & HAMZAOĞLU,
A RECENTLY DESCRIBED ENDEMIC FROM TURKEY

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Abstract

Turkey is one of the richest countries in the world in terms of plant diversity and is home to many endemic plant species. On the other hand, the fact that 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 both 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 107 taxa, of which 46% 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. *Hypericum turcicum* Özbek & Hamzaoglu was reported as a new species growing in the salty areas of gypsum hills in Northwest Anatolia in 2019. As a part of our ongoing studies on *Hypericum* species growing in Turkey, in this study we have explained anatomical characteristics and chemical composition of essential oil of *H. turcicum*, which is most recently described and a local endemic species from Turkey.

Key Words: *Hypericum turcicum*, anatomy, essential oil, endemic.

Acknowledgements

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POSTER PRESENTATION

ESSENTIAL OIL COMPOSITIONS OF FLOWERING AND POST
FLOWERING STAGES OF *LAGOECIA CUMINOIDES* L. GROWING
WILD IN NORTHERN CYPRUS

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Abstract

Lagoecia cuminoides L., belonging to the family Apiaceae, is the only species representing the genus and it grows naturally all over the island. Essential oils of the aerial parts of *L. cuminoides* at both flowering and post-flowering stages were isolated by hydrodistillation and analyzed by GC and GC-MS, simultaneously. The yields of the essential oils were found as 1.1% for the flowering stage and 1.34% for the post-flowering stage.

The major compounds were characterized as γ -terpinene (45.4%), thymol (35.4%) and *p*-cymene (11.6%) in order of decreasing abundance in the essential oil obtained from flowering plant material. On the other hand, in the essential oil obtained from the material collected in the post-flowering stage, the major compounds were characterized as thymol, *p*-cymene and γ -terpinene at concentrations of 83.4%, 8.1% and 6.7%, respectively. The essential oil composition of *L. cuminoides* growing in Cyprus was previously reported from a different location (1).

Key Words: *Lagoecia cuminoides*, essential oil, thymol, Apiaceae, Cyprus

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POSTER PRESENTATION

**BIOLOGICAL PROPERTIES OF EXTRACTS DERIVED FROM
TRANSGENIC *SENNA OBTUSIFOLIA* HAIRY ROOTS CULTURED IN
A SPRINKLE BIOREACTOR**

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Abstract

Senna obtusifolia (L.). also known as *Cassia obtusifolia* or "Sicklepod" belongs to the Fabaceae family. It is a common annual plant occurring in the Americas, Asia, Australia, and Africa. This plant is considered as a remedy for many ailments such as: diseases of the digestive system, eyes, psoriasis or rheumatism. Literature data indicate that the extracts exhibit strong antioxidant, hepatoprotective, antibacterial, antifungal, anti-inflammatory and anticancer properties. [1-4]. *S. obtusifolia* is known as a source of many valuable metabolites, such as polyphenols, alkaloids or terpenoids [4]. Nowadays, plant *in vitro* cultures are becoming an increasingly used system for extracting valuable secondary metabolites. Their unquestionable advantages are the absence of adverse environmental interference (which is extremely important from an environmental point of view) or the possibility of modulating the levels of selected secondary metabolites including metabolic engineering. This approach often allows interference with metabolic pathways through transgenesis using different strategies resulting in high-yielding plant cultures [5]. Among the various plant *in vitro* cultures, hairy roots occupy an important place. These roots are characterised by rapid growth rates, the ability to grow on simple substrates without growth regulators and high genetic stability. The present work deals with obtaining transgenic hairy roots of *S. obtusifolia* by agroinfection as well as their cultivation on a larger-scale in a 10 L sprinkle bioreactor using elicitation. We showed that the applied strategy allowed to increase the content of selected secondary metabolites in the obtained roots. Our studies have shown that the extracts can induce apoptosis in cancer cells by altering the mitochondrial membrane potential (MMP), fragmenting chromosomal DNA and activating the caspase cascade.

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POSTER PRESENTATION

ESSENTIAL OIL COMPOSITIONS OF *Mentha* spp. (*M. spicata* and *M. longifolia* ssp. *cypriaca*) GROWING IN NORTHERN CYPRUS

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Abstract

The genus *Mentha* L. (Lamiaceae) represented 4 species and 7 taxa in Cyprus. The aim of this study is to analyze the aerial parts' essential oils of *Mentha spicata* L. and *M. longifolia* ssp. *cypriaca* (Heinr. Braun) Harley) which is an endemic taxa at a flowering stage. They were isolated by hydrodistillation and simultaneously analyzed via GC and GC-MS. The yields of the essential oils were calculated on a dry weight basis as 4% and 3%, comprising 32 and 22 identified compounds, respectively, representing the totality of essential oils. The major compounds of the former were determined as limonene (10.6%) and carvone (67.8%) while 1,8-cineole (10.0%) and pulegone (64.8%) for the latter. This is the first report on the essential oil composition on the *M. longifolia* ssp. *cypriaca*.

Key Words: *Mentha spicata*, *Mentha longifolia* ssp. *cypriaca*, essential oil, Lamiaceae, Cyprus



POSTER PRESENTATION

ASSESSMENT OF THE KNOWLEDGE OF PHARMACISTS AND
PHYSICIANS ON HERBAL MEDICINE FOR LIVER AND BILIARY
DISEASES IN NORTHERN CYPRUS

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Abstract

The herbal medicine market was valued at 151.91 billion USD in 2021, significantly expanded by the COVID-19 pandemic across the world. A cross-sectional study was conducted by interviewing with a structured questionnaire among 97 out of 343 licensed community pharmacists and 45 out of 95 physicians who registered as internal medicine specialists and/or internal medicine endocrinology and metabolic diseases specialists working privately or in a hospital in Northern Cyprus. The most advised plant was *Cynara scolymus* L., however, the pharmacists were significantly more likely to advise than physicians ($p < 0.05$). It is worthy of concern that the suggestion of *Helichrysum* sp. and *Taraxacum officinale* L. were affected by the work experience of pharmacists which was 12.4% and 16.5%, respectively, though none of the physicians advised. In addition, *Glycyrrhiza glabra* L. and *Rosmarinus officinalis* L. were both not advised by physicians while pharmacists advice was positively correlated with work experience for both plants ($p < 0.05$). The least suggested plant was *Artemisia absinthium* L. with no difference between groups ($p < 0.05$). The aim of this study is to assess knowledge of healthcare professionals (pharmacists and physicians) working in Northern Cyprus on herbal medicines used as prophylactic and curative agents in liver and biliary diseases in comparison with their work experience by year. This is, as far as the author's are aware, the first study that discusses this topic.

Key Words: Herbal Medicine, Liver disease, Biliary disease, Healthcare Profession, Cyprus



POSTER PRESENTATION

PHYTOCHEMICAL STUDIES ON *MYRTUS COMMUNIS* L.

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Abstract

Objective: *Myrtus communis* L. (myrtle, murt), a member of the Myrtaceae family, is a non-endemic medicinal plant. It is an evergreen plant that grows naturally and is cultivated in most Mediterranean countries and Turkey.

Many phenolic compounds, including myricetin and its glycosides, quercitrin, gallic acid, quercetin, chlorogenic acid, caffeic acid, and tannins, were isolated from the leaves of *M. communis*. These compounds are known to have antioxidant, anti-inflammatory, and neuroprotective effects.

This study aims to carry out phytochemical studies on *M. communis* to obtain some of its active antioxidant, neuroprotective, and anti-inflammatory principles. In continuation, we will attempt to show neuroprotective compounds' mechanisms and prove their effects in vivo.

Methods: The plant was collected from Hatay (Turkey) in July 2022. Two hundred grams of plant material was macerated in 80% EtOH solution for 12 hours in 3 repetitions. Isolation and purification studies were performed using liquid-liquid partitioning, open-column chromatography, and thin-layer chromatography. The structures of the pure molecules were determined by spectral methods (NMR and MS).

Results: Based on the spectral data, the compounds were established as an acyl phloroglucinol (gallomyrtucommulone C) and two flavonol glycosides (myricitrin, quercetin-3-*O*- α -L-rhamnopyranoside).

Conclusion: Three known compounds, previously reported from the *Myrtus* genus or Myrtaceae family, were obtained. In due course, we will continue our phytochemical studies and test all compounds with sufficient quantity for their biological activities mentioned above.

Key Words: *Myrtus communis*, phytochemistry, phenolic compounds, flavonoid, phloroglucinol



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FULL PAPERS



FULL TEXT–ORAL PRESENTATION

***H. PERFORATUM* L. AFFECTS LOCOMOTOR ACTIVITY, BODY WEIGHT AND BLOOD GLUCOSE**

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Abstract

Hypericum perforatum is a perennial herb that is widely distributed in all Mediterranean and mainly contains hyperforin and hypericin. It is commonly used as an antidepressant. In rats fed a high-fat diet, *H. perforatum* was found to prevent weight gain. It has been shown to cause the total glucose level to remain elevated for six weeks. We investigated how different doses of *H. perforatum* extract affected Syrian hamster locomotor activity, body weight, food consumption, and blood glucose levels. The aerial parts of *H. perforatum* were subjected to maceration in ethanol using a Soxhlet apparatus. Syrian hamsters received injections of plant extract at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg. The animals' daily body weights and food intake were calculated, along with their locomotor activity for the course of the 10-day experiment. Blood glucose levels were measured twice, before and after the extracts were injected. Results showed that as doses increased, animals' locomotor activity, body weight, and food intake decreased. After being administered into hamsters, an extract at a dose of 300 mg/kg considerably raised blood glucose levels. High doses of *H. perforatum* extract can therefore be thought of as both an antidepressant and an anti-obesity medication. The decrease in daily activity rhythms, in particular, may be an indication of *H. perforatum*'s sedative effect.

Key Words: *H. Perforatum*, locomotor activity, body weight, food intake, blood glucose

1. Introduction

Hypericum perforatum L., a plant used for medicine that can be found in Europe, Asia, North Africa, America, and the Mediterranean, has been utilized for healing by many ancient civilizations. *H. perforatum* (St. John's wort) is known as an herbal medicine for anxiety disorders, seasonal affective disorder, and depression.

Numerous bioactive substances, such as, flavonoids, phloroglucinols, naphthodianthrones, phenylpropanes, tannins, amino acids, essential oils, xanthenes, and other substances, have been reported to be present in *H. perforatum*. These substances have a variety of biological properties and can be used as antioxidants (Li et al., 2008; Al-Fatimi et al., 2007; Arda et al., 2002, and Vorbach et al., 1997). *H. perforatum* affects serotonergic, dopaminergic, noradrenergic, and GABA receptors and reuptake of neurotransmitters, owing primarily to the presence of hyperforin and hypericin (Nathan, 2002). *H. perforatum* is a plant that increases the circadian rhythm's sensitivity to light, making it employed as an antidepressant in situations of mood disorders (Harrer, 2000; Boivin, 2000).

H. perforatum inhibited both locomotor hyperactivity at doses of 25, 50, and 100 mg/kg, especially after 2 and 6 hours of ethanol abstinence (Coskun et al., 2006). However, while repeated doses of *H. perforatum* had anxiolytic and anti-panic effects, they did not cause any change in locomotor activity (Bejjamini and Andreatini, 2003). Additionally, it was claimed that *H. perforatum* could not alter the circadian system's sensitivity to light because it did not alter the activity levels of rats when it was applied, and the antidepressant effect may not be connected to this (Francis, 2005). In fact, the



observation of a significant reduction in locomotor activity at a dose of 200 mg/kg shows that the findings in the literature on locomotor activity are incompatible (Diana et al., 2007).

It was shown that *H. perforatum* reduced insulin resistance in rats fed a fructose diet and reduced weight gain in rats fed a high-fat diet (Husain et al., 2011). Contrary to these findings *H. perforatum* has been found to have significant impact on adipocyte-related disorders by including insulin resistance in white adipose tissue cells (Amini et al., 2009). In studies with humans, however, *H. perforatum* increased the total glucose level in male individuals and even caused it to remain elevated for 6 weeks. Due to the fact that long-term *H. perforatum* therapy decreased insulin secretion and worsened glucose tolerance in this investigation, it was assumed that this impact was likely mediated by beta cells (Stage et al., 2011).

With all of this information mentioned above, the study's objective is to evaluate the effects of various doses of this plant, which has been used extensively in the health industry, particularly on the circadian rhythm, as well as changes in blood glucose, food intake, and body weight in Syrian hamsters with strong photoperiodic properties.

2. Materials and Methods

2.1. Collection of Plant Material

Plant materials were collected in Ida Mountain (Kazdagları-Canakkale) from 342 meters (geographical coordinates; N 39° 45' 08", E 026° 48' 54") in the area of Northwest Turkey in May 2022. Voucher specimens have been deposited.

2.2. Preparation of Crude Extract

Aerial parts were dried for 10 days at room temperature in sterile condition. A total of 20 g of each dried material was extracted for 24 hours in 300 ml 70 % ethanol using a Soxhlet apparatus. The solvent was evaporated under a vacuum and then dissolved DMSO (Dimethyl Sulfoxide).

2.3. Animals

Twenty adult male Syrian hamsters (*Mesocricetus auratus*) were obtained from the Hamster and Gerbil unit of Çanakkale Onsekiz Mart University. Hamsters were subjected to 16 hours of light and 8 hours of darkness in ventilated rooms at a temperature of 22 ± 2 °C. Weighing 100-120 g animals were housed in plastic cages (16 x 31 x 42 cm). Animals had free access to food pellets and tap water. The procedures used in this study were approved by the Institutional Animal Ethics Committee of Çanakkale Onsekiz Mart University (2019/10-09). A cool-white, fluorescent lighting system controlled by automatic programmable timers was provided.

2.4. Experimental Groups

The study was carried out under four groups. The first group (n=5) was given only DMSO as the control group. In the second group, 100 mg/kg *H. perforatum* extract was administered intraperitoneally. In the third group, 200 mg/kg *H. perforatum* extract was administered intraperitoneally and in the fourth group 300 mg/kg *H. perforatum* extract was administered. Injections were administered every day at the same time (12:00 - 13:00 h) during the 10-day experiment.

2.5. Locomotor Activity

Before starting the experiment, animals were placed into the activity wheels for 5 days. Animals that showed distinct circadian rhythms were selected for the experiment. Animals with normal locomotor activities were placed on running wheels and included in the 10-day experiment. The number of wheel revolutions was automatically recorded per 15 min interval. Vital View Data Acquisition Software (Mini Mitter Company, Inc. Bend, OR USA) was used to analyze stored results. The activity was represented as a double-plotted actogram. Since the last measurement values (excluding locomotor activity) were taken on the 10th day, the 10th day locomotor activity is not available in the graphs.

2.6. Measurement of Food Intake, Body Weight, and Blood Glucose

To estimate the food consumption a weighed amount of food pellets was placed into cages. After 24 hours, the food pellets and fragments that remained in the cages were weighed. Food intake was calculated every day and 10-day average consumptions were given. To measure their body weight, each

animal was weighed daily prior to injections throughout the experiment. Accu-Check blood test strips were used to determine the blood glucose level. On the first and tenth days of the experiment, blood (0.1 – 0.2 ml) was drawn from the orbital sinus of the eye using a hematocrit capillary tube before (11:00 - 12:00 h) and after (13:00 - 14:00 h) injections.

2.7. Statistical Analysis

Statistical analysis was performed by using SPSS 22.0 and Sigma plot 14.0. Data were examined by the Kruskal-Wallis's test and comparisons between groups were analyzed with t-tests. $p < 0.05$ was considered statistically significant.

3. Results

The locomotor activity of the control and 100 mg/kg, 200 mg/kg, and 300 mg/kg dose-injected hamsters over the course of 10 days are shown in Figure 1. As the dose increases, the activity of the animals also decreases. This reduction occurs as soon as the injections start. In particular, the efficacy of 300 mg/kg dose was the highest in reducing locomotor activity. Comparing the 100 mg/kg dose to the control group and other doses, the activity of the animals was higher.

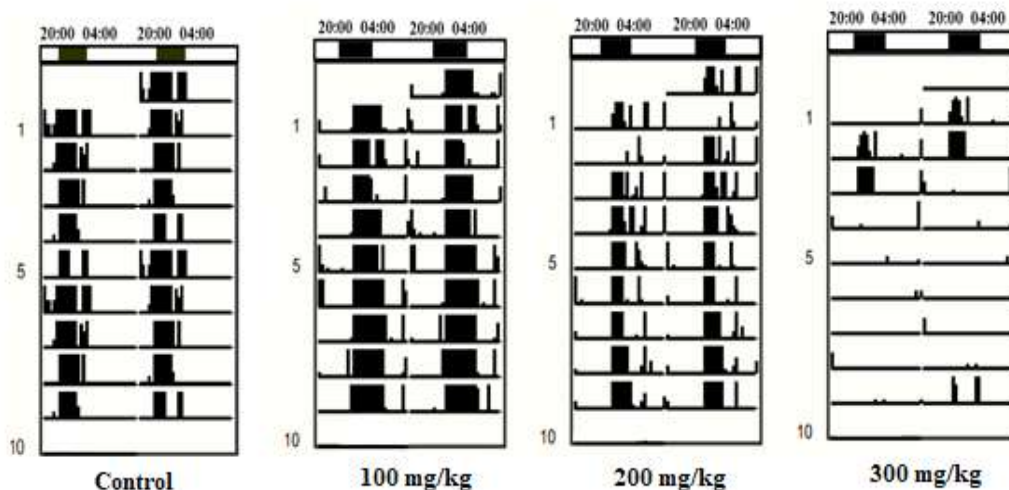


Figure 1. A representative actogram from the groups of control, 100 mg/kg, 200 mg/kg, and 300 mg/kg plant extracts. Lights are on at 04:00 h and light off at 20:00 h. The black bars at the top indicate the dark phase of the day. Each actogram shows two consecutive days.

Figure 2 illustrates the variations in the animals' body weights. The body weight of animals was significantly affected by different doses of plant extracts ($p < 0.001$). Pairwise comparison analysis showed that the body weight of animals injected with 200 mg/kg and 300 mg/kg of the extracts was significantly lower than the control group ($p < 0.001$). The group receiving 300 mg/kg had the lowest body weight ($p = 0.003$).

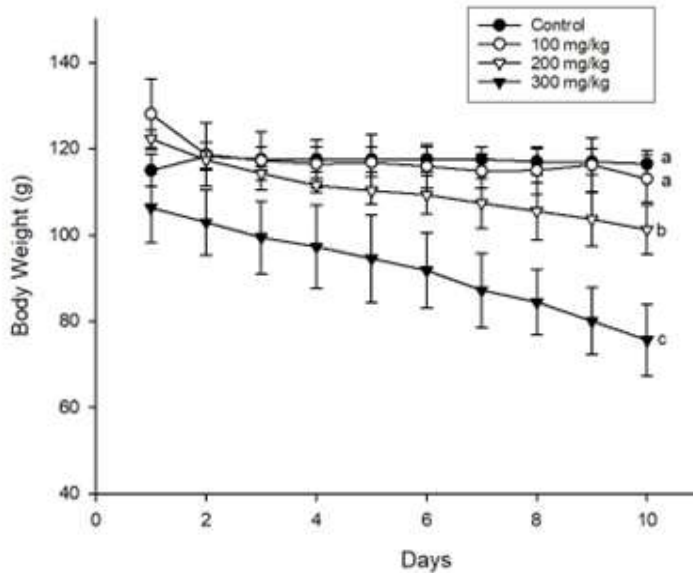


Figure 2. Hamster body weight after injections of *H. perforatum* plant extracts at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg.

Figure 3 displays the average food consumption values during the course of the 10-day experiment. The results shows that the hamsters consume statistically less food at doses of 200 mg/kg and 300 mg/kg than at the control and 100 mg/kg levels ($p < 0.001$).

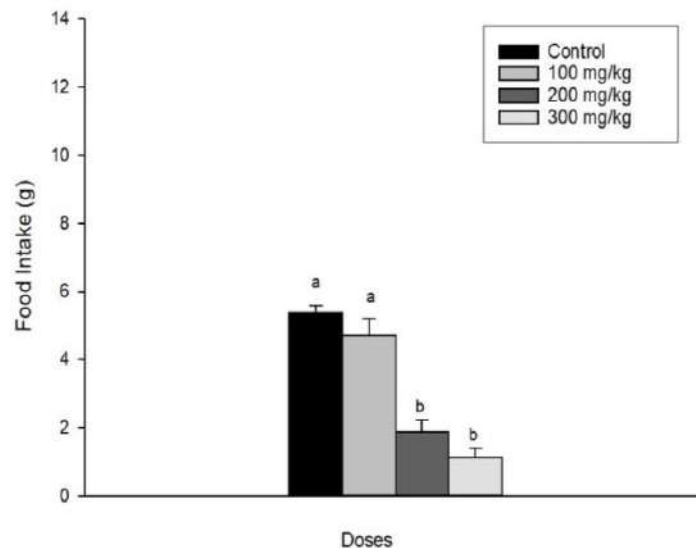


Figure 3. Average food intake following injections of 100 mg/kg, 200 mg/kg, and 300 mg/kg *H. perforatum* plant extracts.

When the glucose levels on the first day were examined, all of the animals in the group had normal (60 mg/dl) glucose levels prior to the injection (Figure 4). However, while the dose of 300 mg/kg after injection increased the glucose level (90 mg/dl), other doses did not make any difference ($p = 0.05$). When the glucose levels in the blood after injections were compared at the end of the experiment (Day

10), it was seen that the glucose levels in the 100 and 200 mg/kg doses did not differ from the control group, whereas the 300 mg/kg dose statistically increased the blood glucose more than the other groups.

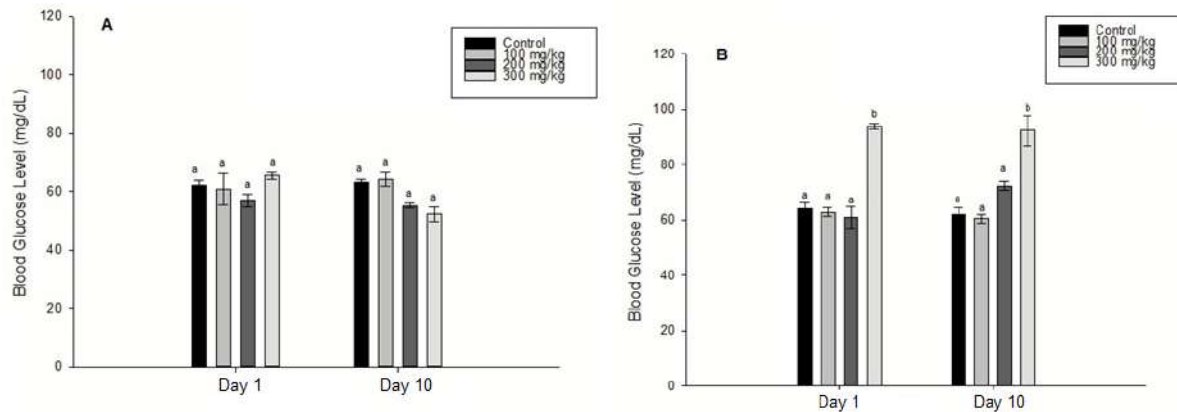


Figure 4. Blood glucose level before (A) and after (B) administration of *H. perforatum*

4. Discussion

The current study found that high doses of *H. perforatum* suppressed locomotor activity in hamsters and reduced body weight, daily food intake, and increased blood glucose levels. While *H. perforatum* doses of 200 mg/kg and 300 mg/kg decreased locomotor activity, doses of 100 mg/kg increased activity. Our findings contradict the literature's findings that high doses of *H. perforatum* increase activity, while other studies show that it decreases activity overall (Bejiamini & Andreatini, 2003). This discrepancy may be explained by variations in the animal species used (Vandenbogaerde et al., 2000; Uzbay et al., 2007) and variations in the animal activity times throughout the day. Knowing the physiological characteristics of the species that will receive the extracts prepared at various concentrations is important for evaluating the impact of plant-derived extracts, particularly on behavior and locomotor activity. For instance, a low or high dose may increase activity in one species while suppressing it in another. In such locomotor activity studies, the photoperiodic feature of animals is taken into consideration. As a result, the animal we chose is the hamster, which regulates its activity based on the light-dark cycle. For example, it sleeps during the day and is active at night. Figure 1 shows that our control group is active in the dark and inactive in the light, i.e. sleeping. In comparison to the control group, *H. perforatum* 100 mg/kg given in the light phase (12:00 – 13:00) increased activity in the dark. We observe a significant reduction in activity at dosages of 200 mg/kg and, more significantly, 300 mg/kg. The 300 mg/kg dose affects animals right away after the injection begins, which is an important fact. Almost no daytime activity in the animals could be calculated after the third day. In people, particularly at large doses, we observe a comparable impact. A dose of 300 mg/kg or greater is thought to have antidepressant properties in humans, whereas doses of 100 mg/kg or less are thought to have no adverse effects on humans (Cervo et al., 2002, Hirano et al., 2004). However, these high doses imply that *H. perforatum* can be used in clinical trials for those with severe depression. The effects of these doses should be carefully evaluated due to the reducing effect of high doses on locomotor activity, as well as body weight, food intake, and blood glucose rise.

The SCN (Suprachiasmatic nuclei), a structure in the brain's hypothalamus region, is at the epicenter of these effects, generating daily locomotor activity and dictating its rhythm. SCN lesions cause disruption of almost all biological rhythms. Every factor that influences the SCN also has a direct or indirect impact on locomotion. The chemical serotonin is primarily responsible for the effects of high doses of extracts obtained from the aerial parts of the *H. perforatum* plant (Cervo et al., 2002, Hirona et al., 2004). *H. perforatum* may inhibit serotonin reuptake. *H. perforatum* has been shown to increase the levels of serotonin, noradrenaline, dopamine, and gamma-aminobutyric acid (GABA) in the central



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nervous system (Muller et al., 1998). *H. perforatum* inhibits the binding of serotonin to presynaptic membrane-adrenergic receptors. As a result, the level of serotonin in the synaptic cleft rises (Cui and Zheng, 2016).

SCN contains one of the densest serotonergic terminals plexes in the brain. The majority of research were conducted in typical 12-hour light/12-hour darkness settings and did not specifically examine how the serotonergic system affected circadian rhythm. The points mentioned about the serotonin system must be carefully reviewed; it is crucial when serotonin influences the SCN, particularly in terms of locomotor activity. The time period while serotonin and its associated receptors are both active plays a major role in the formation of this effect. According to studies, serotonin is a significant neurotransmitter during the day-time. The serotonergic system may have been stimulated by the daytime administration of *H. perforatum* extracts, indicating the plant's sedative properties. It is crucial to investigate if administering *H. perforatum* to animals during the dark phase as opposed to during the day has a different impact in light of the literature and our findings.

It is still unclear which bioactive substances, including phenylpropanes, flavonol derivatives, phloroglucinols, biflavones, xanthenes, proanthocyanidines, some amino acids, naphthodianthrones, and essential oil constituents, could be entirely or partially responsible for the antidepressant activity of *perforatum* (Nahrstedt & Butterweck, 1997). *H. perforatum* species have been widely studied for the presence of specific contents, such as hypericins, to which some authors attribute antidepressant activity (Kitanov, 2001). Small concentrations of hypericin have stimulating or antidepressant effects in the brain (Hudson and Towers, 1994). Large quantities, however, would be depressant. It's interesting to note that this impact is not light-dependent. The behavioral pharmacological effect is not lost upon removing the other significant active component, hyperforin (Butterweck et al., 2003).

In addition, studies have shown that treatment with antidepressants affects food intake in both animals and humans (Gafoor et al., 2018). Serotonin administration reduces food intake (Fletcher et al., 1986). According to our findings and those of others, high serotonin levels caused by *H. perforatum* reduces food intake and suppress appetite in animals, resulting in weight loss. Thus, increased serotonin production and utilization maybe connecting molecule between the antidepressant and antiobesity effects of *H. perforatum*.

H. perforatum plant has a very variable impact on blood glucose levels. Studies have shown both a reduction in blood glucose level and an increase in its effect. The issue to be considered here is where the extract is obtained from the plant and how it is applied. Arokiyaraj et al. (2011) indicated the effect of *H. perforatum* on blood lipid and blood glucose levels in diabetic rats induced with streptozotocin. The administration of increasing doses of *H. perforatum* extract reduced the blood glucose levels of diabetic rats. On the other hand, in some studies on rats, *H. perforatum* administration did not cause a decrease in blood glucose in diabetic animals (Moghadam et al., 2017). Although insufficient results in the literature make commenting on the effects of *H. perforatum* on blood glucose difficult, we can say that the results of our study have an effect on raising blood glucose. A situation we observed in our study is that especially in animals injected with a dose of 300 mg/kg, the blood glucose rises very much in the first 15 minutes after the injection and then decreases to the values we measured (90-100 mg/dl) within 1 hour (13:00 - 14:00). Stage et al. (2011) also demonstrated that using large amounts of the extract causes increased blood glucose levels. Although excess glucose in the blood is shown as the reason for this sudden increase in blood glucose, the source of this glucose should be determined. Since all experimental groups received the same diet, it seems doubtful that the excess glucose came from food. These groups also consume the least amount of food and lose the greatest weight. This extra glucose may be mostly the result of a process that promotes its quick synthesis from lipids or from glycogen stored in the liver and skeletal muscles. In general, different circumstances, such as how the extracts are obtained from various *H. perforatum* parts and how they are administered to the species (single dose, numerous doses per day, continuous dose, etc.) may produce various outcomes (Rafailovska et al., 2022).



As a result, *H perforatum*, especially at high doses, has an effect on parameters such as locomotor activity, nutrition, body weight, and glucose metabolism. More research is required to determine the active ingredient(s) of *H. perforatum* and the daily circadian mechanisms involved.

Conflict of Interest

None

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FULL TEXT–ORAL PRESENTATION

CONTENT OF ESSENTIAL AND FATTY OIL OF *Foeniculum vulgare* L.
(FENNEL) GROWN UNDER INORGANIC AND ORGANIC
CONDITIONS

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Abstract

In recent decades, the over-use of chemical fertilizers has imposed many environmental challenges worldwide. Nowadays, the use of vermicompost manure (VM) and sheep manure (SM) of great interest to environmental security and are effective as a good nitrogen source for sustainable crop production. Therefore, determining the effective doses of VM and SM that will be an alternative to chemical fertilizers, are also important to improve soil fertility and produce healthy products. This study aimed to determine the effects of VM, SM and ammonium sulfate (AS) fertilizers on the fatty oil and essential oil content of fennel (*Foeniculum vulgare* L.). Three levels of VM applications (1000-V1, 1500-V2 and 2000-V3 kg da⁻¹), SM (1000-S1, 1500-S2, 2000-S3 kg da⁻¹) and AN (4-A1, 8-A2, 12-A3 kg da⁻¹) with a control (no fertilizer or manure) were placed in the experimental farm. Essential oil yield showed differences depending on the dose of VM, SM and AS fertilizer applications. The essential oil content range was found to be 2.40 to 3.35% in fennel fruits, and the highest essential oil content was obtained from the treatment of V3. In addition, the fatty oil content range was found to be 17.39 to 33.61% in fennel fruits, and the highest fatty oil content was obtained from the treatment of V3. V3 increased the essential oil and fatty oil yield of fennel, which may be related to the presence of more nutrients or effects of organic manure on soil structure. The findings of the study suggest that the application of VM has promising effects on fennel fruit and can be considered as a suitable substitute for chemical fertilizers when growing fennel, a plant with increasing importance and demand.

Key Words: Fennel, Essential oil, Fatty oil, Organic manures

1. Introduction

Organic agriculture production is an important topic for the growth the medicinal and aromatic plants (MAPs). The previous studies revealed that organic manures have positive effects on the yield and quality properties of MAPs. So, the quality of MAPs is depending on the global and sustainable approach, and establishment of the organic system of MAPs management. A few previous studies were conducted to compare the organic and inorganic fertilizer on the growth and yield of the MAPs (Sartip et al., 2015).

Fennel is one of the plants to grow as MAPs under organic farming system. Fennel (*Foeniculum vulgare*) is an aromatic Mediterranean plant belonging to the Apiaceae family. It is grown in tropical and temperate regions of worldwide and has pharmacological properties like anti-inflammatory, antimutagenic, cardiovascular, chemo modulatory, antitumor, and memory enhancing property (Yaldiz and Camlica, 2019).

The essential oil contents and extracts of fennel are used to control stored food mites (Lee et al., 2006). In addition, the high antioxidant levels in fennel essential oil enhance the importance to use as anti-heart illness and anti-cancer (El-Awadi and Esmat, 2010). Moreover, it was reported that fennel fatty oil is used to benefit role for engines by reducing vibration and noise (Tuccar, 2018).

The principal aim of fennel cultivation is to obtain high yield and quality properties. However, these values can show variability depending on different genotypes, growing conditions and ecological

factors. So, organic manures can be used to have high yield and quality properties in fennel production (Yaldiz and Camlica, 2019).

The aim of the study is to determine the chemical properties of fennel fruit, such as essential oil and fatty oil contents under organic manures and inorganic fertilizers.

2. Material and Methods

2.1. Plant Material, Experimental Design and Growing Conditions

Plant material were obtained from Tefenni, Burdur, Turkey. The study was conducted between March to September in 2020 year. Seeds were sown in March 2020, and experiment was conducted in the Completely Randomized Blocks Design with three replications under field condition. Each experimental plot consisted of five rows, with a distance of 40 cm between each row and 20 cm between each plant. For the distance between the plots, a meter block was considered.

Soil properties of experimental area were as follows: rich in phosphorus (21 ppm), potassium (272 ppm) and organic matter (1.2%); sandy clay-loam and slightly alkaline (pH=8.1). In this context, two different organic manures as sheep (1000, 1500 and 2000 kg da⁻¹), vermicompost (1000, 1500 and 2000 kg da⁻¹) with ammonium sulphate fertilizer doses (4, 8 and 12 kg da⁻¹) with a control (no manure or fertilizer) and conventional fertilizer were used (Table 1).

Table 1. The design of field experiment

No	Application name	Amount of application	Code
1	Control	-	Control
2	Vermicompost-1	1000 kg da ⁻¹	V1
3	Vermicompost-2	1500 kg da ⁻¹	V2
4	Vermicompost-3	2000 kg da ⁻¹	V3
5	Sheep manure-1	1000 kg da ⁻¹	S1
6	Sheep manure-2	1500 kg da ⁻¹	S2
7	Sheep manure-3	2000 kg da ⁻¹	S3
8	Ammonium sulphate-1	4 kg da ⁻¹	A1
9	Ammonium sulphate-2	8 kg da ⁻¹	A2
10	Ammonium sulphate-3	8 kg da ⁻¹	A3

Detailed chemical properties of applied organic manures were given in Table 2.

Table 2. Chemical analysis of different organic manures

Organic manure	Unit	Vermicompost	Sheep manure
Organic matter	%	42.50	90.00
pH (potentiometric)		44779,00	18476,00
EC (1/10)	ms cm ⁻¹	19146,00	23894,00
Total nitrogen	%	2,40	0.60
Phosphorus	%	1,70	0.90
Potassium	%	1.00	0.42
Calcium	ppm	50000,00	60200,00
Magnesium	ppm		8800,00
Iron	ppm		1000,00

Zinc	ppm		210,00
Manganese	ppm		400,00
Copper	ppm		130,00
Moisture	%	19,00	5,00

As the base fertilizer, 15 kg da⁻¹ diammonium phosphate was applied only Ammonium sulphate application area. Laboratory analyses were performed on the fruits of fennel.

2.2. Essential oil content and fatty oil

Essential oil content and fatty oil were determined as reported by Yaldiz and Camlica (2019). Dried fruits (200 g) were put to water distillation using a clevenger-type apparatus with the TS 8882 method during 3 h. Anhydrous sodium sulfate was used to dry isolated essential oils and was kept at 4 °C until further usage. Essential oil yields was estimated following each sample dried weight. For fatty oil analysis, fennel fruits were grinded as 5 g and they were extracted at 60°C by Soxhlet extractor during 8 h, using n-hexan as a solvent. After oil extraction the solvent was removed by a rotary evaporator.

2.3. Statistical analysis

The obtained data were analyzed using JMP-13 statistical software and results were compared via least significant difference test (LSD) in order to find differences among the organic manure and inorganic fertilizer applications in fennel at p<0.05.

3. Results and Discussion

3.1. Essential oil content

The essential oil contents (EOC) changed between 2.40-3.35% under different organic manure and inorganic fertilizer applications (Figure 1). The highest essential oil content was found from V3 (2000 kg/da vermicompost manure) application and followed by S1 and A2 applications. The lowest essential oil content was observed in the control application and followed by S2 and V1 (Figure 1). The essential oil content of fennel fruit increased depending on increasing vermicompost applications (V1, V2 and V3) in field experiment.

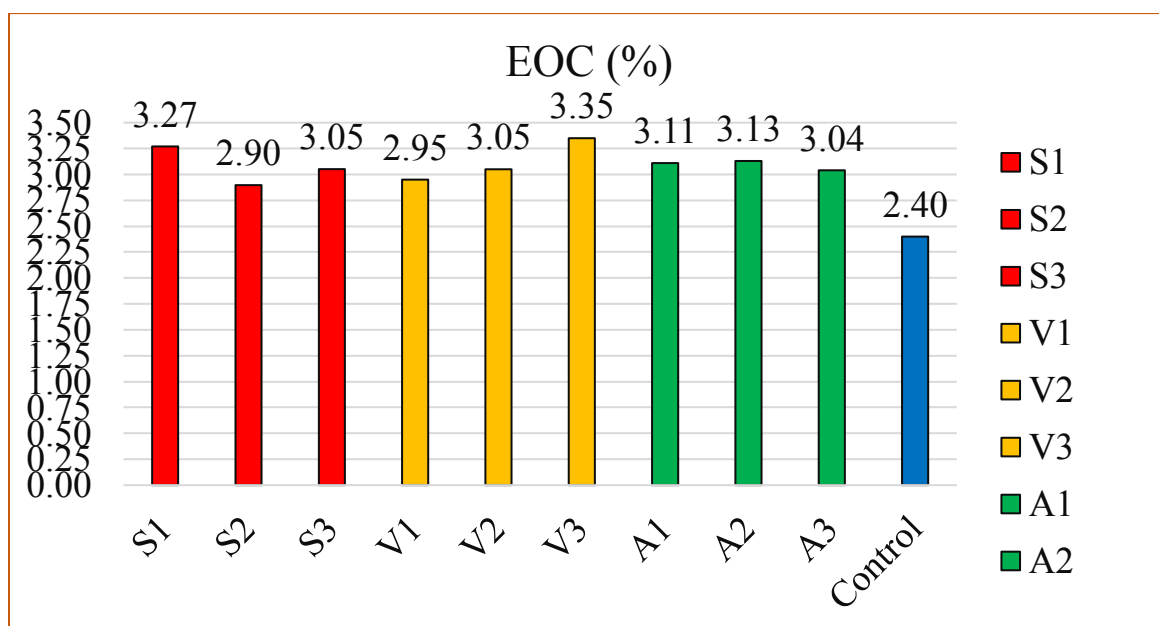


Figure 1. Essential oil content of fennel fruit under organic manure and inorganic fertilizer

3.2. Fatty oil content

Significant differences were found among the organic manures and inorganic fertilizers in fennel fruits in terms of fatty oil. The fatty oil content (FOC) ranged from 17.39% to 33.61%, and the highest fatty oil contents were found from A3, Control and S1 applications. The lowest fatty oil contents were noted in V3, A1 and S2 applications (Figure 2).

Fatty oil content decreased depending on the vermicompost applications (V1, V2 and V3). However, it increased depending on the increasing ammonium sulphate doses (A1, A2 and A3).

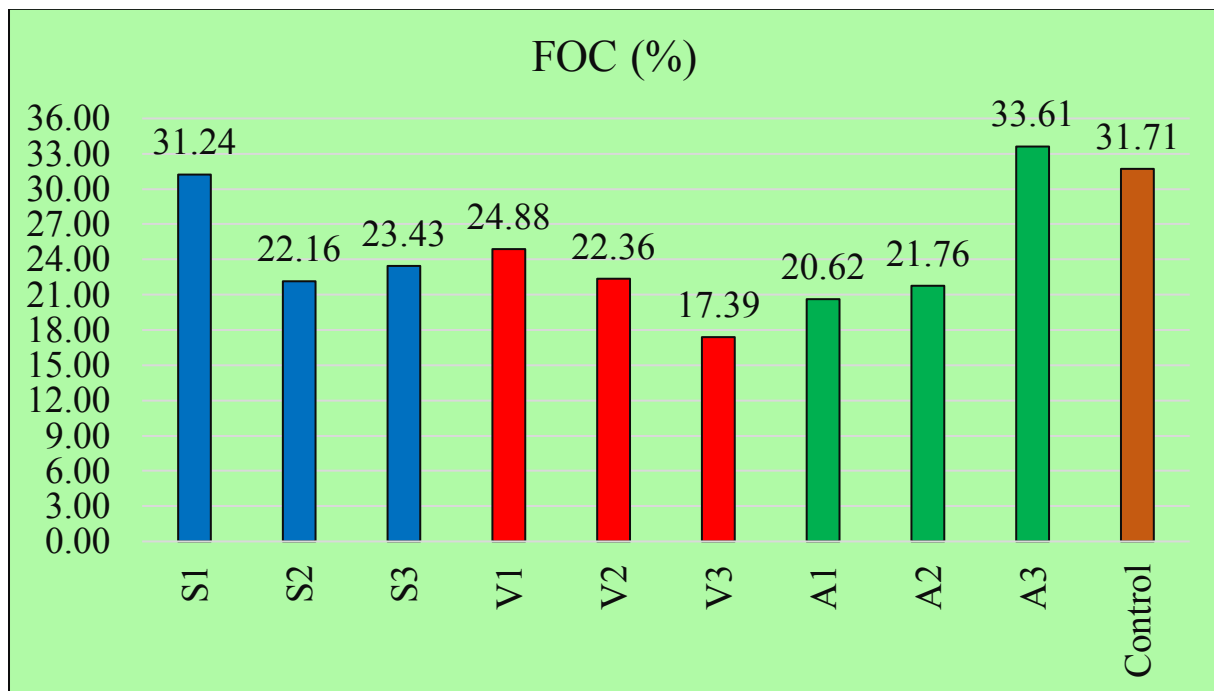


Figure 2. Fatty oil content of fennel fruit under organic manure and inorganic fertilizer

Our essential oil content results (2.40-3.35%) were found similar reported by Erhan (2022) (2.88-3.27%) under organic manure doses, Moradi et al. (2011) (2.20-2.90%) and Mohamed and Abdu (2004) (2.30-3.53%). The fatty oil result (17.39-33.61%) obtained from study was found similar with Erhan (2022), and it was found lower than Ayırtman (2015) and Kalkan (2015) findings. The obtained differences according to previous studies can be explained depending on the cultural practices such as fertilizer diversity, climatic conditions, cultivation techniques and irrigation methods (Moradi et al. 2011).

4. Conclusions

The examined quality properties of fennel fruit showed differences under different organic manures and inorganic fertilizer applications. The applications of organic manures and inorganic fertilizers had positive effects on fennel fruit in that the essential oil content was high compared to control applications. The essential oil content increased depending on the increasing vermicompost (V1, V2 and V3) applications.

The results of the present research are also promising that the genetic variation found among different genotypes of fennel supports the idea of production and improvement of this crop for sustainable crop production systems.



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FULL TEXT-ORAL PRESENTATION

INFLUENCE OF DIFFERENT ORGANIC MANURES ON THE COLOR CHARACTERISTICS OF *Salvia officinalis* L. (SAGE) UNDER FIELD CONDITIONS

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Abstract

Salvia officinalis L.(sage) is a medicinal plant extensively used as a raw material in the medicine, pharmaceutical, food, and perfume industry. Due to the economic values of sage, it has attracted the interest of producers. Organic farming is becoming more integrated in the agro-food chain and the global market, while environmental, food safety regulations contribute to making agriculture more sustainable and producing food that is safe for the consumer. Therefore, it is necessary to grow sage in accordance with the standards and of high quality for sustainable agriculture worldwide.

The present study aimed to evaluate the influence of doses of organic manures as sheep (500, 750, 1000, 1250 kg/da), chicken (500, 750, 1000, 1250 kg/da) and vermicompost (50, 100, 150, 200 kg/da) with a control (no manure) and conventional fertilizer on the color parameters of sage under field conditions. Furthermore, our study was undertaken to determine relationships between color measurements and pigment concentrations. According to statistical analysis results, there were significant differences in terms of L*, a*, b*, C*, and h°. In the case of sage leaves showed L* values in the range 51,93 and 66,66, a* values among 2,79 and 17,35, b* values among 12,89 and 17,35, C* values among 15,12 and 24,09 and h° values among 30,60 and 77.80. Among the organic manure applications, the highest L* and C* were found from 500 kg/da chicken manure dose and the highest a* and b values were noted as 17,35 under different organic manure doses. The lowest b* and h° values were observed from 500 kg/da chicken manure application. The lowest L*, a* and C* values were found from different organic manure applications. In conclusion, chicken and sheep manure applications had positive effects on leaf color parameters sage under field conditions, and 500 kg/da chicken manure application had the best result in field conditions.

Key Words: Sage, Leaf color parameters, Organic manures, *Salvia officinalis* L.

1. Introduction

Sage (*Salvia officinalis*) is one of the important medicinal and aromatic plants and it is cultivated in many parts of the world (Yaldiz and Camlica, 2021). Some metabolites such as alkaloids, fatty acids, phenolic compounds and steroids are the predominant medicinally valuable (Hernandez-Saavedra et al., 2016).

This plant is used for the preparation of many foods, and traditionally, it has been used in the treatment of dyspepsia (such as heartburn and swelling), excessive sweating, ulcers, gout, rheumatism, inflammation, age-related cognitive disorders, sore throat and skin inflammation (Zargari, 1990).

Irradiation affects the morphological and physiological characteristics of plants, and it is one of the major environmental factors to affect the growth, yield, distribution and survival of plants (Keller et al., 2005; Kumar et al., 2011). In addition, plant growth stages, cultivar, leaf thickness, plant population

and different soil and climatic factors causing the leaf chlorosis effected the leaf color values of plant (Turner and Jund, 1994).

It was reported that leaf color can be used to determine the stress conditions of the plants to adapt the environmental factors (Brand et al., 1998). Moreover, the leaf color formation occurs based on comprehensive effect of pigments like chlorophyll, anthocyanin and carotenoid (Fan and Huang, 2013). It was also reported that the variation in ratio of anthocyanin content to chlorophyll content effected the colorful leaves of plants (Juan et al., 2008; Wang et al., 2012).

Organic manures supply plant nutrient and increase soil organic matter content as a natural, renewable source in sustainable agricultural system (Foissy et al., 2013). It is noted that organic manures can be used as an alternative instead of chemical fertilizer (Ozliman et al., 2021).

Furthermore, cultivation of medicinal and aromatic plants (MAPs) under integrated management with organic and inorganic fertilizers is the best present strategy. Because, the yield and active compounds of MAPs increase under grown these fertilizers (Malik et al., 2011; Yaldiz et al., 2019).

Many studies were conducted on the variability of morphology, yield and essential oil content of sage under grown different organic manures. However, no study cannot be found on sage leaf color under grown organic manures. Firstly, this study was conducted to determine the leaf color variability of sage grown under different organic manures.

2. Material and Methods

2.1. Plant Material, Experimental Design and Growing Conditions

Plant material were obtained from Atatürk Horticultural Central Research Institute, Yalova, Turkey. The study was conducted between May to September in 2020 year.

Seeds were sown in plastic pots (400 mm diameter) filled with 4 kg of field soil in 20 November 2019. During the growing period, no fertilizer or chemical application was applied to the plant. Plants in all pots were up to 10 cm in May 2020, and experiment was conducted in the Completely Randomized Blocks Design with three replications under field condition. Each experimental plot consisted of five rows, with a distance of 60 cm between each row and 45 cm between each plant, and five rows each having six plants were maintained in the plot. For the distance between the plots, a meter block was considered. Two cuttings were carried out on 9 June and 22 August 2020, respectively (Figure 1).

Soil properties of experimental area were as follows: rich in phosphorus (12.19 ppm), potassium (51.04 ppm) and organic matter (3.1%); clay-loam and slightly alkaline (pH=7.6). In this context, four different organic manures as sheep (500, 750, 1000, 1250 kg da⁻¹), chicken (500, 750, 1000, 1250 kg da⁻¹) and vermicompost (50, 100, 150, 200 kg da⁻¹) with a control (no manure) and conventional fertilizer were used.

Table 1. The design of field experiment

No	Application name	Amount of application	Code
1	Conventional fertilizer	15 kg da ⁻¹ AS+12 kg da ⁻¹ DAP	IOF
2	Control	-	Control
3	Vermicompost-1	50 kg da ⁻¹	V1
4	Vermicompost-2	100 kg da ⁻¹	V2
5	Vermicompost-3	150 kg da ⁻¹	V3
6	Vermicompost-4	200 kg da ⁻¹	V4
7	Chicken manure-1	500 kg da ⁻¹	C1

8	Chicken manure-2	750 kg da ⁻¹	C2
9	Chicken manure-3	1000 kg da ⁻¹	C3
10	Chicken manure-4	1250 kg da ⁻¹	C4
11	Sheep manure-1	500 kg da ⁻¹	S1
12	Sheep manure-2	750 kg da ⁻¹	S2
13	Sheep manure-3	1000 kg da ⁻¹	S3
14	Sheep manure-4	1250 kg da ⁻¹	S4

Compared to organic manures, SM has the highest organic matter, calcium, magnesium, iron, copper and pH content, and CM has the highest zinc and manganese content. However, VM had the highest total nitrogen, phosphorus, potassium and moisture content compared to SM and CM (Table 2).

Table 2. Chemical analysis of applied organic manures

Organic manure	Unit	Vermicompost	Chicken manure	Sheep manure
Organic matter	%	42.50	89.00	90.00
pH (potentiometric)		6-8	7.08	8.50
EC (1/10)	ms/cm	6.52	6.88	6.65
Total nitrogen	%	2.40	0.70	0.60
Phosphorus	%	1.70	0.13	0.90
Potassium	%	1.00	0.44	0.42
Calcium	ppm	50000	43200	60200
Magnesium	ppm		7700	8800
Iron	ppm		900	1000
Zinc	ppm		480	210
Manganese	ppm		570	400
Copper	ppm		70	130
Moisture	%	19	6	5

As the base fertilizer, 15 kg da⁻¹ diammonium phosphate, and 6 kg da⁻¹ ammonium sulphate (21% N) were used and then 6 kg da⁻¹ AS was applied as top fertilizer before flowering date. The color parameters were measured before harvest.



Figure 1. The pictures in experiment area

2.2. Determination of leaf color

Color determination of the skin surface for sage leaves were performed with colorimeter under grown with different organic manures. Uniform CIELAB space parameters; lightness (L^*), redness/greenness (a^*), yellowness/blueness (b^*), Chroma (C^*) and hue angle (H) were obtained. These characteristic values were read directly from the device and their means were given. The properties were classified as follows: L^* : Lightness (black: 0, white: 100), a^* : Green (-60) and red (+60) color directions, b^* : Blue(-60) and yellow (+60) color directions, C^* : Chroma value (0-60), h° : Hue angle (red:0°, yellow: 90°, green: 180°, blue: 270°).

2.3. Statistical analysis

The obtained data were analyzed using JMP-13 statistical software and results were compared via least significant difference test (LSD) in order to find differences among the organic manure applications in sage at $p < 0.05$.

3. Results and Discussion

3.1. L^* (Lightness)

The L^* values changed between 51.93-66.66 under different organic manure applications with 57.82 mean. The highest L^* value was found from C1 (500 kg/da chicken manure) application and followed by control and V4 applications. The lowest L^* values was observed in the V2 application and

followed by V1 and V3 (Table 3). All of the L* values of sage under different organic manures were found to be close to white over 50.

3.2. Redness/greenness (a*)

The lowest application of S1 (13.36) and C1 (12.57) with control applications (17.35), compared with the other experiments, resulted in the highest a* values. C1, and C2 applications contained the highest a* values compared with the other organic manure applications, except S1. The a* values were found as positive, and it can be said that a* values of sage under different organic manures are red. This study clearly demonstrated that the application of chicken manure had a positive impact on leaf color parameters (Table 3).

3.3. Yellowness/blueness (b*)

There was found significant differences among the different organic manure applications on sage in terms of b* values (Table 3). The b* values were found between 12.97-17.35 and mean was notes as 14.68. The highest b* value was found from S4 applications and followed by C4 (16.18) and S2 (15.37). The lowest value was observed in C1 and C3. According b* values, the leaf colors of sage was determined as yellow depending on the evaluation of the color parameters.

3.4. Chroma value (C*)

Significant differences were found among the organic manure applications interms of C* values (p<0.05). It changed between 15.12-24.09 and the highest C* values were found from C1, control and V4 applications. The lowest values were observed from V1, S2 and V3 organic manure applications (Table 3).

3.5. Hue angle (h°)

There was found large variability in Hue angle values. It varied from 30.60-77.80 and the highest value was found from S2 application and followed by S4 (77.78), V3 (77.02) and C4 (73.58). The lowest values were noted in C1, V4 and control applications (Table 3). Generally, hue angle values were found between red-yellow and three applications were found to be close to red (<50°).

Table 3. Leaf color parameters of sage under different organic manure

Applications/ parameters	L*	a*	b*	C*	h°
IOF	59,03cde	6,54bcd	14,30c-f	16,08de	66,33abc
Control	64,70ab	17,35a	14,83c-f	22,83ab	40,68de
S1	60,61bcd	13,36ab	13,83efg	20,15a-d	50,20cde
S2	55,62d-h	3,31d	15,37bc	15,43e	77,80a
S3	56,07d-h	8,83a-d	15,20bc	18,24b-e	62,41abc
S4	57,41d-g	2,79d	17,35a	17,78cde	77,78a
C1	66,66a	12,57ab	12,97g	24,09a	30,60e
C2	58,28c-f	12,50abc	13,92d-g	19,63a-e	52,51bcd
C3	56,17d-h	11,66a-d	12,89g	18,33b-e	51,86bcd
C4	53,95e-h	4,80bcd	16,18ab	16,95de	73,58a
V1	53,02gh	4,83bcd	14,33c-f	15,12e	71,52ab
V2	51,93h	5,44bcd	15,18bcd	16,07de	71,65ab
V3	53,37fgh	3,35cd	15,10b-e	15,85de	77,02a
V4	62,63abc	11,97a-d	13,70fg	22,03abc	40,22de
Mean	57,82	8,52	14,65	18,47	60,3
LSD (5%)	5,19	9,19	1,27	4,71	20,06



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Previous study was reported that color sage leaves grown under full sunlight (0%), and 30%, 50%, and 70% shade changed between 49.02-52.89 for L*, between 9.96-15.02 for C* and 98.44-112.26 for h° values (Rezai et al., 2018). L* and C* values obtained from our study were found higher, and h° value was found lower than Rezai et al. (2018). The differences can be explained depending on genotype difference, environmental factors and growing conditions.

4. Conclusion

The leaf color parameters of the sage showed differences under different organic manure applications.

V1 (50 kg/da) and V2 (100 kg/da) applications had dark green leaves with the lowest C* (15.12) and L* (51.93) values for leaf color, respectively. Overall, the results of this study were suggested that applications of organic manures have different effect on leaf color of sage. As suggested by our study, suitable applications of different organic manures can increase the L* and C* values of sage. a* b* and H values can increase depending on the control, S4 and S2 applications, respectively.

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FULL TEXT–ORAL PRESENTATION

EVALUATION OF SOME BIOLOGICAL ACTIVITIES OF *SALVIA SERICEOTOMENTOSA* EXTRACTS PREPARED BY DIFFERENT EXTRACTION METHODS

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Abstract

Since ancient times, plants, herbs and spices have been used for their several biological activities and medicinal properties. Plants' importance for contemporary life is increasingly being recognized. Therefore, traditionally used medicinal plants also represent potential sources for developing new drugs [1]. *Salvia* species, known as "sage" in Turkey, are used to treat many diseases such as colds, tooth and throat aches, menstrual problems, Alzheimer's disease and diabetes. Various biological properties of extracts and essential oils obtained from *Salvia* species, such as antitumor, antioxidant, antimicrobial, antidiabetic and anti-inflammatory activities, have been reported before [2]. *Salvia sericeotomentosa* is an endemic plant for our country. In this study, the cytotoxic effects and total phenolic contents of the extracts prepared by three different methods (infusion, maceration and soxhlet extraction) using the aerial parts of the endemic *Salvia sericeotomentosa* plant were determined comparatively. Cytotoxicity was determined by the MTT test and total phenolic determination was made by the Folin Ciocalteu test. The obtained data revealed that the infusion extract was the most effective extract both in terms of cytotoxicity and total phenolic content. This study is a pioneering study for future studies, and it is very important to evaluate cytotoxicity in different cell lines in a broad perspective.

Key Words: Cytotoxicity, nürşalbası, Turkey.

1. Introduction

People have needed plants to meet many basic needs for centuries. Despite this, only 1-10% of the approximately 500,000 plant species found in the world are used by humans (Kunzelman et al., 2005). Plant extracts are an important source of bioactive compounds for many drug development programs, and a large number of important drugs have been obtained and identified from plants. Medicinal plants in particular are used for various purposes, for example; it is extracted and made functional for direct consumption as herbal or traditional medicine, or prepared for use for experimental purposes (Abubakar& Haque 2020). Medicinal plants have been the source of many biologically active compounds for centuries and are widely used as raw materials for treating various disease states. Medicinal plants come to the fore mainly due to their use as a source of therapeutic compounds that can shed light on the development of new drugs. Many of these compounds, especially phenolic compounds and flavonoids, have been reported to be important for human health, including cancer prevention and cancer therapy (Wahle et al., 2010; Venugopal & Liu, 2012).

The extraction of medicinal plants is the process of separating active plant materials or various secondary metabolites using an appropriate solvent and extraction procedure. In general, polar solvents such as methanol, ethanol and water are used in the extraction of polar compounds, while non-polar solvents such as dichloromethane and hexane are used in the extraction of non-polar compounds (Sasidharan et al., 2011; Pandey& Tripathi, 2014; Altemimi et al., 2017). Considering the



phytochemicals to be obtained from a medicinal plant, the extraction method chosen for the plant and the choice of solvent are very crucial.

Salvia is the largest genus of the Lamiaceae family, which includes more than 900 species of medicinal and aromatic plants (Askari et al., 2021). Many *Salvia* species are native to the Mediterranean region, and some *Salvia* species are used worldwide as spices, in addition to their use as traditional herbal medicines. *Salvia* species are traditionally used for asthma, bronchitis, cough, digestive and circulatory disorders, angina, mouth and throat inflammation, skin diseases, depression and many other diseases (Khalil et al., 2011; Walch et al., 2011; Khan et al., 2011).

Salvia sericeotomentosa is a plant species endemic to our country and the number of studies conducted on the species has been quite limited.

The objective of this study is to determine the cytotoxic effects and total phenolic content of extracts prepared by three different methods (infusion, maceration and soxhlet extraction) using the aboveground parts of the endemic *Salvia sericeotomentosa* plant comparatively.

Material and Methods

1.1. Plant material collection and extract preparation

The plant material was collected from Hatay Kisecik village, Amanos mountain, the path to the radar on May 2021. The collected material was cleaned and dried without direct sunlight. After the material was pulverised via grind and extracted with three different techniques as infusion, maceration and soxhlet extraction. Methanol was used as a solvent in maceration and Soxhlet extraction and water used for infusion method. The extracts were separated from their solvent with a rotary evaporator. Crude extracts were stored at -20°C until use.

1.2. Cell Culture

The human acute promyelocytic leukaemia cell line HL60 was kindly provided by Dr Zerrin CANTURK, and they were used for cytotoxicity assignment. 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% CO₂ and 37 °C.

1.3. Cytotoxicity Assay

Extracts prepared with various concentrations (0.0625-1 mg/ml) and the applied cells incubated for two-time intervals (24-48h). Cytotoxicity experiments were carried out via MTT assay. Viable tumor cells were counted thanks to their ability to reduce the yellow MTT reagent to a blue formazan crystal (Mossman, 1983). The absorbance of the plates was then measured at 570 nm. Each experiment was performed in three replicates, at least three wells. Cell viability (%) values were calculated by proportioning the absorbance of the wells to which the extract was applied to the control group.

1.4. Total Phenolic Content Determination (TPC)

The total phenolic content (TPC) of the extracts was evaluated according to the previous method with some minor modifications (Slinkard et al., 1977; Ahmed et al., 2015). Each extract was prepared at 1 mg mL⁻¹ concentration. Folin-Ciocalteu reagent was used for the determination of total phenolic content. And then samples were covered with aluminium foil and incubated in a water bath at 40 °C for 30 minutes. Gallic acid was used as a standard and the absorbance of the standard and extracts were determined at 765 nm.

2. Results

2.1. Cytotoxicity Assay Results

The MTT assay was used to reveal the cytotoxic effects of the extracts. The absorbance values were compared with the control group and % viability values were calculated and graphs were created

(Figure 1). When we evaluated the MTT test results over a 24-hour period, it was found that all extracts showed a dose-dependent cytotoxic effect. However, it is clearly seen that the most effective of the extracts is the infusion extract (IC₅₀: 0,115 mg/ml). When we evaluate the 48-hour results of the extracts, we can likewise mention the presence of a dose-dependent cytotoxic effect and clearly state that the most effective extract is the infusion extract (IC₅₀: 0,071 mg/ml). If we evaluate our extracts in general for both time intervals, we can say that they show a dose- and time-dependent cytotoxic effect. From the point of view of cytotoxicity, it has been found that extracts prepared in different ways have a close cytotoxic effect on each other, and especially the form that we consume as tea is more effective.

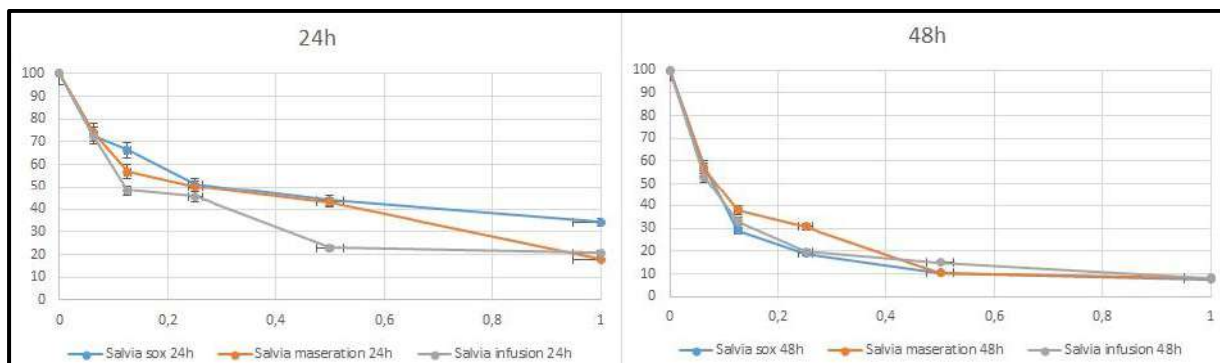


Figure 1. MTT Assay graphs of applied *Salvia* extracts

2.2. Total phenolic content (TPC)

It is known that various phenolic substances found in plants are highly valuable in cancer studies. We used a Folin assay to determine whether the extraction technique had an effect on the total phenolic content. The data obtained showed that the total phenolic content changed as infusion > soxhlet > maceration. This condition is compatible with the cytotoxic effect. The observed cytotoxic effect may be due to phenolic components. This will become clear in our future studies when we conduct more detailed studies of the chemical composition and phenolic content of the extracts.

3. Discussion

With this study, it has been shown once again how important plants are in the treatment of cancer. In particular, the study of wild, previously unstudied species is very important in this regard. The unique aspect of our study is that there are no reports on the biological effects of the *Salvia serotomentosa* species we used in the study. Although not related to this species, there are numerous studies evaluating the various biological activities of different *Salvia* species.

S. officinalis extract was reported to inhibit angiogenesis in vivo, which could be a new starting point for the development of a new anti-angiogenic drug (Keshavarz et al., 2011). And also some diterpenoids isolated from the roots of the plant have been reported to have DNA-damaging and cytotoxic activity in Hepg2cells and Caco-2 cells (Hadri et al., 2010). In another study, Ly, Te and Ca found in *Salvia libanotica* were reported to cause synergistically induced cell cycle arrest and apoptosis on HCT-116 without any effect on the development of standard human intestinal cell lines. As a result, it was underlined that the components of sage oil promise chemotherapeutic agents against colon cancer (İtani et al., 2008).

Ethanollic extracts of *Salvia dominica*, *Salvia hierosolymitana*, and *Salvia indica* were evaluated for their anti-proliferative activity on a MCF-7 (breast cancer) cell line. And it was reported these extracts exhibited high cytotoxic activity (Abu Dahab et al., 2007). In another study conducted earlier, the antitumor activity of methanolic *Salvia menthifolia* extracts upon DBTRG-05MG cell line (human glioblastoma) has been investigated. As a result of the study, it was reported that the plant is a source of antitumor agents (Fiore et al., 2012). It was reported that *S. leriifolia* exhibited a strong inhibitory



activity on ACHN, COR-L23, C32, and A375 respectively. The cytotoxic activity of *Salvia verticillata* essential oil was investigated against Caco-2, HT-29, T47D and NIH-3T3 cell lines. Consequently, the essential oil of *S. verticillata* showed a higher cytotoxic effect on the Caco-2 cell line (Khosravi et al., 2014).

In conclusion, the anticancer effects of different *Salvia* species are known. With this study, a new one has been added to the information in this field. Our study is a pioneering study and our studies will continue with different cell lines, primarily to be able to see the cytotoxic effect from a broader perspective. Then, we will try to clarify at the molecular level what the mechanism that causes cell death (apoptosis or necrosis) is. The active substance/substances will be determined by performing a content analysis of the obtained extracts.

Conflict of Interest

The authors declare that there is no conflict of interest.

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FULL TEXT–ORAL PRESENTATION

**KARYOTYPE ANALYSIS OF *HYACINTHELLA LINEATA* AND
HYACINTHELLA ACUTILOBA (ASPARAGACEAE) IN TURKEY**

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Abstract

The genus *Hyacinthella* Schur is a member of the Asparagaceae family, which includes aromatic and medicinal plants. *Hyacinthella* is represented by 17 species in the world. The genus consists of 12 species, 10 of which are endemic in Turkey. The aim of this study is to determine the chromosome number and morphology of *Hyacinthella lineata* (Steud. ex Schult. & Schult.f.) Chouard and *Hyacinthella acutiloba* K.Perss. & Wendelbo. Chromosome counts were made by germinating bulbs belonging to taxa collected during field studies. The squashing technique was used for chromosome studies and counts were performed at somatic metaphase. The karyotype measurements of the examined taxa were carried out with the KAMERAM program and the taxa were compared in terms of their karyomorphology using asymmetry indices (CV_{CL} , CV_{CI} , AI and M_{CA}). The chromosomal counts confirmed the previous reports of $2n=16$ and $2n=18$ given for *H. lineata* and *H. acutiloba*, respectively. In conclusion, this study is the first report for chromosomal asymmetry indices of analyzed taxa.

Key Words: Chromosome count, endemic, karyomorphology, asymmetry, Turkey.

1. Introduction

Turkey is one of the important biodiversity centers in the world. The sources of this richness include having different soil types and topography, hosting three different floristic geographies and feeling different micro-macro climate structures (Karaköse 2019). Our country has a very rich gene source in terms of *Hyacinthella* species. While the genus is represented by 17 species in the world, 10 of them are endemic for our country (Güner et al. 2012). *Hyacinthella* has been included in the Asparagaceae s.l. family with the arrangements made within the scope of APG III (Chase et al. 2009). The genera in this family are very interesting as natural ingredient sources in the pharmaceutical field. Biological activity and phenolic contents were determined in some species belonging to the *Hyacinthella* (Aydın and Mammadov 2017). There are morphological, palinological, anatomical and karyological studies on this genus (Persson and Wendelbo 1981, 1982, 1988; Persson and Persson 1992; Kandemir et al. 2000; Selvi et al. 2008; Çelik et al. 2004; Akpulat and Çelik 2005; Tekin and Meriç 2013; Acar Şahin and Eroğlu 2022). The use of karyological data in taxonomy contributes to the assessment of the genetic relationship among species or populations (Greilhuber and Ehrendorfer 1988; Guerra 2008). In the literature, the chromosome numbers of the species belonging to the genus *Hyacinthella* have been reported as $2n=16$, 18, 20, 22 and 24 (Persson and Wendelbo 1981, 1982, 1984; Persson and Persson 2001; Gedik and Kocabaş 2020). The aim of this study is to determine the chromosome number and

morphology of *Hyacinthella lineata* (Steud. ex Schult. & Schult.f.) Chouard and *Hyacinthella acutiloba* K.Perss. & Wendelbo.

2. Material and Methods

Chromosome counts were made by germinating bulbs belonging to taxa collected during field studies (Table 1). The squashing technique was used during chromosome research and the counts were carried out in the somatic metaphase (Golblatt 1996). For this, meristems at the root tips of germinated bulbs were used. Root tips were first pretreated with 8-hydroxyquinoline at 4°C for eight hours, then the material was fixed with Karnoy at low temperature for 24 hours. Before staining, the material was hydrolyzed with 5M HCl for one hour at room temperature. For the material dyeing process was stained with 1% aceto-orsein. After obtaining the appropriate metaphase, the chromosome images were taken with an Olympus BX53 microscope-Olympus DP72 digital camera. The karyotype measurements of the taxa examined in this study were carried out with the KAMERAM program and the taxa were compared in terms of their karyomorphology using various symmetry indices (Levan et al. 1964; Zarco 1986; Paszko 2006). In addition, the mean centromeric index (M_{CA}) was calculated according to Peruzzi and Eroğlu (2013).

Table 1. Localities of taxa studied

Taxa	Locality
<i>Hyacinthella lineata</i>	Denizli, Çay-Denizli Yolu, 1200m, yolun sağındaki sarp kayalıklar üzeri, 24 v 2019, <i>T.Uysal</i> 3927 (KNYA).
<i>Hyacinthella acutiloba</i>	Sivas, Beypınar köyü civarı, Tohma çayı, Kaynarca köyüne varmadan 500 m önce, kremi-sarı kalker, 1700 m, 20 iv 2019, <i>T.Uysal</i> 3871 (KNYA).

3. Results and Discussion

In this study, chromosome numbers, karyograms, idiograms and karyotype asymmetries of *Hyacinthella lineata* and *Hyacinthella acutiloba*, which are endemic to our country, were determined. *H. lineata* ($2n=16$) and *H. acutiloba* ($2n=18$) have diploid chromosome numbers (Tables 2 and 3; Figure 1). The chromosome numbers for these species were previously reported as $2n=16$ and $2n=18$ by some researchers (Persson and Wendelbo 1981, 1982; Gedik and Kocabaş 2020).

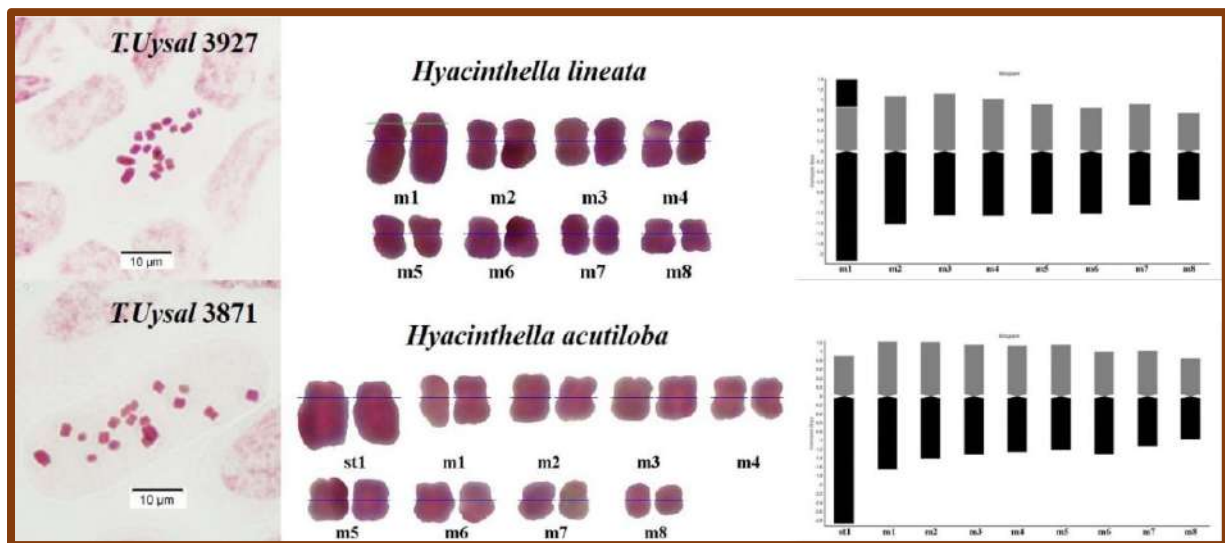


Figure 1. Mitotic metaphase chromosomes, karyograms, and idiograms of *H. lineata* and *H. acutiloba*

Table 2. The chromosome features of *H. lineata* and *H. acutiloba*. 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, CF: chromosome formula, m: metacentric, st: subtelocentric.

Collector numbers	Taxa	2n	x	R (SC-LC) μm	Ratio LC/SC	p (μm) mean ($\pm\text{SD}$)	q (μm) mean ($\pm\text{SD}$)	CL (μm) mean ($\pm\text{SD}$)	TCL	CI mean ($\pm\text{SD}$)	CF
<i>T.Uysal 3927</i>	<i>H. lineata</i>	16	8	1.71 – 3.55	2.079	1.01 (± 0.19)	1.32 (± 0.34)	2.32 (± 0.52)	18.574	44 (± 0.02)	2m ^{SAT} +14m
<i>T.Uysal 3871</i>	<i>H. acutiloba</i>	18	9	1.83 – 3.79	2.078	1.08 (± 0.13)	1.46 (± 0.53)	2.54 (± 0.52)	22.876	44 (± 0.07)	16m+2st

Table 3. The karyotype indices of *H. lineata* and *H. acutiloba*. A₁: intrachromosomal asymmetry index, A₂: interchromosomal asymmetry index, CV_{CL}: coefficient of variation of chromosome length, CV_{CI}: coefficient of variation of centromeric index, AI: karyotype asymmetry index, M_{CA}: mean centromeric asymmetry.

Collector numbers	Taxa	A ₁	A ₂	CV _{CL}	CV _{CI}	AI	M _{CA}
<i>T.Uysal 3927</i>	<i>H. lineata</i>	0.224	0.223	22.323	5.706	1.274	13.30
<i>T.Uysal 3871</i>	<i>H. acutiloba</i>	0.197	0.205	20.546	16.429	3.376	14.96

In previous studies, chromosome formula of *H. acutiloba* was reported as $n=9=6m+3sm$ (Kahramanmaraş; Gedik and Kocabaş 2020). Unlike the previous study reported (Gedik and Kocabaş 2020), the chromosome formula of *H. acutiloba* was determined as $2n=18=16m+2st$ (Sivas; Table 1). Similarly, the chromosome formula of *H. lineata* is $2n=16=2m^{\text{SAT}}+14m$ (Denizli; Table 1). This formula is inconsistent with previous studies (Manisa; Persson and Wendelbo 1981, 1982). It is reported that the karyotype formulas in different populations of a species may be different (Konichenko et al. 2014; Demirci Kayıran and Özhatay 2017). The difference habitats may have caused morphological differences or even genetic differences (Demirci Kayıran and Özhatay 2017). In addition, the satellites in the genus is quite common (Persson and Wendelbo 1982). Satellite was also detected in *H. lineata* in this study. The studied species have different basic chromosome numbers ($x=8$ and 9). The different counts ($x=8, 9, 10, 11$ and 12) detected within the genus are consistent with previous reports (Persson and Wendelbo 1982; Persson and Persson 2001). In fact, Persson and Wendelbo (1982) indicated that higher numbers ($x=10, 11$ and 12) are obviously derived and so are smaller (evident especially in some of the species with $x=11$ and 12) and more asymmetric chromosomes. Additionally, they emphasized that structural mutations such as translocations and inversions may be effective in the transition from symmetry to asymmetry within the genus. The presence of symmetrical and asymmetrical karyotypes indicates chromosomal rearrangements and different evolutionary processes (Uysal et al. 2017). In the karyotypes of the analysed species have a predominance of metacentric chromosomes. The asymmetry indices of *H. lineata* and *H. acutiloba* are detected as $CV_{CL}=22.32$; $M_{CA}=13.30$ and $CV_{CL}=20.54$; $M_{CA}=14.96$, respectively (Table 3). Similar results have been reported in the Muscari genus, closely related to *Hyacinthella*, in previous studies (Demirci Kayıran and Özhatay 2017; Bozkurt 2020; Uysal et al. 2021). Because *H. lineata* and *H. acutiloba* have the less basic chromosome number ($x=8$ and 9) and symmetrical karyotypes, we can infer that they are more primitive or ancestral taxa (Stebbins 1971; Persson and Wendelbo 1982; Uysal et al 2017; Demirci Kayıran and Özhatay 2017; Bozkurt 2020; Uysal et al. 2021). Persson and Wendelbo (1982) emphasized that *H. lineata* and *H. acutiloba* are closely related in terms of cytological and gross morphological characters and they have been reported that evolution in these taxa follows the process of increase and decrease in the number of chromosomes. In conclusion, it would be beneficial to evaluate cytological and molecular studies together for a better understanding of the evolutionary mechanism of the genus, completed largely karyology.



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FULL TEXT–ORAL PRESENTATION

THE PERFORMANCE AND SAFETY OF BIOAPIFIT® ANTI
HEMORRHOIDAL OINTMENT IN PREMARKET/POSTMARKET
STUDY IN THE TREATMENT OF HEMORRHOIDAL DISEASE

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Abstract

Objective / Purpose: Hemorrhoids are a very common anorectal condition defined as the symptomatic enlargement and distal displacement of the normal anal cushions. The objective of this study was assessment of the efficacy and safety of Bioapifit® anti hemorrhoidal ointment consisted of honey, *Cera flava*, glycerin, the oil macerates of *Achillea millefolium* L., *Plantago major* L., *Quercus robur* L., *Salvia officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Calendula officinalis* L., *Matricaria chamomilla* L., essential oils of *Melaleuca alternifolia*, *Thymus vulgaris* ct. Thymol and *Origanum vulgare* for the treatment of hemorrhoids of grade 1 to 3.

Materials and methods: 40 participants for premarket and 35 per year for post market investigation recruited. Bioapifit® ointment was 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: The external and rectal application of Bioapifit® ointment resulted in significant decrease of all the symptoms of haemorrhoidal disease both in premarket and post market studies at third day of the treatment ranging from 58% in premarket application to 61% in post market study. In the end of the treatment overall subjective discomfort decreased for 96% in premarket and up to 96.4% in post market investigation. There was no significant difference in the product efficacy between premarket and post market investigation. Clinical cure rate was observed in 85% of the patients both in premarket and post market investigation. None of the patients experienced any discomfort or adverse effect including allergic reaction, worsening of the existing or the occurrence of new symptoms during the treatment.

Conclusion / Discussion: Physical parameters like low pH, high osmolarity/low water activity, high viscosity, greasiness, coating and lubricating effect resulted in significant decrease of the symptoms of hamorrhoidal disease such as bleeding, itching, irritation, and pain as well as were wound infection.

Keywords: hemorrhoidal disease, honeybee's products, herbal macerate

1.INTRODUCTION

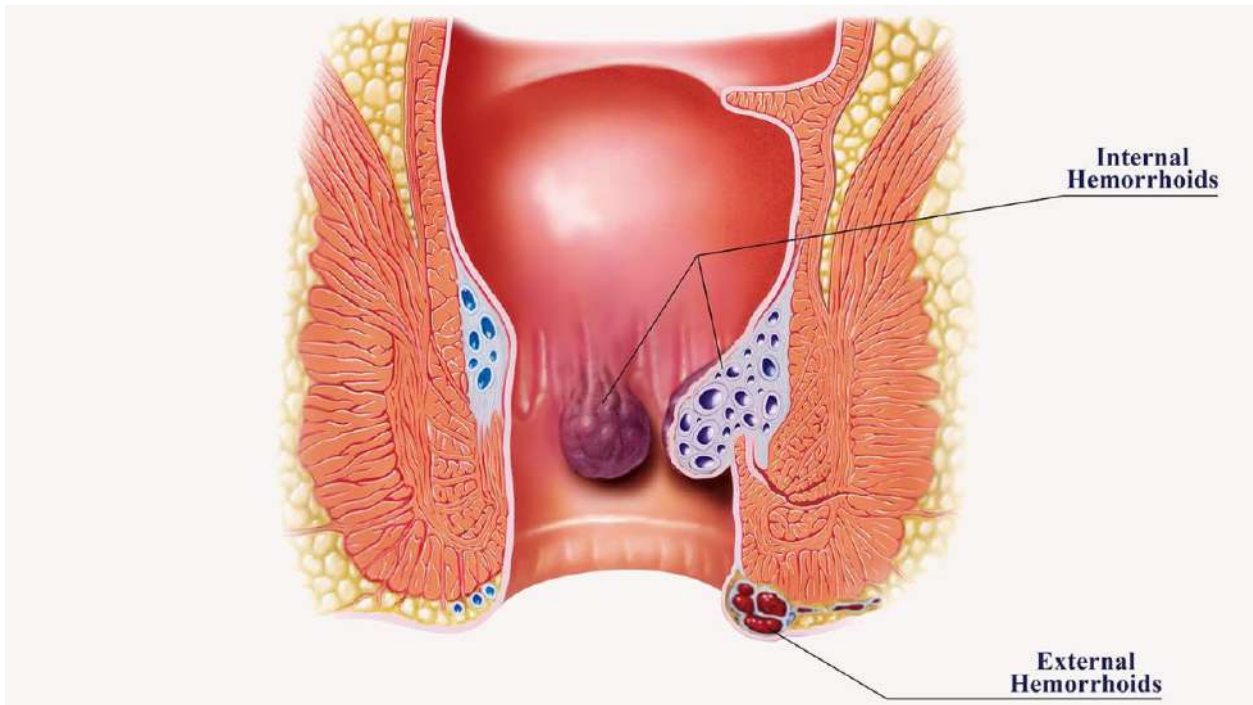
Hemorrhoids are a very common anorectal condition defined as the symptomatic enlargement and distal displacement of the normal anal cushions. (1)

Hemorrhoids (lat. *noduli haemorrhoidales*) are swollen, enlarged veins that form inside and outside the anus and rectum. More than half of the population of both sexes aged 50 and older will develop hemorrhoid symptoms in their lifetime. Hemoorrhoids cause symptoms when they become enlarged, inflamed, thrombosed, or prolapsed. (2) Risk factors are straining and constipation, pregnancy, obesity,

familial tendency, ages between 45 and 65 years old, loss of rectal muscle tone or Inflammatory bowel disease. (3)

Hemorrhoids are divided into internal and external. Signs of **external** hemorrhoids include: itchy anus, hard lumps near the anus that feel sore or tender, pain or ache in the anus, especially when you sit and rectal bleeding.

Internal hemorrhoids cannot cause cutaneous pain, because they are above the dentate line and are not innervated by cutaneous nerves. However, they can bleed, prolapse and cause perianal itching and irritation.



The diagnosis is made on the basis: physical examination (visual inspection of the rectum, digital rectal examination), anoscopy, proctosigmoidoscopy or colonoscopy. (3)

In the differential diagnoses they can be: anal cancer, anal fissures, anal fistulae; polyps; perianal abscesses; pruritus ani; prostatitis; proctitis, rectal prolapse, condyloma; inflammatory bowel disease; colorectal tumors.(4)

Degrees of hemorrhoidal disease:

1st degree: There are no visible signs, hemorrhoids are usually manifested only by bleeding.

2nd degree: It is accompanied by the sliding of hemorrhoids through the anal opening, especially when bending the body forward or when straining, and it spontaneously returns to the intestine.

3rd degree: In addition to bleeding in several places, there is an extensive spontaneous discharge of hemorrhoids through the anus. At this stage of the disease, the patient has to push the hemorrhoidal nodes back into the intestine with his own hand.

4th degree: Untreated hemorrhoids stretch more and more and start to come out. Despite being pushed back into the gut they always come out. (5)

Standard treatment approaches included: increased fiber and liquid intake, warm baths or avoidance of straining and prolonged toilet sitting. Unless it gets better than that used creams and ointments. If this does not help to solve hemorrhoidal disorders then we solve it surgically with the following techniques: rubber band ligation, sclerotherapy, electrocoagulation or hemorrhoidectomy. (6)



2. MATERIALS AND METHODS

Study Design

The objective of this study was assessment of the efficacy and safety of Bioapifit® anti hemorrhoidal ointment consisted of honey, *Cera flava*, glycerin, the oil macerates of *Achilea millefolium* L., *Plantago major* L., *Quercus robur* L., *Salvia officinalis* L., *Olea europaea* L., *Polygonum aviculare* L., *Calendula officinalis* L., *Matricaria chamomilla* L., essential oils of *Melaleuca alternifolia*, *Thymus vulgaris* ct. Thymol and *Origanum vulgare* for the treatment of hemorrhoids of grade 1 to 3.

Intended use. Bioapifit® anti hemorrhoidal ointment is intended for the alleviation of symptoms of hemorrhoids, fissures, *pruritus ani* and of Untreated hemorrhoids stretch more and more and start to come out. Despite being pushed back into the gut they always come out. her non-infective ano-rectal conditions such as: rectal bleeding, itching and irritation in the anal region, swelling around the anus, pain, defecation discomfort.

General description. Bioapifit® anti hemorrhoidal ointment is homogeneous, greasy, viscous mass of characteristic herbal odor and olive green color. Bioapifit® anti hemorrhoidal ointment is composed of honey, *Cera flava*, glycerin, the oil macerate of *Achilea millefolium* L., the oil macerate of *Plantago major* L., the oil macerate of *Quercus robur* L., the oil macerate of *Salvia officinalis* L., 7% of the oil macerate of *Olea europaea* L., the oil macerate of *Polygonum aviculare* L., the oil macerate of *Calendula officinalis* L., the oil macerate of *Matricaria chamomilla* L.; essential oils: *Melaleuca alternifolia*, *Timus vulgaris* ct. Thymol, *Origanum vulgare*.

Intended use. Bioapifit® anti hemorrhoidal ointment is intended for the alleviation of symptoms of hemorrhoids, fissures, *pruritus ani* and other non-infective ano-rectal conditions such as: rectal bleeding, itching and irritation in the anal region, swelling around the anus, pain, defecation discomfort.

Mode of action. Bioapifit® anti hemorrhoidal ointment relieves the symptoms of hemorrhoidal disease such as bleeding, itching, irritation and pain. The product is classified as medical device based on its physico-mechanical mode of action including:

- the creation of the protective coating on the damaged perianal and rectal mucosa, enabling its recovery and preventing further irritation and infection by the contact with stool;
- the creation of unfavorable conditions for the growth, adhesion and multiplications of the pathogens by restoring and maintaining acidic wound milieu and creating the environment with low water content;

pain and discomfort relief during defecation due the presence of the emollients and lubricants effects. The investigator will recruit patients during regular office visit, following the predefined inclusion and exclusion criteria. In total, 75 patients will be included. After the informed consent has been signed at Visit 1, Day 1 and the questionnaire filled in by each individual patient, all the participants will be subjected to clinical examination in order to confirm the grade of the hemorrhoidal disease, signs and symptoms. The questionnaire data and the clinical study results will be recorded in source data. Patients will be given of Bioapifit® anti hemorrhoidal ointment and instructions for use.

Diagnosis will be made based on the clinical examination and selfassessment of the severity of the symptoms at day 0, 3, 5 and 10. Should patients come back to the investigator exhibiting symptoms of their initial disease or report an AE after their follow-up visit, but within 30 days after the initial administration of Bioapifit® anti hemorrhoidal ointment, this will be recorded in their Case Report Form as an unscheduled visit.



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Method of Blinding and Randomization. The patients will be selected during their regular office visit and no blinding or randomization will be performed.

Medical Device Dose Study, Mode of Administration, and Dosage Requirements. Bioapifit® anti hemorrhoidal will be administered in line with the approved posology. The ointment will be applied externally three times a day onto clean perianal area and rectally once a day and suppositories rectally once a day (before bedtime) for 10 consecutive days.

Duration of Patient Participation Bioapifit® anti hemorrhoidal ointment will be administered for 10 consecutive days. Visit 2/ET will be performed at Day 11±2 days after the last dose has been administered.

Inclusion Criteria. Patients may be included in the study only if they meet all of the following criteria:

- The patients who are ≥18 years of age.
- The patients of both gender with clinically confirmed hemorrhoidal disease of grade 1 to 3 accompanied with one or more of the following symptoms: bleeding, itching, defecation discomfort and pain.
- In the investigator's judgment, the patients should receive local treatment only.
- Signed informed consent.

Exclusion Criteria. Patients will be excluded from participating in this study if they meet any of the following criteria:

- pregnancy,
- breast-feeding,
- presence of malignant or inflammatory bowel diseases,
- history of allergy to any ingredient of the product,
- hemorrhoids of grade 4,
- less than 18 years of age and previous history of anorectal surgery,
- participation in another clinical study,
- Patient is either unwilling or unable to use the investigational medical device.

Measures and Time Points: Visit 1 was performed at inclusion day. Visit 2/ET will be performed at Day 13±2 days.

Primary Measure and Time Point: The primary study objective is to assess the clinical efficacy of a 10-day treatment with Bioapifit® anti hemorrhoidal ointment to alleviate the symptoms of hemorrhoidal disease at Day 11±2 days.

Secondary Measures and Time Points: The secondary objective is to evaluate the safety of Bioapifit® anti hemorrhoidal ointment throughout the study.

Safety Measures and Time Points: Safety measures and time points will include the following: the safety of Bioapifit® anti hemorrhoidal ointment throughout the study, until Visit 2/ET.

Allowed and Disallowed Medications Before and Throughout the Study. Allowed medications: cardiovascular, gastrointestinal, respiratory drugs, drugs in chronic use. hormonal contraception, hormone-replacement therapy. Disallowed, medications: drug or medical device intended for the treatment of hemorrhoidal disease, immunosuppressant, chemotherapeutics, corticosteroids, antimicrobial drugs.



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3.Statistics. Statistical analyses will be performed using STATISTICA 11.0 software package. Frequencies tables and basic statistical parameters together with Post hoc comparison test will be applied.

Safety Analyses. All non-serious and serious adverse events and the related information (seriousness, relation to the investigational medical device, cause of withdrawal from the study, etc.) will be presented in individual data listing tables. The number and percentage of patients by whom at least one adverse event has been recorded will be calculated. The number and percentage of serious and non-serious adverse events will be presented in a summary table.

Adverse events if any are recorded and patients will be withdrawn from the study, as determined by the investigator.

Procedures at Visit 1 – Baseline. Patients will be recruited based during their regular office visit. A signed and dated informed consent form will be obtained before any protocol procedures commence. After the informed consent has been obtained, the patients included will be assigned a permanent identification number (by a nurse).

Patients who have signed the informed consent form and who meet the general inclusion/exclusion criteria, will fill in a questionnaire and undergo clinical examination.

The following procedures will be performed for patients with study indication at Visit 1:

- obtaining written informed consent before any other study-related procedures are performed,
- review of the general inclusion/exclusion criteria,
- filling in a questionnaire,
- review of medical history and allergy data,
- review of medication history,
- determination of the grade of hemorrhoidal disease,
- informing patients of study restrictions and compliance requirements,
- withdrawing from being involved in the medical device testing,
- data on concomitant participation in a clinical study involving any other medical product or device.

A patient who has not been enrolled in the study on the basis of their baseline assessment results will not be reconsidered for enrolment.

Procedures at Visit 2, Day 13±2 (Visit 2/ET). The following procedures/assessments will be performed at day 11±2 (Visit 2/ET):

- determination of the grade of hemorrhoidal disease,
- clinical efficacy assessments,
- treatment/medical device satisfaction assessment,
- adverse event inquiry,
- collecting medical device packaging.

Procedures Following the Investigation of the Medical Devices. Patients who have participated in the study in compliance with the protocol and have used a particular medical device for at least 10 days and



performed Visit 2/ET will be considered to have completed the study. Should a patient return to site within 30 days from Visit 1 and the initial administration of the investigational medical device to report the reoccurrence of hemorrhoidal disease symptoms or to report an adverse event, general data on symptoms will be collected and recorded in source data as unscheduled visit.

Procedures for patients who withdraw prematurely from the study are described.

If necessary, patients should receive treatment at the standard of care upon termination of the study.

4. RESULTS

Description of the Population

The study included 75 patients with hemorrhoidal disease graded from I to III that are treated with Bioapifit® anti hemorrhoidal ointment. Among them 24 (68.6%) were females and 11 (32,4) were male participants. The age of the patients ranged from 27 to 70 years (50.4±10.8). Body mass index ranged from 18.5 to 32.7 kg/m² (25.5±3.4 kg/m²).

According to the education level the majority of them College/University Degree or Master of Arts (48.6%) followed by secondary education (28.6%), Master of Science or Ph.D (20%) and Associate Degree (2.9%). 54.3% of them suffered from constipation and among 85.7% of them sedentary type occupation or lifestyle prevailed. 68.6% of them were not engaged in any sporting activity. Majority of them consumed boiled food preferentially (77.1%). The supplements with laxative effect consumed 65.7% of the population.

Results on clinical performance

Before the treatment 62.9% of the participants had first grade hemorrhoidal disease, 22.9% of them second grade and 14.3% of the participants third grade hemorrhoidal disease. Following the ten days of the treatment with Bioapifit® anti hemorrhoidal ointment complete disappearance of all the symptoms was confirmed in 88% of the patients while in 11.4% of them only mild symptoms persisted classified as first degree hemorrhoids.

Table 1. Mean values and standard deviations of the scores for each symptom of the hemorrhoidal disease and overall subjective discomfort prior and following 3, 5 and 10 days of the treatment with Bioapifit® anti-hemorrhodal ointment

Treatment time (days)	Pain	Defecation discomfort	Bleeding severity	Anal itching severity	Overall subjective discomfort
	$\bar{X}\pm SD$	$\bar{X}\pm SD$	$\bar{X}\pm SD$	$\bar{X}\pm SD$	$\bar{X}\pm SD$
0	5.1±1.4	5.1±1.6	1.6±1.0	2.4±1.0	5.1±1.7
3	1.8±1.1	2.0±1.1	0.5±0.6	1.0±0.7	2.0±1.0
5	0.7±0.7	0.7±0.7	0.0±0.0	0.4±0.7	1.0±1.2
10	0.0±0.2	0.1±0.3	0.0±0.0	0.2±0.4	0.2±0.5

At baseline, the mean values and standard deviations for the symptoms like pain, defecation discomfort, bleeding severity, anal itching severity and overall subjective discomfort were 5.1±1.4, 5.1±1.6, 1.6±1.0, 2.4±1.0 and 5.1±1.7, respectively (Table 1, Fig. 1). Following only three days of the treatment all the parameters decreased significantly to 1.8±1.1 for pain, 2.0±1.1 for defecation discomfort, 0.5±0.6 for bleeding, 1.0±0.7 for anal itching and 2.0±1.0 for overall subjective symptoms with decrease between 57.6% (for anal itching) to 71.4% (for bleeding severity). Further decrease of all the parameters was obtained following the fifth day of the treatment when all 35 participants reported no bleeding while pain was reduced for 86.4%, defecation discomfort for 87.2%, anal itching for 84.7% and overall subjective symptoms for 80%. Median value for pain, defecation discomfort and overall subjective symptoms were 1 and for bleeding and itching 0. At 10th day the median value of all the symptoms

including overall subjective discomfort was 0. The percentages of decrease of pain, defecation discomfort, bleeding severity, anal itching severity and overall subjective discomfort were 99.4%, 97.8%, 100.0%, 92.9% and 96.1%, respectively.

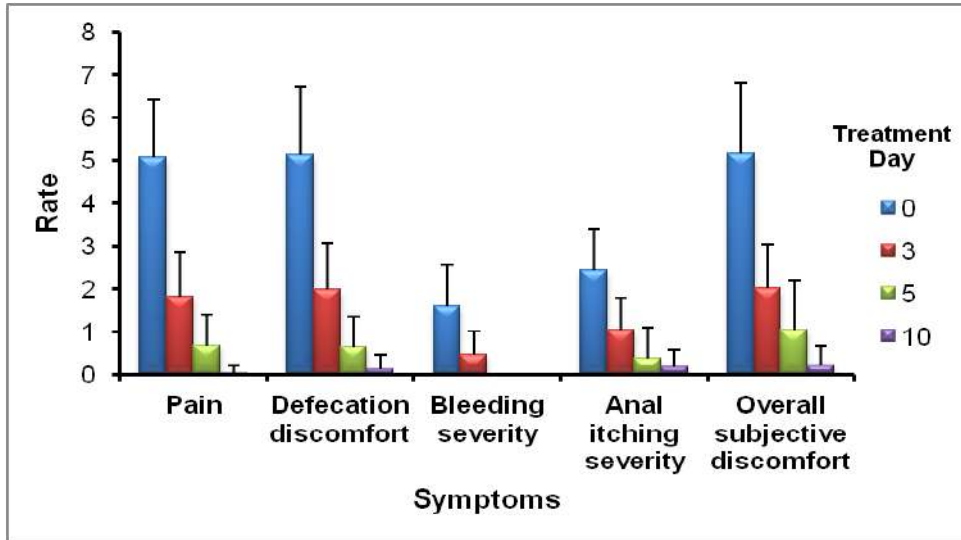


Figure 1. Mean values and standard deviations of the scores for each symptom of the hemorrhoidal disease and overall subjective discomfort prior and following 3, 5 and 10 days of the treatment with Bioapifit® anti-hemorrhoidal ointment

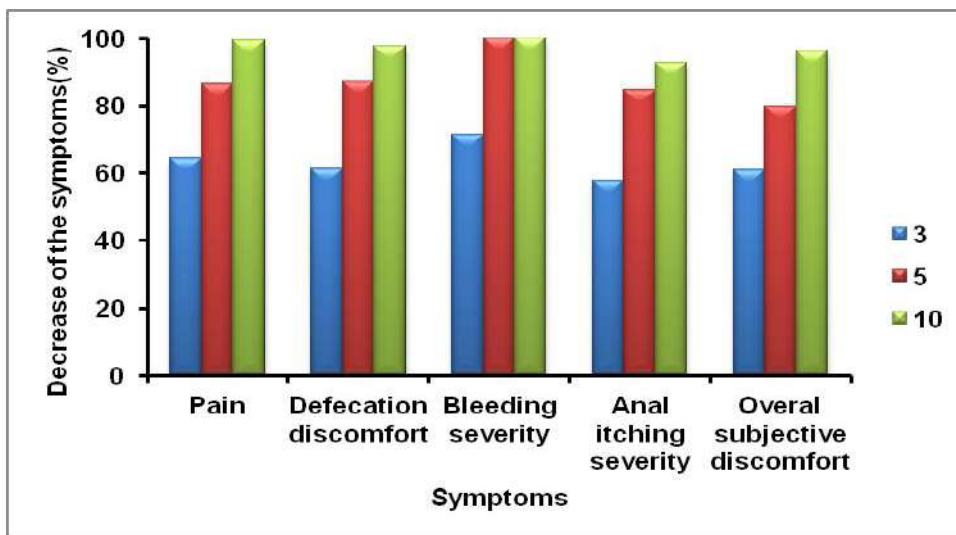


Figure 2. Percentage of decrease of the scores for each symptom of the hemorrhoidal disease and overall subjective discomfort following 3, 5 and 10 days of the treatment with Bioapifit® anti-hemorrhoidal ointment

Results on clinical safety

None of the patients experienced any discomfort or adverse effect including allergic reaction, worsening of the existing or the occurrence of new symptoms during the treatment. This was also confirmed by Principal investigators by physical examination. The patients reported decrease in pain and itching up to 20 minutes after the application of the ointment. After third day of the treatment 20 of 75 patients reported no bleeding while after five days bleeding stopped in all 75 participants. After five days 15 of



75 participants reported no pain and overall subjective discomfort, 16 of them no defecation discomfort and 26 of them no anal itching.

5.CONCLUSION

Efficacy results.

Ten days external and rectal application of Bioapifit[®] ointment resulted in significant decrease of all the symptoms of hemorrhoidal disease at third day of the treatment which ranged between 57.6% and 71.4% compared to baseline (Fig.2). In the end of the treatment overall subjective discomfort decreased for more than 96%. Clinical cure was observed in 88% of the patients.

Based on the results of the current study and premarket data it could be concluded that Bioapifit[®] anti hemorrhoidal ointment is clinically efficient in alleviating the symptoms of hemorrhoidal disease exclusively by physical manner due to coating, pH adjusting, and lubricating effect.

Safety results.

Based on the informations obtained from the patients or by direct examination by Principal investigators it is possible to conclude that tested medical device Bioapifit[®] anti hemorrhoidal ointment does not cause any adverse effect and is safe for perianal and rectal administration externally three times per day and rectally once a day for the period of ten days.

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FULL TEXT–ORAL PRESENTATION

THE FOOD USED IN MATERNAL AND NEWBORN HEALTHCARE IN BISKRA (SOUTHERN EAST OF ALGERIA): THE CASE OF HERBAL SWEET

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Abstract

Background: Food is considered to be the first means to health care, especially when is prepared with special ingredients, so there are dishes associated to special celebration in different civilisation. The birthday of a new born is one of the important celebrations in Algerian’s families that are marked with special food one of them is the herbal sweet of Biskra, served to mothers after the first day of delivery till 40th day, to know its specificity we laid an investigation about its composition. **Methods :**The survey was carried out on a simple of 60 females informants from Biskra region (Southern East of Algeria), during the two academic years 2020/2021 and 2021/2022. The main purpose of this study is to determine the level of women knowledge from Ziban region in maternal and newborn healthcare, to enumerate what are the herbs, spices and medicinal plants used for the preparation of special dishes using a questionnaire prepared in Arabic language. In this case, we study the herbal sweet (R’fiss l’hechahech). **Result :** our investigation in sweet herbal allowed us to inventory about 23 species of medicinal aromatic and herbal plants belonging to 10 botanic families. The oldest informants (grand-mothers and mothers) give us more information than the youngest informants (single and students). The most sited species are *Rosmarinus* and *Thymus* with 100 % of responses. The leaves are the most used part with 56 %, the most medicinal uses of the listed species are : promote lactation, calm abdominal pain and intestinal gas, and help the postpartum recovery. **Conclusion:** There remain, however many dishes that deserve further study, and it is very important to document them because the transfer of knowledge from the old generation to the new generation is diminishing.

Key words: maternal healthcare, investigation, herbs, spices

1. Introduction

Spices and condiments are an important part of human history and nutrition, and have played an important role in the development of most cultures around the world (**García-Casal, 2016**)

Spices are used in many traditional cultures to boost energy, relieve stress, improve the nervous system, aid digestion, relieve cold symptoms and headaches, and treat many diseases. A food creator can explore interesting food concepts that combine taste and “cure” by using authentic spices and methods of preparing and presenting them in meals (**Raghavan, 2007**).

The Zibans region is known for its traditional dishes, also its combination of condiments and sauces, due to the special fragrant herbs and spices grown in the region. These herb and spice- ingredients not



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only improve the taste and the color of the food, but they make these dishes healthier and allow people in the arid and Saharan zona to better adapt to these hard living conditions.

Sweet and cakes have always been the main offering in celebrations and ceremonies in Algerian cultures, plus that, the presence of dates as principal ingredient of those recipe is the characteristic of the Ziban region located in the southen east of Algeria and ranked in the first place for its dates production .

One of this celebrations is the born of new child, **Auballe-Sallinave (1997)** explain that the birth of a child is an important social event that the entourage watches for and which will fundamentally change the status of its mother. Even before birth and during the forty days following childbirth, certain elements of life take on great value, with increased intensity during the first seven days.

The 40 days after childbirth are particularly dangerous, both for the mother and for the child. The proverb says:

gbar nâfsa yibqâ ‘arba’în yûm mah’lûl

The grave of a woman in childbirth remains open for forty days!

It is for this reason that the mother must eat substantial food: eggs, milk, honey, olive oil, fenugreek and chickpeas (**Claus Gilbert, 1997**).

Nutrition and diet therapy includes preparations of medicinal herbs in the form of decoction and infusion. Medicinal plants given during this period are traditionally known to strengthen the body and to prevent disorders such as postpartum depression, insomnia, and constipation. This diet is also helpful for the growth and development of the new born as bioactive molecules pass from mother to the child through lactation (**Ali-Shtayeh et al, 2015**).

Some of the traditional dishes of Algeria explain **Laksir et al (2019)**, are served to the mother after delivery and to the guest who come to celebrate her safety afer giving birth, we cited :

El merdoud (vaiety of berkoukess with herbs), B’ssissa or tomina (fat and sweet with honey, butter and nuts), M’laoui (taditional bead) and Rfiss (traditionnal Algerian cake with lozande shape, made with semolina, dates and butter).

In Zibans region, during the new born celebrations, that correspond to the saventh day after birth, there is always the special hebal sweet made with semolina, date, nuts, vegetable oil, spices and fragrant herbs called R’fiss l’hechahech ,or elklil .

The aim of the sturdy

It is the purpose of this paper to document the herbs, spices and the medicinal plants involoved in the preparation of the herbal sweet.

This study is aimed at contributing to safeguard the gastronomic heritage and document ethnomedicinal uses the plants of the Ziban’s region (Southen Easten of Algeria) in the preparation of the herbal sweet as well as the indigenous knowledge related to the use of this natural resource in healthcare of the maternal and the new born by the local population.

We aimed also to evaluate the inheritance of this knowledge from the last generation to the next one by choosing two cathogories of women’s ages and the level of instruction.

2. Material and methods

The survey was carried out from March –September 2020 (during the confinement of the covid 19 pandemic) on a sample of 30 female informantes from Zibans region (the Southern East of Algeria) for that reason the questionnaire was distributed to the students from the department of agronomic sciences, university of Biskra, by e-mail, and to the older category of women by call-phone interview or using the e-mail of their progeniture.

We prepared a questionnaire in arabic, based on semi-structured questions with multiples choises to collescte informations about the informants , and open answers for the part that deals with the number of medicinal, aromatic plants, spices, herbs (MAHS) used in the herbal sweet (R’fiss el m’rou) recipe.

We documented the medicinal uses of the spices and herbs invontoried during the survey using the handbooks, encyclopedea related to the medicinal plants, spices and condiments, also from reseach aticles that trait one of some of the listed species.

Area of study

Biskra was the capital of the Ziban and the Aures. The study area occupies a privileged position in the south-east of Algeria between 34°52' N, 5°45' E covering an area of 2 167.20 km² (Saad et al, 2019), under a form of an oasis with multi-layered cultivation (date palm, arboriculture, and crops herbaceous or market gardening) or in the form of irrigated perimeters and greenhouse shelters more specialized (date palms, cereals or vegetable crops) (Rechrachi, 2017).

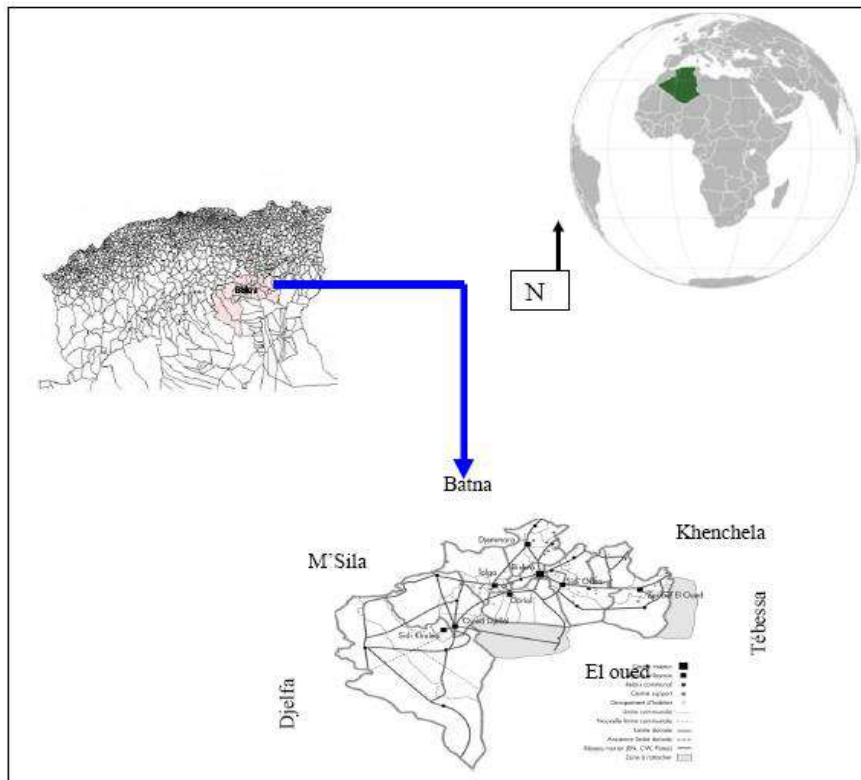


Figure 1: Location of the study area (Biskra , South-East of Algeria)

The Zibans are also known, but in a lesser way, for the cultivation of aromatic plants and condiment (Ramdani et al, 2009).

3. Results and discussion

3.1. Informants women

The average age of the informant's women is 45,33 year, the average age of the 15 students is 23,46, they are all single, and they listed an average of 5,9 of MAHS, in the other hand the average age of the 15 mothers /grandmothers is 67,8, they have all the experience on maternal healthcare their level of instruction is: 40 % were analphabet, 40 % with primary education and the last 20 % were medium, high and university instructions, and they listed an average of 13.9 MAHS with the maximum of 23 species

In this study, 25 MAHS belonging to 11 botanical families were reported to be involved in the preparation of the traditional herbal sweet named locally by R'fiss l'mroure used by the women in maternal and newborn health care.

3.2. Ethnobotanical survey

The participants of this survey give us information about the recipe to prepare the herbal sweet, (figure 2) we can divide the ingredients into 5 categories :

1. HSMP: the Latin, English, and Arabic names of those species, the used part, and the medicinal used are collected in **table 1**.
2. The dough of date: from the fruit of *Phoenix dactylefera*, the Ghars variety. The dough is prepared either from the new production or from the conserved fruit, it needs to be separated from its seeds and crushed to obtained homogenized dough.
3. Semolina: from *Hordium durum*, which had coarser particles, the semolina is roasted and pour hot on the dough of date with the same quantities.
4. Nuts: those ingredients are optional, it depends on the financial ability of the family, those nuts are grouped in **table 2**.
5. Vegetable oil, especially the olive oil from *Olea europea L.* fruit, the oil used to heat **Rfiss l'mroure** when it is served.



Figure 02: the difference between the traditional Algerian sweet R'fiss (losange form above) and the herbal sweet R'fiss l'mroure (circular form)

Table 1: List of the herbs, spices and medicinals plant involved in the preparation of the herbal sweet

Botanic family	Scientific name	English common name	Local name	Part used	Medical uses	References
Lamiaceae	<i>Thymus vulgaris</i>	1. Thyme	Zaater	Leaves	Antispasmodi, carminative, emmenagogu, anthelmintic, spasmodic, laxative, stomachic, tonic, vermifuge Lactagogue, abdominal pain, relaxant	Peter ,2006
	<i>Rosmarinus officinalis</i>	2. Rosmary	Ikilil	Leave, flowers	Facilate delivery, mensruel pain, relaxant, antidepressio, treat abdominal cracks after delivery, restorative.	Ali-Shatayeh et al, 2015
	<i>Mentha piperita</i>	3. Pepper ment	Naanaa	Leaves	Antispasmodi, anti diarrhe, abominl pain	Iserin, 2001
	<i>M. rotundifolia</i>	4. Round leaf ment	Megil sif	Leave, flowers	Stimulant, stomachic, carminative, antiseptic	Peter, 2006
	<i>M. polegum</i>	5. Basilic	Feliou	Leave, flowers	Emmanogogu, reduce fiver, abdominal pain, carminative	Peter, 2006
	<i>Origanum basilium</i>	6. Oregano	Hebak	Leave, flowers	Antispasmodi, carminative, galactagogue, sedative (mild), stomachic, , anti-depressant.	Bartram, 1998
	<i>Marrubium vulgare</i>	7. Horehound	Merewa	Leave, flowers	Cough, apéritif ,arthritis	Iserin 2001, Benarba, 2016
Astraceae	<i>Matrticaria pubescens</i>	8. Chamomille of the sahara	Guertoufa	Leaves, flowers	Fever, cough, eye diseases, digestive disorders, diarrhoea, constipation, toothache facilate childbirth,	Cherif et al, 2017

					fortifying after childbirth	
	<i>Artemisia herba alba</i>	9.	Chih el abyeud	Leaves,	Stomache, ulcer, gastric Abdominal pain, chest pain	Benarba, 2016 Ali-Shatayeh et al, 2015
	<i>Anacyclus pyrethrum var pyrethrum</i>	10.	Guentes	Roots	against the toothache, intestinal pain, colic and personal hygiene for women, the anesthetic effect and antiseptic	Ouarghidi et abbad (2019)
	<i>Artemisia compestris</i>	11.	Etgoufet	Leaves	Antiseptic, stomach pain, fortifiant	Iserin, 2001
Apiaceae	<i>Pimpinella anisum L.</i>	12. Anise, sweet cumin	habbet ehlaw	Seeds	Internal pains, increases menstrual flow, promotes secretion of milk, and dissolves intestinal gas. Antispasmodic antibacterial, diuretic.	Skidmore-Roth, 2010
	<i>Carum carvi L.</i>	3. Caraway	Karawieh	Seeds	Uterus postpartum recovery facilitate delivery antispasmodic, carminative, lactagogue, emmenagogue.	Bartam 1998, Ali-Shatayeh et al, 2015
	<i>Cuminum cyminum L.</i>	4. Cumin	Camoun	Seeds	Abdominal pain; flatulence; lactagogue Stimulant, carminative, stomachic, astringent and antiseptic	Ali-Shatayeh et al, 2015 Peter, 2006
	<i>Coriandrum sativa</i>	5. Coriander	Kousbour	Seeds	appetite stimulant, as a treatment for ar-thritic conditions and	Skidmore-Roth , 2010

					dyspepsia, and as an antiseptic.	
	<i>Foeniculum vulgare</i>	6. Fennel	Besbas	Seeds	increase breast milk and the libido, aid digestion, as a remedy for flatulence, and treat indigestion and menstrual irregularities. diuretic, increase bile production, and relieve gastro intestinal pains.	Skidmore-Roth, 2010
Verbenaceae	<i>Aloysia triphylla</i>	7. Lemon verbena	Louiza	Leaves	Calming an sigestive	Iserin, 2001
Fabaceae	<i>Trigonella foenum-graecum</i>	8. Fenugreek	Holba	Seeds	treat gastrointestinal complaints, including constipation, dyspepsia, and gastritis. promote lactation, for menstrual and menopausal discomfort. promote wound healing .	Skidmore-Roth, 2010
Cupressaceae	<i>Juniperus phenicea</i>	9. Juniper berry	Aaraar	Leaves	Gastritis, Stimulant, diuretic, stomach tonic, pulmonary antiseptic, depurative	Agiel et mericli ,2017 Kheddam, 2003
Lythraseas	<i>Punica granatum L</i>	20. Pomegranate	Roman	Peel of fruit	genital infections, allergic dermatitis, tympanitis, scalds antioxidant	Al-juraifani , 2013
Lauraceae	<i>Cinnamomum caryphyllata</i>	21. Cinnamm	Karfa	Peel of trunk	Lactagogue; anemia; flatulence; postpartum recovery; facilitate	Ali-Shatayeh et al, 2015

					delivery; vomiting; abdominal pain	
	<i>Laurus nobilis</i>	22. Bay laurel	Rend	Leaves	Digestive, carminative, diuritic, antirheumatic, relieve pain in joints, chest, womb.	Ali shatayeh et al, 2003, Raghavan, 2007
capparaceae	<i>Capparis spinosa</i>	23. Caper	Kabar	Leaves, flowers buds	cure wounds and problems in the spleen, liver, kidneys and intestines, dispel gases, treat skin diseases, strengthen teeth and relieve backaches and joint pain.	Faran , 2014
Rosaceae	<i>Rosa gallica</i>	24. Red rose	Wared jouri	Flowers petals	Astringents, lower cholesterol levels.	Iserin, 2001
Rutaceae	<i>Citrus sinensis</i>	25. Orange	Bortukal	Peel of fruit	aromatic, digestive, carminative, anti- inflammatory, antifungal, antibacterial	Bartram, 1998

All those HSMP are treated with the same method, they must be cleaned, dried, and ground to a fine powder till their use. Those results show that from the 8 plant parts used in the preparation of the herbal sweet, the leaves are the most used part of the species with 56 %, the mixed HSMP used in the herbal sweet R'fiss el mrour are used to: promote lactation, calm abdominal pain, intestinal gas and help the postpartum recovery. There are numerous reports on the beneficial effects of spices on health, including as antioxidants, antibiotics, antivirals, anticoagulants, anti-carcinogenic, and anti-inflammatory agents, having potential effects on infection, cancer, diabetes, hypertension, coronary diseases, cataract, neurodegenerative disease, digestive problems, and ulcerative lesions (**García-Casal, 2016**)

Table 2 : Nuts used in herbal sweet recipes

Family	Latin name	English name	Arabic name	Used part
Rosaceae	<i>Prunus dulcis</i>	Almond	Louz	Seeds
Juglandaceae	<i>Juglans regia</i>	Nut	Jouz	Seeds
Fabaceae	<i>Arachis hypogaea</i>	Peanut	Foul soudani/ kawkaw	Seeds
Anacardiaceae	<i>Pistacia vera</i>	Pistachio	Fostok	Seeds
Pinaceae	<i>Pinus coulteri</i>	Pine nuts	Ezgogo	Seeds
Pedaliaceae	<i>Sesamum indicum</i>	Sesame	Jeljelan/ semsem	Seeds



Most edible "nuts" contain concentrated food reserves for future generations of plants and provide valuable sources of energy, protein, oils, minerals, and vitamins suitable for human consumption. Others have their food reserves in the form of starch instead of protein (Wichens, 1995).

A study done by Aubaile-Sallinave (1997), on food for the new mother in the Arabic Moslem Mediterranean world going back to the tenth century and of recent ethnographic descriptions shows that this diet is subject to specific rules: it must be very hot and therefore energetic it must contain source of sugars (honey, dates, fruits and more recently sugar), flour (cereals - barley, wheat hard-, dates), fats (melted butter, olive oil, sesame, poultry broth), with dried fruits (almonds, walnuts, hazelnuts, pine nuts), with spices (cinnamon, saffron, pepper, chili, cumin, etc.) and condiment (fenugreek because it makes overweight, garden cress, seeds of fennel, green anise, thyme, etc.), we can conclude from this study that the herbal sweet is one of the gastronomic histories from Ziban region, it must be protected as a non-material heritage.

4. Conclusion

Despite the development of the health services in Algeria on mothers and newborns, the mothers stay attached to the persuasion that they must stay at home during the postpartum recovery period which lasts 40 days. During what, the relative's women who care for the postpartum mother and the new baby have to take care of them, referring to traditional medicine and healthy meals.

Our results show that in Biskra region, many herbs spices, and medicinal plants are still in uses by old women for the healthcare of maternal and newborn

The herbal sweet " R'fiss l'mrou", the aim of this study, is one of the gastronomic ethnic of Biskra contain more or less 20 herbs spices, and medicinal plants that give this dish its medicinal value in improving the health of the mother and the newborn as well.

There remain, however many dishes that deserve further study, and it is very important to document them because the transfer of knowledge from the old generation to the new generation is diminishing.

A knowledge

The authors would like to thanks all the informants who participate to this survey.

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FULL TEXT - ORAL PRESENTATION

**A HYDROPHILIC DEEP EUTECTIC SOLVENT-BASED METHOD
FOR THE RECOVERY OF NATURAL PRODUCTS FROM *HIBISCUS
SABDARIFFA***

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Abstract

Alternative nature antioxidant sources are of great importance for the development of novel functional food products. Citric acid-based deep eutectic solvent (DES) was integrated into automatic solvent extraction (ASE) to obtain high added-value substances from *Hibiscus sabdariffa*. Based on the earlier reports [1,2], a DES combination including citric acid as hydrogen bond acceptor and ethylene glycol as hydrogen bond donor was employed with a molar ratio ¼. This green solvent integrated extraction system was optimized by response surface method (RSM). Box-Behnken design type of RSM produced 17 experimental runs with 3 independent variables (water level in DES, extraction time and mass of the raw material) and 3 levels (20-50%, 30-60 min and 0.5-1.5 g). The ASE system was optimized to achieve the highest total phenolic content (TPC) and total anthocyanin content (TAC). The extract obtained under the optimal conditions were compared to those of conventional solvents such as ethanol, methanol and aqueous mixtures of them. Additionally, antioxidant activity of the *Hibiscus sabdariffa* extracts was also measured depending on *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazil) assay. The relationships between the TPC/DPPH findings and TAC/DPPH findings were also established.

Key Words: Green chemistry, green solvents, deep eutectic solvent, anthocyanin, antioxidant, multivariate optimization methods.

1. Introduction

Hibiscus sabdariffa is a special medicinal plant cultivated in generally Africa, America and South East Asia [3]. It has been reported to possess various bioactive properties (antioxidant, antitumoral, antihypertensive and anti-hyperlipemic effects) due to its phytochemical substances as well as its nutritonal value [4, 5]. Antimicrobial effects have been also demonstrated by *in vitro* studies [6]. *In vivo* studies also verified the antioxidant activity of the H. sabdariffa extract [7]. So, recovery of high added value substances from this antioxidant source is of great value. Emerging technologies are recommended as alternative to conventional methods. Green technologies ensure a safe extract and quality in addition to reduction and/or elimination of energy and petroleum-based solvent consumptions [8]. On the other hand, investigation of alternative media instead of petroleum-based solvents commonly used in chemical processes has become an important research topic. Deep eutectic solvent (DES) has been proposed as biocompatible innovative solvents recently [9]. Alañón et al. prepared choline chloride as hydrogen bond acceptor (HBA) and oxalic acid, lactic acid, ethylene glycol, 1,2-Propanediol, urea and sugars (fructose, maltose and sucrose) as hydrogen bond donor (HBD) [10]. They used microwave-assisted extraction (MAE) for the bioactive ingredients from H. *sabdariffa*. Oxalic acid based DES gave the best yield for particularly anthocyanins. Kurtulbaş et al. applied citric acid-based DES for the

anthocyanin recovery from *H. sabdariffa* by means of MAE [2]. Şahin et al. used citric acid/ethylene glycol as DES to extract similar chemicals from the current raw material by means of ultrasound-assisted extraction [11]. Zannou et al. prepared a DES mixture containing sodium acetate (HBA) and formic acid (HBD), and compared the results with the conventional solvents such as water, 70% ethanol and 80% methanol solutions [4].

In the present study, a DES combination including citric acid as hydrogen bond acceptor and ethylene glycol as hydrogen bond donor was employed with a molar ratio $\frac{1}{4}$, taking into account previous works [1, 2]. Environmentally friendly automatic solvent extraction (ASE) method was employed to obtain high added-value substances from *Hibiscus sabdariffa* for the first time. The process conditions were optimized by means of statistically experimental design method (Response Surface Method-RSM). Effects of the process parameters were also identified by means of analysis of variance test (ANOVA) by means of RSM.

2. Material and Methods

2.1. Plant and Chemical Materials

Plant samples were purchased from a local market and used after they were dried. Folin-Ciocalteu, DPPH (2,2-diphenyl-1-picrylhydrazil), trolox, gallic acid, ethanol, methanol, citric acid, Na_2CO_3 and HCl were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. ASE

Figure 1 demonstrates the ASE system (Velp Scientifica, Usmate, Italy).

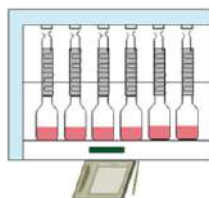


Figure 1. Automatic solvent extraction system

2.3. Bioactivity assays

Total phenolic content (TPC), total anthocyanin content (TAC) and DPPH radical scavenging activity were measured by spectrophotometric analysis via UV-vis spectrometer (PG Instruments, T60/Leicestershire, England). TPC was measured at 765 nm depending on the earlier study [12], while DPPH measurements were recorded at 517 nm [13]. TAC results were quantified after the samples were measured at 520 and 700 nm [14], respectively.

2.4. Statistical experimental design

Box-Behnken design as a subtype of RSM was applied to the ASE system with 3 variables and 3 levels (Table 1).

Table 1. Process parameters of ASE for the extraction of bioactive substances from *Hibiscus sabdariffa*

Process Parameter	Units	Symbol	Coded levels		
			-1	0	1
Water level	%, v/v	A	20	35	50
Time	min	B	30	45	60
Mass	g	C	0.5	1.0	1.5

Design-Expert software (12.0.1.0 version, StatEase Inc., USA) was used to employ RSM. TPC, TAC and antioxidant activity findings were dependent variables (responses), while water level in the DES system, extraction time and raw material mass were independent variables.

3. Results and Discussion

3.1. Extraction of bioactive substances from *Hibiskus sabdariffa*

Table 2 shows the design matrix of ASE of bioactive substances from the selected plant material based on the Box-Behnken design of RSM. Total phenolics changed between 37.05 and 74.79 mg-GAE/g-DP, whereas TAC was between 2.13 and 10.48 mg-C3G/g-DP. Antioxidant activity values ranged from 1.67 to 5.93 mg-TEAC/g-DP, corresponding to over 80% of inhibition of DPPH radical.

Table 2. Experimental results formed by Box-Behnken design for the ASE of bioactive substances from *Hibiskus sabdariffa**

Run	A: Water level (% _{v/v})	B: Time (min)	C: Mass (g)	TPC (mg-GAE/g-DP)	TAC (mg-C3G/g-DP)	DPPH scavenging activity (mg-TEAC/g-DP)
1	35	45	1	51.20±0.001	4.91±0.000	2.69±0.001
2	35	60	1.5	51.82±0.000	2.83±0.000	1.67±0.000
3	50	45	0.5	73.23±0.003	10.48±0.001	4.79±0.001
4	35	30	1.5	37.05±0.001	5.89±0.000	1.77±0.000
5	20	60	1	56.70±0.001	2.13±0.000	2.16±0.001
6	35	60	0.5	74.79±0.001	7.10±0.001	5.72±0.001
7	35	45	1	49.57±0.001	4.73±0.001	2.53±0.001
8	35	45	1	52.69±0.001	4.82±0.001	2.98±0.001
9	20	30	1	41.22±0.001	3.07±0.001	2.63±0.001
10	50	45	1.5	57.63±0.001	3.52±0.000	1.84±0.000
11	35	45	1	50.26±0.001	4.93±0.001	2.67±0.000
12	35	45	1	52.16±0.001	4.32±0.001	2.64±0.001
13	20	45	1.5	47.83±0.000	3.41±0.001	1.71±0.000
14	50	30	1	50.09±0.001	6.99±0.000	2.57±0.001
15	35	30	0.5	50.10±0.001	6.42±0.002	5.93±0.001
16	20	45	0.5	73.93±0.000	Not detected.	5.75±0.001
17	50	60	1	70.63±0.001	8.01±0.001	2.52±0.000

* Data are given as the arithmetic mean of 3 replicates.

3.2. Modelling results

Quadratic models proposed by Box-Behnken design for each response (Y_{TPC} , Y_{TAC} and $Y_{Antioxidant\ activity}$) are given below:

$$Y_{TPC} = 51.18 + 3.99 A + 9.44 B - 9.71 C + 1.26 AB + 2.63 AC - 2.48 BC + 6.60 A^2 - 3.11 B^2 + 5.38 C^2 \quad (1)$$

$$Y_{TAC} = 4.74 + 2.55A - 0.29 B - 1.05 C + 0.49 AB - 2.59 AC - 0.94 BC - 0.45 A^2 + 0.76 B^2 + 0.06 C^2 \quad (2)$$

$$Y_{Antioxidant\ activity} = 2.70 - 0.07 A - 0.10 B - 1.90 C + 0.11 AB + 0.27 AC + 0.03 BC - 0.24 A^2 + 0.01 B^2 + 1.06 C^2 \quad (3)$$

All models (Eqs.1-3) are found statistically significant as seen in ANOVA tables (Table 3). Mass amount was the most significant parameter on the response TPC, followed by time at $p < 0.0001$. Regarding TAC, water amount was the most effective independent variable for the current system. Raw material amount, interactions between A and C, and B and C, and B^2 were also statistically significant for the response TAC. As for antioxidant activity, C, C^2 are significant model terms, since p values greater than 0.1 indicate the model terms are not significant.

Table 3. ANOVA for TPC, TAC and antioxidant activity in *Hibiskus sabdariffa* extract

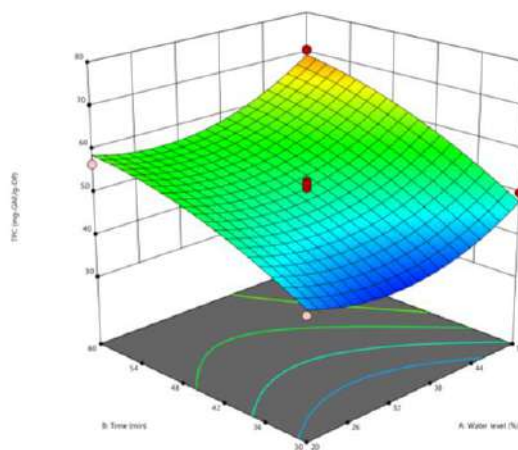
	Source	Sum of Squares	df	Mean Square	F-value	p-value	
TPC	Model	2000.41	9	222.27	43.62	< 0.0001	significant
	A-Water level	127.22	1	127.22	24.97	0.0016	
	B-Time	712.33	1	712.33	139.80	< 0.0001	
	C-Mass	755.04	1	755.04	148.18	< 0.0001	
	AB	6.40	1	6.40	1.26	0.2994	
	AC	27.60	1	27.60	5.42	0.0528	
	BC	24.64	1	24.64	4.83	0.0638	
	A^2	183.34	1	183.34	35.98	0.0005	
	B^2	40.84	1	40.84	8.01	0.0254	
	C^2	121.81	1	121.81	23.91	0.0018	
	Residual	35.67	7	5.10			
	Lack of Fit	28.98	3	9.66	5.78	0.0616	not significant
	Pure Error	6.69	4	1.67			
	Cor Total	2036.08	16				
<i>C.V.: 4.08%</i>		<i>R²=0.9825</i>		<i>Adjusted R²=0.9600</i>			
TAC	Model	95.94	9	10.66	58.45	< 0.0001	significant
	A	52.06	1	52.06	285.45	< 0.0001	
	B	0.6570	1	0.6570	3.60	0.0995	
	C	8.74	1	8.74	47.91	0.0002	
	AB	0.9560	1	0.9560	5.24	0.0558	
	AC	26.87	1	26.87	147.34	< 0.0001	
	BC	3.50	1	3.50	19.21	0.0032	
	A^2	0.8515	1	0.8515	4.67	0.0675	
	B^2	2.43	1	2.43	13.30	0.0082	
	C^2	0.0158	1	0.0158	0.0869	0.7767	
	Residual	1.28	7	0.1824			
	Lack of Fit	1.03	3	0.3429	5.53	0.0659	not significant
	Pure Error	0.2478	4	0.0620			
	Cor Total	97.21	16				
<i>C.V.: 8.69%</i>		<i>R²=0.9869</i>		<i>Adjusted R²=0.9700</i>			
Antioxidant activity	Model	34.28	9	3.81	57.72	< 0.0001	significant
	A	0.0361	1	0.0361	0.5477	0.4833	
	B	0.0870	1	0.0870	1.32	0.2887	
	C	28.90	1	28.90	438.02	< 0.0001	
	AB	0.0451	1	0.0451	0.6834	0.4357	
	AC	0.3007	1	0.3007	4.56	0.0702	
	BC	0.0028	1	0.0028	0.0431	0.8415	
	A^2	0.2431	1	0.2431	3.68	0.0964	
	B^2	0.0002	1	0.0002	0.0029	0.9587	
	C^2	4.75	1	4.75	71.93	< 0.0001	
	Residual	0.4619	7	0.0660			
	Lack of Fit	0.3502	3	0.1167	4.18	0.1004	not significant
	Pure Error	0.1117	4	0.0279			
	Cor Total	34.74	16				

*C.V.:*8.31% *R*²=0.9867 *Adjusted R*²=0.9696

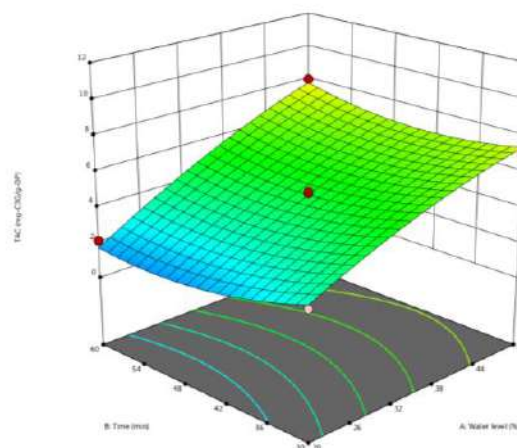
Model fitting can be evaluated with lack of fit, coefficient of variance (C.V.), R^2 , and adjusted R^2 values. As seen in Table 3, R^2 values for all the systems are close to 1, while adjusted R^2 values are in good agreement with R^2 . Additionally, non-significant lack of fit is desirable since the model is wanted to fit. Moreover, C.V. values for the 3 systems are calculated less than 10%, which indicates the reliability of the experimental data.

3.3. Effect of ASE parameters

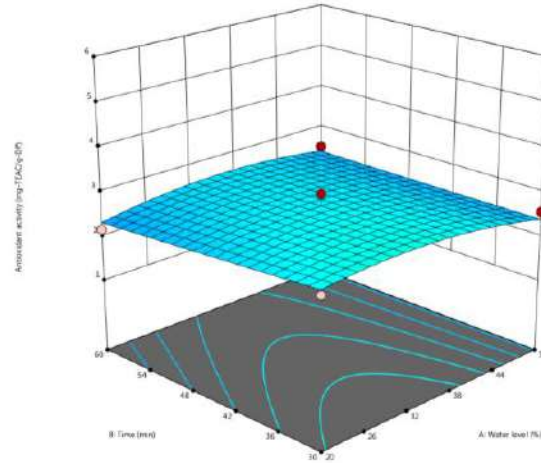
Design-Expert software also produces three dimensional (3D) plots as seen in Figures 2, 3 and 4. Figures 2a, 2b and 2c display the effect of time and water on the TPC, TAC and antioxidant activity of the extract samples. Time had generally positive effect on the yields. This result is acceptable, since the contact time between the solute and the solvent increases. On the other hand, antioxidant activity was not affected significantly.



a



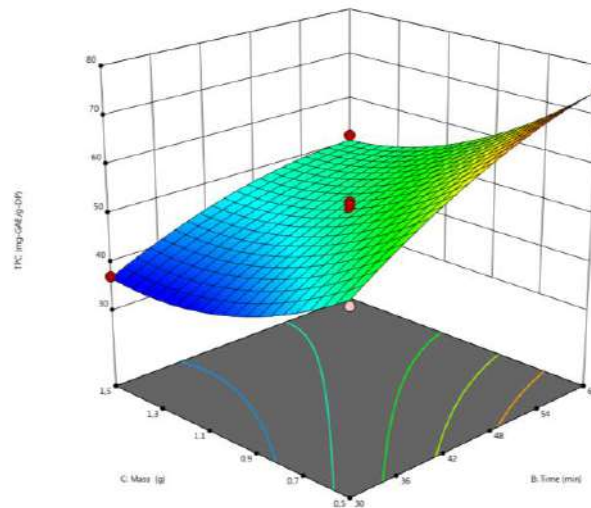
b



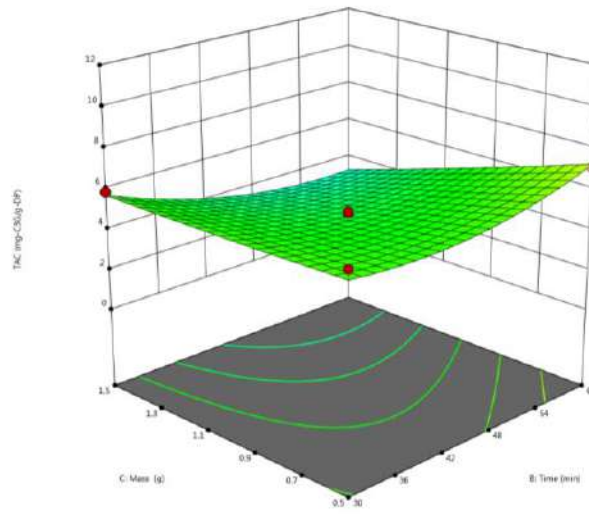
c

Figure 2. Effects of water level and time on the (a) TPC yield, (b) TAC yield and (c) antioxidant activity in *Hibiskus sabdariffa* extract.

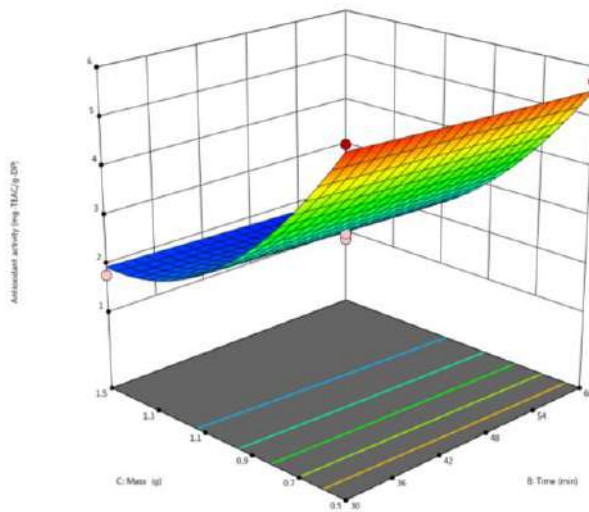
Figures 3a, 3b and 3c show the impacts of mass and extraction time. Increasing the raw material affected the system negatively, except for the TAC yield. Depending on the mass transfer principles, the surface area decreased by mass increase while the solvent volume was constant.



a



b



c

Figure 3. Effects of mass and time on the (a) TPC yield, (b) TAC yield and (c) antioxidant activity in *Hibiskus sabdariffa* extract.

Expectedly, increasing the water volume in the DES system increased the TPC and TAC yields (Figure 4). Actually, this is the result of polarity increase, viscosity decrease and surface tension improvement [15]. On the other hand, antioxidant activity was not influenced significantly as already seen in ANOVA table (Table 3).

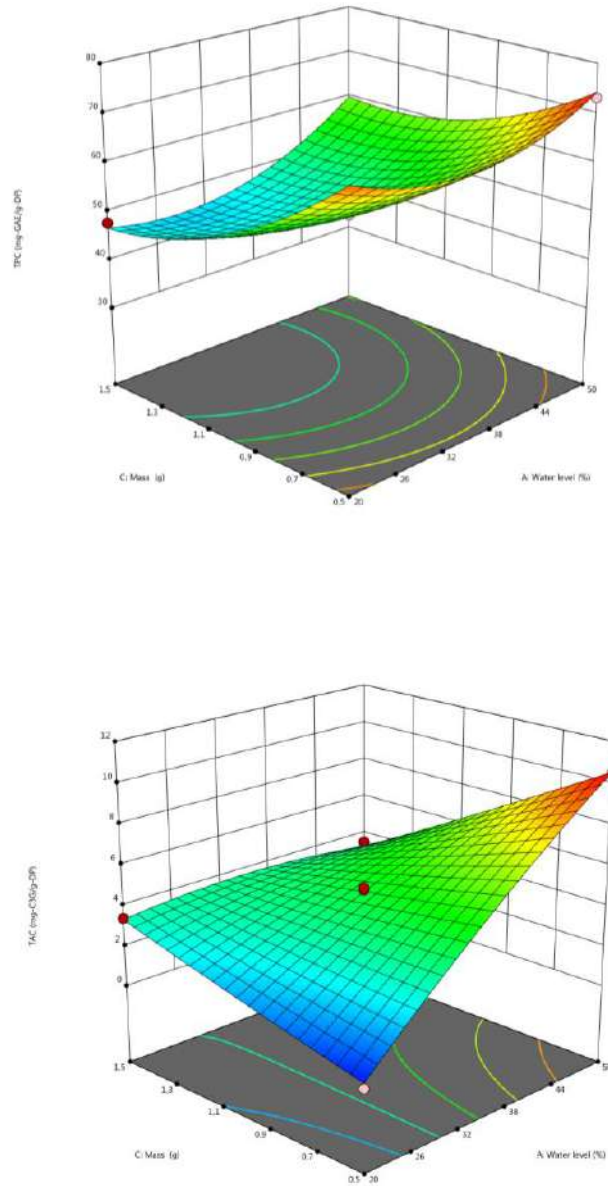
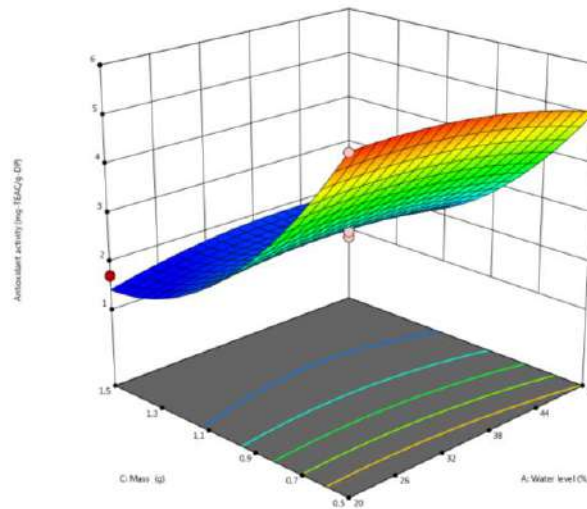


Figure 4. Effects of mass and water level on the (a) TPC yield, (b) TAC yield and (c) antioxidant activity in *Hibiskus sabdariffa* extract.



3.4. Optimization results

Optimum conditions are calculated as 44% water level in the DES, 60 min of extraction time and 0.5 g plant sample in order to extract maximum TPC (79.05 mg-GAE/g-DP), TAC (10.48 mg-C3G/g-DP) and antioxidant activity (5.31 mg-TEAC/g-DP) from *Hibiskus sabdariffa*. Kurtulbaş et al. used the same DES system with 50% water, and extracted 31.89 mg-GAE/g-DP of TPC and 2.96 mg-C3G/g-DP of TAC from the same material with MAE. TPC increased almost 2.5 times, while TAC yield enhanced by 3.5 times. Ultrasound-assisted extraction with the same DES system also gave poorer TPC yield comparing to that of ASE (79.05 versus 22.77). As a result, ASE, an alternative method developed very recently as a green technology, performed much better than the previous developed methods.

4. Conclusion

Hibiskus sabdariffa. has shown to be a powerful source of bioactive ingredients depending on the *in vitro* analyses. The proposed method including citric acid-based deep eutectic solvent integrated into automatic solvent extraction has been applied to the dried plant successfully. The model study has indicated that the experimental data are compatible with the produced quadratic models according to the statistical indicators (F, p, coefficient of variation, lack of fit and coefficients of determination values). The extract samples scavenged the free radicals significantly (almost 90%), indicating that the current biomass might be natural alternative additive for the development of functional food formulations. However, the most important issue to be considered is the safety of the food product. Therefore, more work needs to be done to scale and commercialize the applications of natural substances in food formulations. The synergistic effects of the individual components with each other should also be examined.

Conflict of Interest

The author declares that there is no conflict of interest in writing upon submission of the manuscript.



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FULL TEXT - ORAL PRESENTATION

ENCAPSULATION OF ROSESHIP SEED EXTRACT IN ALGINATE
BASED MICROBEADS: OPTIMIZATION OF IONIC GELATION
SYSTEM BY RESPONSE SURFACE METHOD

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Abstract

Natural products obtained from various biowaste are prone to adverse effects of environmental factors such as oxidation, humidity and light. In order to deal with such drawbacks, microencapsulation is an alternative preservation method. In the current study, ionic gelation has been used for trapping the active materials of roship seed in alginate beads. The performance of the produced beads were evaluated based on encapsulation efficiency in terms of total phenolic content (EE_{TPC}) and antioxidant activity. The process parameters of the ionic gelation system was selected as gelling medium (calcium chloride) concentration (2–15%, w/v), wall material (sodium alginate) concentration (1-2%, w/v) and hardening time (15-45 min). EE_{TPC} and antioxidant activity were the response variables for the optimization of the ionic gelation system by application of Box-Behnken design of Response Surface Method (Box-Behnken-RSM). 17 experimental runs with 3 variables and 3 levels were carried out depending on Box Behnken-RSM. The maximum EE_{TPC} and antioxidant activity yield were found as 54.655% and 4.221 mg trolox equivalent per gram dried capsule under the optimal ionic gelation conditions (8% $CaCl_2$, 1.66% sodium alginate and 26 min of hardening time). Additionally, antioxidant activity of the beads was determined by DPPH (2,2-diphenyl-1-picrylhydrazil) test. Depending on the analysis of variance test (ANOVA) of Box-Behnken-RSM, the most effective process parameter was found as the calcium chloride concentration followed by the second power sodium alginate concentration ($p < 0.0001$), whereas the most significant parameter for the antioxidant activity was the second power of calcium chloride concentration, followed by sodium alginate concentration ($p < 0.0001$).

Key Words: Biopolymer, alginate beads, ionotropic hydrogel, biowaste, antioxidant activity, Box-Behnken-RSM.

1. Introduction

Functional foods have become quite popular in recent years thanks to their positive effect on health and natural nutritional properties. Functional food ingredients are incorporated into food products in order to enrich their nutritional values and flavour. These ingredients can be used to create new functional foods and make serious advantages in terms of shelf life, nutritional value and customer satisfaction [1]. Bioactive compounds in food products shows functional properties as well, such as antioxidant(phenolics), antimicrobial (essential oils), probiotic and aromatic [2]. However, it should not be forgotten that the direct application of these natural ingredients to the products may lead to the deterioration of the taste of the product and also the loss of the function of the bioactive component [3]. It may also interact with other components in the food matrix, resulting in a reduction in the quality of functional food products. Biologically active compounds are extra nutritional components that typically occur in very small amounts in foods. In general, these compounds are found in millions of species of plants, animals, marine organisms and microorganisms and can be obtained by various methods



(extraction and biotechnological) [4]. One property of the biologically active compounds which shows health benefits like vitamins, probiotics, polyphenols, omega-3-fatty acids is that their sensitivity against air, light, heat and moisture [5]. Therefore, if a biologically active compound forms a decomposition product like flavorless, colorless or carcinogenic compounds, shelf life of the product will be affected drastically. In order to overcome these aforementioned problems and improve the product's properties, encapsulation method is a powerful tool because of its ability to embed various bioactive compounds in a protective matrix [6]. Encapsulation applications are used in pharmaceutical, nutraceutical and food industries [7]. Microencapsulation can be achieved by wrapping an active substance with one or more coating material [8]. The encapsulation of natural antioxidants such as jujube extract [9], hibiscus extract [10], chokeberry extract [11], grape marc extract [12], green tea extract, [13], stevia extract [7], olive leaf extract [14], lemon balm extract [15] and haskap berry extract [16] have been studied widely in recent years.

One of the most used methods of encapsulation is ionic gelation with formation of alginate gels by ionic cross-linking with Ca^{++} (multivalent cation) [17]. Ionic gelation is a highly efficient, simple and inexpensive encapsulation method which not requires a specialised equipment, organic solvents or high temperature. These properties makes ionic gelation perfect for both hydrophobic or hydrophilic compounds. Since the heat is a factor which reduces effectiveness of the active material and the coating material, heat free application is advantageous [18]. Since sodium alginates shows ionic gelation properties and biological compatibility, they are considered as all-purpose, natural anionic polymers which used in wide area as an encapsulation agent of biological active compounds, including plant extracts polyphenols [7]. Sodium alginate can form a gel structure named as “egg-box model” upon replacing sodium ion by a divalent cation such as calcium [19]. In this study, rosehip seed extract has been used as active material source to form microbeads. Various studies showed that rosehips are rich in polyphenols in particular catechin, gallic acid, quercetin, and cyanidin. However, some of these important biologically active compounds are not stable for extended periods of time. So, these useful properties could be lost. Encapsulation method provide the prevention of the active materials from oxidation, and improve biological availability of the polyphenolic compounds [20].

In this study, Box Behnken-RSM was applied as a statistical experimental design method in order to optimize EE_{TPC} and antioxidant activity using three independent variables; gelling medium (CaCl_2) concentration, wall material (sodium alginate) concentration and hardening time of microbeads. In order to determine the encapsulation efficiency of the produced microbeads, total phenolic content (TPC) and surface phenolic content (SPC) were measured spectrophotometrically. Furthermore, antioxidant activity of the microbeads were also evaluated by *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazil) assay.

2. Material and Methods

2.1. Plant material and chemicals

In September 2021, rosehip samples were collected from Çankırı in Turkey. The seeds were removed from fresh fruits of rosehips. After the seeds were cleaned, they were dried at ambient conditions. Sodium alginate, calcium chloride dihydrate, Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazil), trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ethanol (>99.5%), methanol (>99.8%), sodium carbonate (Na_2CO_3), acetic acid (>99.7%) and gallic acid monohydrate were provided from Sigma-Aldrich (St. Louis, MO, USA). Calcium chloride were used as crosslinkers.

2.2. Extraction procedure

Automatic solvent extractor (Velp Scientifica, Usmate, Italy) ensuring extraction of up to 6 samples simultaneously was used to extract biologically active materials from rosehip seed. In this

method, firstly, 1 g of rosehip seed samples were weighed and put in cellulose extraction thimbles (single thickness, 33 mm × 80 mm, Whatman, Maidstone, UK). Then, the cellulose extraction thimbles were placed into extraction cups, containing 80 mL of 80% v/v aqueous ethanol. The operating conditions were: 20 min of immersion time, 40 min of washing time, 30 min of recovery time, 2 min of cooling time. The extract was stored in glass bottle at -4°C until encapsulation. In this method, more than 90% of the solvent is recovered in the internal recovery tank.

2.3. Production of alginate beads

Rosehip seed extract was used as the active material, while sodium alginate was used as the wall material. Three different concentrations of sodium alginate solutions (1, 1.5 and 2%, w/v) and three different concentrations of calcium chloride solutions (2% w/v, 8.5% w/v and 15% w/v) were prepared by dissolving them in distilled water. The rosehip extract and sodium alginate solution were combined in a volume ratio of 1:2. Calcium chloride solution (2, 8.5 and 15%, w/v) was used as gelling bath for the microencapsulation of the rosehip seed extract. The mixture of active material and wall material was added dropwise to calcium chloride solution while stirred with magnetic stirrer (Model MSH-20D, DAIHAN Scientific Co. Ltd., Korea) under ambient conditions to form alginate-based microbeads (Figure 1). The formed microbeads were kept for a certain period (15,30 and 45 min) in the gelling bath to harden and filtered. Then the microbeads were washed with distilled water and dried. As seen in Table 1, various factors were evaluated to optimize the parameters affecting the formulation of the microbeads: sodium alginate concentration, calcium chloride concentration and hardening time.



Figure 1. The schematic representation of alginate beads preparation by ionic gelation method

Table 1. Process parameters and levels selected for the ionic gelation of rosehip seed

Process Parameter	Units	Symbol	Coded levels		
			-1	0	1
Calcium Chloride	(%, w/v)	A	2	8.5	15
Sodium Alginate	(%, w/v)	B	1	1.5	2
Time	min	C	15	30	45

2.4. Physicochemical analysis

2.4.1. Determination of total phenolic content (TPC)

100 mg of microcapsule was weighed and dissolved in 3 mL ethanol:acetic acid:water mixture (50:8:42, v/v). This mixture was agitated by vortex for 1 min and immersed in an ultrasonic bath at 25 °C for 20 minutes. It was then passed through 0.45 µm syringe filters. The TPC of the prepared mixture was determined spectrophotometrically depending on Folin-Ciocalteu method. For this purpose, 100 µL of microcapsule mixture was taken and 2000 µL of Folin-Ciocalteu reagent (10%, v/v) was added to it. After the mixture were kept in the dark for 5 minutes, 1800 µL of sodium carbonate solution (7.5%, w/v) was added into the mixture, and mixed. After incubation of the obtained mixture in the dark for 1 hour, the absorbance of the mixture were measured at 765 nm in UV spectrophotometer (PG Instruments, T60 / Leicestershire and England) (Tolun et al., 2016). Results were expressed as mg gallic acid equivalent (mg-GAE) per liter (ppm).

2.4.2. Determination of surface phenolic content (SPC)

In order to determine of surface phenolic content of microcapsules, 100 mg microcapsule were weighed and dissolved in 3 mL ethanol-methanol solution (1:1, v/v) by immersing in an ultrasonic bath (Protech, Istanbul, Turkey) for 5 min at ambient temperature (Tolun et al., 2016). The solutions were filtered through 0.45-µm syringe filters. The SPC was calculated according to the same method aforementioned for TPC determination. SPC results were also expressed as ppm unit.

2.4.3. Determination of DPPH antioxidant activity

The antioxidant activity of encapsulated rosehip seed extract was determined by *in vitro* DPPH (2,2-diphenyl-1-picrylhydrazil) assay. 15 mg of microcapsules were dissolved in 3 mL of ethanol: acetic acid: water mixture (50:8:42, v/v). This mixture was mixed in a vortex for 1 min, and immersed in a water-bath at 40°C for 20 min. Then, it was passed through 0.45 µm syringe filters prior to DPPH assay. Briefly, for the DPPH assay, 100 µL of microcapsule mixture was taken and 3.9 mL of 25 mg L⁻¹ DPPH radical solution was added to it. After the resulting mixture kept in the dark 1 hour, absorbance values were recorded at 517 nm (Tolun et al., 2016). Antioxidant activity values of microcapsules were expressed as mg trolox equivalent per gram dried matter (mg-TEAC/g-DM).

2.4.4. EE of microbeads

Encapsulation efficiency (EE) refers to the percentage of total phenolics which is successfully embedded into an encapsulated product. In order to determine the encapsulated phenolic content, the difference between the TPC and SPC are calculated. The EE of microbeads was calculated with the following equation:

$$EE (\%) = \frac{TPC - SPC}{TPC} * 100 \quad (1)$$

2.5. Experimental design and statistical analysis

Box-Behnken-RSM was used to investigate the effect of the independent variables in the ionic gelation process on the response variables via Design-Expert software (version 12.0.1.0, StatEase Inc., USA). Additionally, modeling and optimization of the ionic gelation process for the entrapping of the active material of rosehip seed extract into alginate beads were also implemented with the same approach. EE and DPPH free radical scavenging activity of the microbeads were selected as response variables (Y) of the ionic gelation system, whereas calcium chloride concentration, sodium alginate concentration and hardening time were selected as independent variables.

3. Results and Discussion

3.1. Ionic gelation of rosehip seed extract in alginate beads

Table 2 presents the encapsulation efficiency and antioxidant activity of the microbeads under the given ionic gelation process conditions based on Box-Behnken-RSM. As seen in Table 2, 17 experimental studies with 5 center points (8.5% calcium chloride, 1.5% alginate and 30 min) produced by Design-Expert software (version 12.0.1.0, Stat-Ease Inc., USA) were performed. Encapsulation efficiency of produced beads varied from 7.35 to 57.95%, while antioxidant activity of the produced beads varied from 3.47 to 4.23 mg-TEAC per gram dried capsule.

Table 2. Experimental findings generated by Box-Behnken design for the ionic gelation of active substances from rosehip seed extract

Run	A: Calcium Chloride (%, w/v)	B: Sodium Alginate (%, w/v)	C: Time (min)	EE _{TPC} (%)	DPPH (mg-TEAC/g-DM)
1	2	1.5	45	41.40	3.64 ± 0.001
2	8.5	15	30	57.95	4.09 ± 0.003
3	8.5	2	15	37.47	4.13 ± 0.001
4	8.5	1.5	30	54.64	4.16 ± 0.001
5	8.5	1.5	30	49.65	4.20 ± 0.002
6	2	1.5	15	52.56	3.72 ± 0.001
7	8.5	2	45	36.23	4.23 ± 0.002
8	2	2	30	47.62	3.85 ± 0.006
9	15	2	30	26.71	3.96 ± 0.005
10	8.5	1	15	35.26	4.06 ± 0.002
11	15	1.5	45	15.42	3.71 ± 0.004
12	8.5	1.5	30	50.52	4.22 ± 0.002
13	15	1	30	14.66	3.75 ± 0.001
14	8.5	1.5	30	49.69	4.22 ± 0.005
15	8.5	1	45	7.35	3.58 ± 0.001
16	2	1	30	37.29	3.47 ± 0.006
17	15	1.5	15	32.57	4.04 ± 0.003

* Data are given as the arithmetic mean of 3 replicates.

3D (three dimensional) surface plots generated by Design-Expert software also enabled to visualize interactions between the process parameters. Figure 2-4 shows the effects of process parameters on the encapsulation efficiency (a) and antioxidant activity (b) of the alginate beads which includes rosehip seed extract. Figure 2 shows the impacts of calcium chloride and sodium alginate concentrations on the EE_{TPC} (a) and antioxidant activity (b) at constant hardening time (30 min). It was observed that the sodium alginate concentration increase in the encapsulation of rosehip seed extract with ionic gelation method has a positive effect in terms of EE_{TPC}. As seen in Figure 2a, EE_{TPC} increased

continuously until the sodium alginate concentration was %1.66. After that optimal point, an increase in sodium alginate concentration had decreased the encapsulation efficiency. This effect may be attributed to the increase in viscosity due to higher concentration of sodium alginate. In the literature, it is reported that at least 1.5% of alginate concentration is required in order to produce spherical microbeads. On the other hand, sodium alginate concentrations over 20 g/L in most cases adversely affect the process due to various problems such as high viscosity, difficulty in pumping and non-Newtonian behavior [21]. Similar effect was also observed in case of antioxidant activity of the capsules. The wall material concentration enhanced the antioxidant activity of the capsules all through the way, as the thickening of the surrounding capsule would prevent leakage of the biologically active material to the environment (Figure 2b) [22].

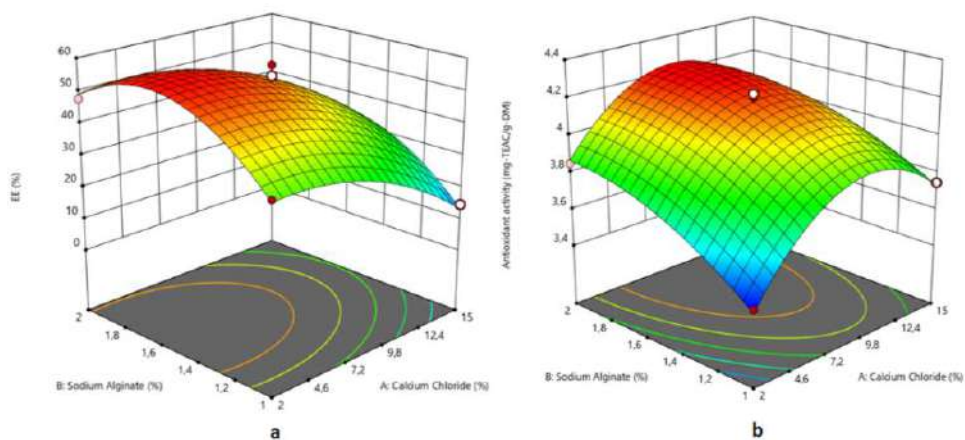


Figure 2. Effects of calcium chloride concentration to alginate concentration on the (a) encapsulation efficiency and (b) antioxidant activity of the microcapsules including rosehip seed extract

Figure 3 shows the effects of calcium chloride and hardening time on the EE_{TPC} (a) and antioxidant activity (b) at constant sodium alginate concentration (1.5 %). As seen in Figures 3a and 3b, the EE_{TPC} and antioxidant activity of the capsules increased continuously up to a certain point and then decreased as the calcium chloride concentration increased. This finding can be explained by the fact that divalent cations such as Ca^{++} ions are necessary to form gel structure in the ionic gelation system. After the optimum $CaCl_2$ concentration is reached, the active Ca binding sites in the polymer chain may be saturated [23].

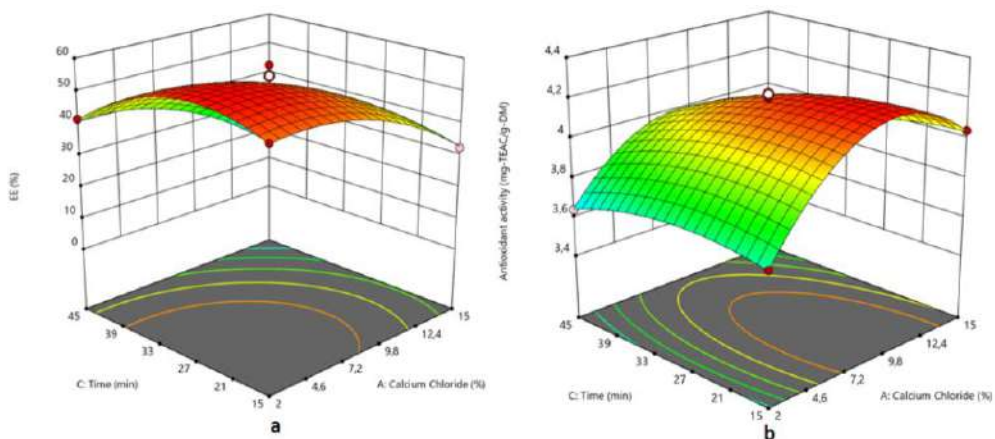


Figure 3. Effects of calcium chloride concentration to time on the (a) encapsulation efficiency and (b) antioxidant activity of the microcapsules including rosehip seed extract

Figure 4 shows the hardening time effect on the EE_{TPC} and antioxidant activity. As the increase in the hardening time, the EE_{TPC} and antioxidant activity increased at first. After optimal hardening time was achieved, the EE_{TPC} and antioxidant activity were decreased as seen in Figure 4a and 4b. This effect may be attributed to that the core material passes into the gelling bath solution ($CaCl_2$) and the effectiveness of the capsule decreases in relation to excessive exposure in the $CaCl_2$ solution [16].

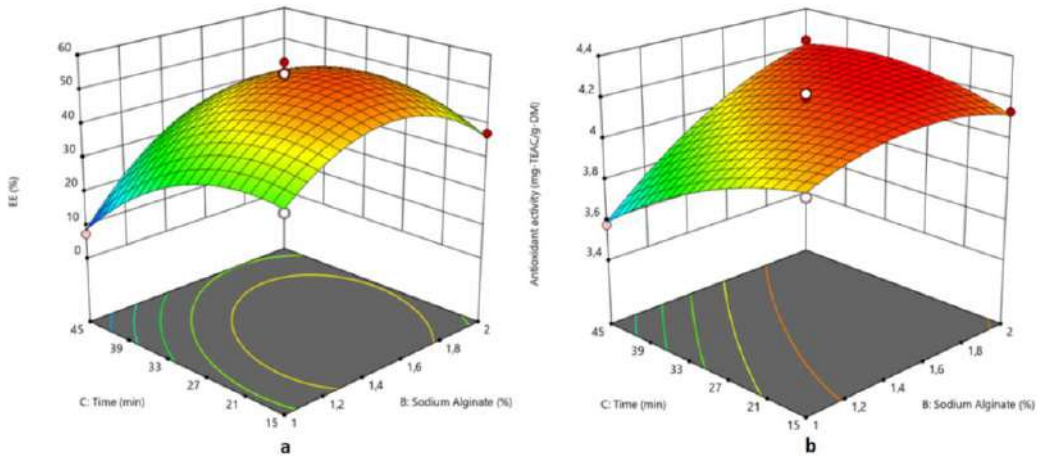


Figure 4. Effects of sodium alginate concentration to time on the (a) encapsulation efficiency and (b) antioxidant activity of the microcapsules including rosehip seed extract

3.2. Model fitting

Analysis of variance (ANOVA) test was also provided by using Box-Behnken-RSM study. Depending on ANOVA test results, two empirical relationships (Y_{EE} and $Y_{\text{antioxidant activity}}$) that relate the response and independent variables were obtained. The quadratic polynomial equations (Eqs.2 and 3) in terms of coded factors for each response (Y_{EE} and $Y_{\text{antioxidant activity}}$) are given below:

Response	Model
$Y_{EE} =$	$52.49 - 11.19 A + 6.68 B - 7.18 C + 0.4305 AB - 1.50 AC + 6.67 BC - 7.26 A^2 - 13.66 B^2 - 9.75 C^2 \quad (2)$
$Y_{\text{antioxidant activity}} =$	$4.18 + 0.0971 A + 0.1652 B - 0.0997 C - 0.0427 AB - 0.0620 AC + 0.1457 BC - 0.3232 A^2 - 0.0989 B^2 - 0.0781 C^2 \quad (3)$

On the other hand, the fitness of the proposed models were investigated by the lack-of-fit test. The lack of fit value for the proposed models were calculated as greater than 0.05 (non-significant). This situation supports that the proposed models fit the data adequately. The quadratic polynomial equations generated for the proposed models were found to be statistically significant ($p < 0.0001$, $F=42.43$ and 52.60) (Tables 3 and 4). ANOVA results also showed that the values of R^2 close to 1 for the proposed models. According to the high coefficient of determination values ($R^2 > 0.98$, adjusted $R^2 > 0.95$ and predicted $R^2 > 0.93$), it can be said that there is a satisfactory relationship between the experimental and predicted values of the responses. Predicted R^2 and adjusted R^2 values calculated as 0.9304 and 0.9589 for EE_{TPC} ; 0.9435 and 0.9667 for antioxidant activity respectively. These results shows that the difference is less than 0.2 which indicates there is an adequate relationship between predicted R^2 and adjusted R^2 values. Additionally, the coefficient of variation values of the proposed models were found

lower than 10% (Tables 3 and 4). There is a relationship between coefficient of variation value and reliability of the results, where this value was low, precision and reliability will be higher.

Table 3. Analysis of variance findings on encapsulation efficiency value of microcapsules

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3517.57	9	390.84	42.43	< 0.0001	significant
A-Calcium Chloride	1001.59	1	1001.59	108.72	< 0.0001	
B- Sodium Alginate	357.20	1	357.20	38.77	0.0004	
C- Time	412.67	1	412.67	44.80	0.0003	
AB	0.7413	1	0.7413	0.0805	0.7849	
AC	9.00	1	9.00	0.9767	0.3559	
BC	177.90	1	177.90	19.31	0.0032	
A ²	221.65	1	221.65	24.06	0.0017	
B ²	786.08	1	786.08	85.33	< 0.0001	
C ²	399.87	1	399.87	43.41	0.0003	
Residual	64.49	7	9.21			
Lack of Fit	10.30	3	3.43	0.2534	0.8558	not significant
Pure Error	54.19	4	13.55			
Cor Total	3582.05	16				
<i>C.V.: 7.98% R²=0.9820 Adjusted R²=0.9589 Predicted R²=0.9304</i>						

Table 4. Analysis of variance findings on antioxidant activity value of microcapsules

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.02	9	0.1135	52.60	< 0.0001	significant
A-Calcium Chloride	0.0755	1	0.0755	34.99	0.0006	
B- Sodium Alginate	0.2185	1	0.2185	101.27	< 0.0001	
C- Time	0.0795	1	0.0795	36.87	0.0005	
AB	0.0073	1	0.0073	3.38	0.1087	
AC	0.0154	1	0.0154	7.14	0.0319	
BC	0.0850	1	0.0850	39.39	0.0004	
A ²	0.4398	1	0.4398	203.88	< 0.0001	
B ²	0.0412	1	0.0412	19.08	0.0033	
C ²	0.0257	1	0.0257	11.89	0.0107	
Residual	0.0151	7	0.0022			
Lack of Fit	0.0024	3	0.0008	0.2547	0.8548	not significant
Pure Error	0.0127	4	0.0032			
Cor Total	1.04	16				
<i>C.V.: 1.18% R²=0.9854 Adjusted R²=0.9667 Predicted R²=0.9435</i>						

Considering the effects of process variables depending on p values, all of the selected independent parameters were found statistically significant for the ionic gelation system ($p < 0.05$). As seen in Table 3, the interactions were not found to be statistically effective except for the interaction between sodium alginate concentration and hardening time (BC) for EE_{TPC} ($p > 0.05$). In addition, the second power of all the selected parameters were statistically influential for EE_{TPC} ($p < 0.05$). The most significant parameter on EE_{TPC} were calcium chloride concentration and the second power of sodium alginate concentration ($p < 0.0001$) followed by C, C², B, A², BC ($p < 0.05$). When ANOVA findings are

considered in terms of the other response (antioxidant activity), the most effective parameter was the second power of calcium chloride concentration and sodium alginate concentration ($p < 0.0001$). As seen in Table 4, all the selected parameters, their interactions and second powers were found to be statistically significant except for the interaction between calcium chloride concentration and sodium alginate concentration (AB) for antioxidant activity ($p < 0.05$).

3.3. Validation study

Optimization study was also carried out in order to maximize EE_{TPC} and antioxidant activity in the microencapsulation of rosehip seed extract in alginate microbeads (Table 5). All experiments were performed in triplicate and ANOVA results were reported as mean standard deviation. Verification of the optimum process conditions were also performed. According to validation study, the difference between the experimental and the calculated results have been found lower than 2%, which indicates that the reliability of the second order model equations derived by Box-Behnken-RSM.

Table 5. Optimum ionic gelation conditions for the highest encapsulation efficiency and antioxidant activity

Optimum Conditions			Response	Predicted	Experimental	Error (%)
A (%, w/v)	B (%, w/v)	C (min)				
7.982	1.664	25.992	EE_{TPC}	54.653	53.665	1.81
			Antioxidant activity	4.221	4.156	1.54

4. Conclusion

In this present study, rosehip seed extract prepared by a promising “green” extraction technology, rich in polyphenols, was encapsulated in alginate beads. Microencapsulation of rosehip seed extract was achieved through ionic gelation technique, a very simple, inexpensive, easy to control in terms of production, non-toxic and organic solvent-free method. The encapsulation of rosehip seed extract in calcium-alginate beads was optimized using Box-Behnken design. Three process parameters (calcium chloride concentration, sodium alginate concentration and hardening time) were investigated and their effects on encapsulation efficiency and antioxidant activity were determined. Based on Box-Behnken-RSM, the optimum conditions for the preparation of alginate microbeads by ionic gelation method were obtained at: 7.98% calcium chloride concentration, 1.66% sodium alginate and 26 min of hardening time. All selected independent variables were found statistically influential for both responses ($p < 0.05$). The suggested quadratic polynomial equations are strongly adequate to describe the experimental data according to several statistical indicators of ANOVA such as F-value, p-value, C.V. (coefficient of variance), non-significant lack of fit, R^2 , adjusted R^2 and predicted R^2 . This study showed that rosehip seed extract rich in polyphenol and antioxidant activity were suitable active material to obtain promising substances for food application.

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Conflict of Interest

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

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FULL TEXT - ORAL PRESENTATION

INVESTIGATION OF BIOACTIVE PROPERTIES OF *TEUCRIUM CHAMAEDRYS* LEAVES: PROCESS OPTIMIZATION USING CENTRAL COMPOSITE DESIGN

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Abstract

Teucrium chamaedrys leaves have been reported as alternative herbal medicine rich in phytochemicals such as phenolics and flavonoids [1]. Therefore, the selected biomass has been investigated in order to develop a green extraction method for recovery of its bioactive substances. Homogenizer-assisted extraction (HAE) method was used by means of a GRAS (generally recognized as safe) solvent (aqueous ethanol). Central composite design of Response Surface Method (RSM) was used to optimize the HAE system through 20 experimental runs. Ethanol concentration (30-90%, v/v), extraction time (30-90 sec) and homogenizer speed (4000-10000 rpm) were independent process parameters. Responses (dependent variables) of the system were total phenolic content (TPC), total flavonoid content (TFC), individual phenolic compound (4-hydroxycinnamic acid) and antioxidant activity of the extracts. DPPH (2,2-diphenyl-1-picrylhydrazil) assay was used to measure the antioxidant activity. In order to identify the significance of the process parameters, their interactions and second powers, analysis of variance test (ANOVA) was employed via RSM. Model statistics such as p and F values, lack of fit, coefficient of variation (C.V.), coefficient of determination (R^2) and adjusted R^2 findings have demonstrated the reliability of the second order models produced for the present system.

Key Words: Green chemistry, teucrium chamaedrys, antioxidant, multivariate optimization methods.

1.Introduction

Plants have traditionally been utilized to cure a variety of ailments, particularly in East Asian countries. Alternative medicine's popularity has risen again in recent years. According to surveys performed in Australia and the United States, over 48.5 and 34% of respondents, respectively, have tried at least one kind of unorthodox therapy, including herbal medicine. Due to a dearth of safe contemporary pharmaceuticals, the World Health Organization (WHO) has also urged that effective plants be evaluated for illnesses such as diabetes. [2]

Teucrium species, often known as Germanders, are perennials, subshrubs, or shrubs with fragrant simple or lobed leaves that can be evergreen or deciduous.[3] Teucrium species contain a high concentration of neo-clerodane diterpenoids. Despite the fact that various furanoid diterpenes with a neo-clerodane skeleton have been identified from a variety of plants, the genus Teucrium is the most prolific natural source of these components. As a result, Teucrium species are now recognized as chemotaxonomic indicators for neo-clerodanes. Although not as plentiful as in the genus Salvia from the same family, flavonoids and aromatic compounds have been discovered in the genus Teucrium [4]. This plant has been used for the treatment of diabetes, gastric inflammation and convulsion [5, 6]. Teucrium



species possess free radical and hydroxyl radical scavenging activity as well as anti-oxidant activity in vitro[7].

Green technologies for the extraction require advanced efforts without too much solvent and energy necessities by application of alternative bio-based solvents and biomass in order to produce speciality products[8]. In this study, homogeneous assisted extraction will be used as green extraction for obtaining a renewable natural product from a sustainable raw material. Therefore, the leaves of *Teucrium chamaedrys* have been preferred as sources of antioxidants in the proposed paper, since they are biomass with potential antioxidant source [9-12]. In order to optimize the HAE process, central composite design (CCD) of response surface approach (RSA) was used. The system was optimized to maximize the phenolic content (TPC), total flavonoid content (TFC), individual phenolic compound (4-hydroxycinnamic acid) and antioxidant activity of the extracts. DPPH (2,2-diphenyl-1-picrylhydrazil) content in the extract. This statistical approach also provided the design of experimental study, modeling the HAE, and analyzing the experimental data. Furthermore, antioxidant activity of the extracts was evaluated by several in vitro measurements to produce more precise and reliable findings. So, the current study will be the first to examine the HAE of bioactive substances from *Teucrium chamaedrys* leaves in terms of several respects such as statistical experimental design, modeling, and optimization.

2. Material and Methods

2.1. Plant and Chemical Materials

Teucrium chamaedrys leaves were brought from Palestine. Green leaves were selected without green spots. The samples were washed in three steps. It was first washed using clean drinking water to remove impurities. The leaves were washed twice in distilled water. They were then laid out on a clean cloth in a room where the sun's rays did not directly enter room temperature so that their nutritional value and vitamins would not be lost and protected from dust and vermin to prevent contamination. They were turned daily to ventilate, thus preventing mold growth.

In this project ethanol was chosen as a polar, non-toxic and inexpensive solvent. Folin-ciocalteu reagent, sodium carbonate ($\geq 99.0\%$), (+)-catechin, hydrochloric acid, sodium hydroxide, 2,2-diphenyl-1-picrylhydrazil (AA-DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and gallic acid monohydrate ($\geq 98.0\%$) Obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol ($\geq 99.8\%$), methanol ($\geq 99.9\%$), aluminum chloride, sodium nitrite, and potassium persulfate were supplied by the Deionized purified water Millipore Milli-Q purification system from Merck (Darmstadt, Germany).

2.2. HAE

Figure 2.1 demonstrates the HAE system (IKA T25 ULTRA TURRAX). The extraction was carried out in this approach by taking a set amount of leaves with a particle size of 2000 μ m. On the other hand, three variables were investigated: homogenizer speed (rpm), solvent concentration% (v/v), and extraction time (sec). A homogenizer was used to perform the HAE procedure (IKA T25 ULTRA TURRAX). The extracts were filtered using a 0.45 μ m syringe filter.



Figure 0.1: Homogeneous Assisted Extraction

2.3. Bioactivity assay

Total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical scavenging activity were measured by spectrophotometric analysis via UV–vis spectrometer (PG Instruments, T60/Leicestershire, England). Gallic acid or the equivalent of another phenolic component is used to determine the overall TPC of plants and foods. The phenol solution is bright yellow in color, known as FCR. However, it is thought to contain phosphomolybdic/phosphotungstic acid complexes that are reduced to form a blue chromophore with maximum absorption at 765 nm[13], while TFC was determined by UV spectrophotometer (PG Instruments, T60/Leicestershire, UK) at 510 nm. Details of the method have been established in previous studies. These recorded results were calculated in terms of catechin equivalents (mg-CE/L) per (+) *Teucrium chamaedrys* extract[14], Antioxidant activity (AA) measurement by AA-DPPH method was determined spectrophotometrically at 517 nm[15]. HPLC results were quantified after the samples High Performance Liquid Chromatography. technique [16], respectively.

2.4. Statistical Experimental Design

The response surface method (RSM) is a combination of statistical and mathematical techniques that are important for improving, improving and optimizing processes. The answer is the term performance indicators for quality attributes. In this research, RSM was used to optimize the amount of bioactive compounds using different extraction methods. On the other hand, these surfaces were used to explain how independent variables affected their responses, to provide information about the relationships between independent variables, and to explain the combined effect of all independent variables on the response. central composite design as a subtype of RSM was applied to the HAE system with 3 variables and 3 levels (Table 2.1).

Table 0.1: Process independent parameters of HAE for the extraction of bioactive substances from *Teucrium chamaedrys*.

Process independent Parameter	Units	Symbol	Coded levels		
			-1	0	1
Solvent concentration	%, v/v	A	30	60	90
Extraction time	sec	B	30	60	90
Mixing speed	rpm	C	4000	7000	10000

In this research, RSM was used to optimize the total amount of phenolics, flavonoids, DPPH and HPLC using the Design-Expert (version 12).

3.Result and Discussion

31.Extraction of bioactive substances from *Teucrium chamaedrys*.

Table 0.1: shows the experimental and predictive data (in terms of TPC, TFC, DPPH and HPLC) obtained under the CCD designed conditions.

Ru n	Solvent concentrati on (%v/v)	Extraction time (sec)	Mixing speed (rpm)	TPC (mg- GAE/g-DP)	TFC (mg- CE/g-DP)	DPPH (mg-TEAC/g- DP)
1	30	30	4000	18.798±0.007	32.832±0.000 7	6.898± 0.001
2	60	60	7000	19.017±0.012	45.708±0.000 1	7.694± 0.001
3	30	60	7000	23.096±0.004	38.686±0.000 7	7.294± 0.007
4	90	30	10000	9.597±0.005	46.968±0.000 7	11.309± 0.001
5	60	60	7000	19.064±0.002	48.705±0.000 7	7.703± 0.001
6	60	60	7000	20.084±0.005	47.202±0.000 7	8.238± 0.007
7	90	90	4000	12.068±0.003	44.179±0.001 4	14.331± 0.003
8	90	60	7000	11.488±0.002	46.884±0.000 7	14.542± 0.002
9	30	90	10000	28.121±0.003	44.519±0.000 7	7.891± 0.007
10	60	90	7000	24.607±0.011	57.555±0.000 1	7.917± 0.007
11	90	90	10000	14.180±0.002	65.374±0.000 7	14.485± 0.009
12	90	30	4000	10.557±0.001	32.181±0.001 4	14.625± 0.001
13	30	30	10000	21.788±0.012	24.000±0.000 1	7.485± 0.002
14	30	90	4000	28.892±0.013	50.635±0.000 7	6.182± 0.001
15	60	60	7000	19.106±0.003	48.705±0.000 1	7.473± 0.007
16	60	60	4000	18.902±0.004	45.635±0.002 8	8.110± 0.002

17	60	60	7000	19.285±0.006	45.735±0.000	8.013±0.001
					1	
18	60	60	10000	19.822±0.003	48.368±0.002	7.650±0.001
					8	
19	60	60	7000	17.616±0.004	45.726±0.000	7.774±0.001
					1	
20	60	30	7000	19.050±0.002	42.602±0.000	7.413±0.003
					1	

*Data are given as the arithmetic mean of 3 replicates.

TPC, TFC and AA-DPPH measurements of the HAE system designed by CCD under various conditions are given in Table 3.1. TPC values measured in HAE-extracted samples ranged between 9.597 and 28.892 mg-GAE/g-DP, while TFC varied between 24 and 65.374 mg-CE/g-DP as shown in Table 3.1. On the other hand, the results of AA-DPPH study on the antioxidant capacity of *Teucrium chamaedrys* leaf extract prepared by HAE method are included.

As shown in Table 3.1, the scavenging activity of the samples against AA-DPPH free radicals ranged from 6.182 to 14.625 mg-TEAC per gram of dried samples.

In addition, figure 3.1 also shows the correlations between TPC and TFC, AA-DPPH. Antioxidative activity results of AA-DPPH showed a higher positive correlation with TPC in HAE method ($r = 0.6239$ vs. 0.023). This indicates that phenols have a greater effect on the antioxidant activity of *Teucrium chamaedrys* leaf extract.

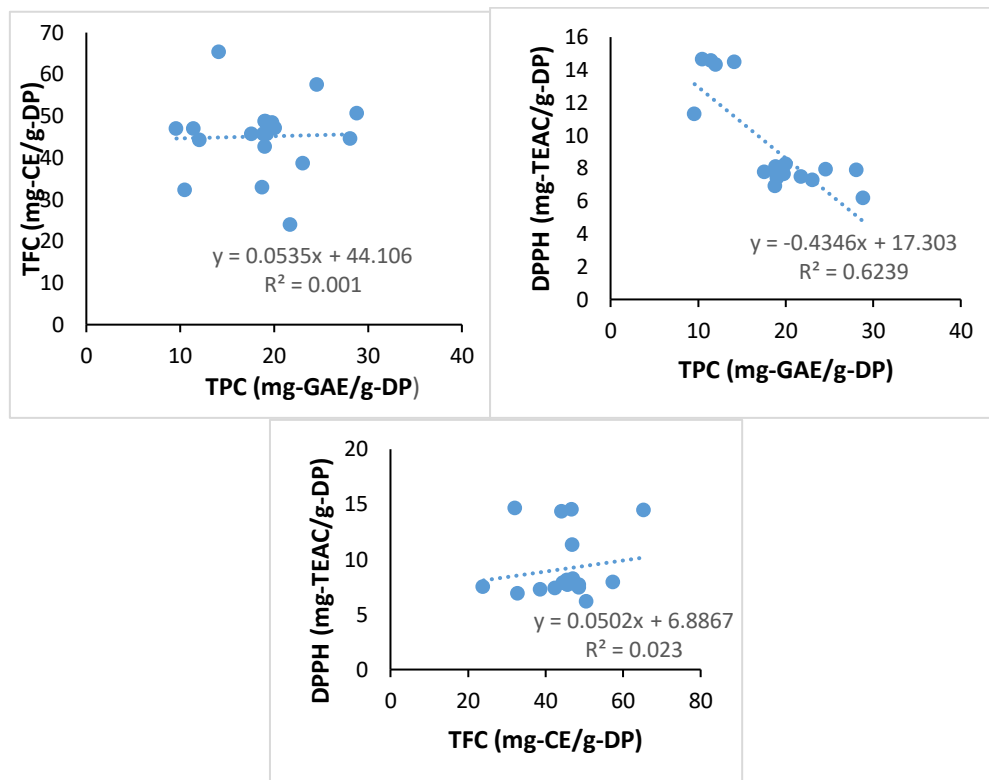


Figure 0.1: the correlations between TPC and TFC, AA-DPPH.

3.2. Modeling Study and Effect of Process Parameters

The quadratic models for the related systems (TPC, TFC and AA-DPPH) extracted by the HAE method are expressed by the following equations:

$$\text{HAE-TPC (mg-GAE/g-DP)} = 19.38 - 6.28A + 2.81B + 0.4291C - 1.29AB - 0.1334AC - 0.0861BC - 2.62A^2 + 1.92B^2 - 0.5485C^2$$

$$\text{HAE- TFC (mg-CE/ g-DP)} = 47.45 + 4.49A + 8.37B + 2.38C - 0.9898AB + 6.37AC + 1.14BC - 5.40A^2 + 1.90B^2 - 1.18C^2$$

$$\text{HAE-DPPH (mg-TEAC/g-DP)} = 7.88 + 3.35A + 0.3076B - 0.1326C + 0.3990AB - 0.6823AC + 0.5740BC + 2.95A^2 - 0.3074B^2 - 0.0924C^2$$

Statistical analysis (ANOVA) findings produced by CCD of RSM using the Design-Expert program are presented in Table 3.2 for each response, along with appropriate statistical variables.

Table 0.2: Analysis of variance test results for TPC, TFC and AA-DPPH obtained from Teucrium chamardrys leaves using HAE method.

	Source	Sum of Squares	Df	Mean Square	F-value	p-value	
TPC	Model	515.03	9	57.23	47.07	< 0.0001	significant
	A-Solvent concentration	394.45	1	394.45	324.46	< 0.0001	
	B- Extraction time	78.84	1	78.84	64.85	< 0.0001	
	C- Mixing speed	1.84	1	1.84	1.51	0.2466	
	AB	13.35	1	13.35	10.98	0.0078	
	AC	0.1423	1	0.1423	0.1171	0.7393	
	BC	0.0593	1	0.0593	0.0488	0.8296	
	A ²	18.86	1	18.86	15.51	0.0028	
	B ²	10.12	1	10.12	8.32	0.0163	
	C ²	0.8273	1	0.8273	0.6805	0.4286	
	Residual	12.16	10	1.22			
	Lack of Fit	8.97	5	1.79	2.82	0.1339	not significant
Pure Error	3.18	5	0.6365				
Cor Total	527.18	19					
<i>C.V.: 5.88%</i>		<i>R²=0.9769</i>		<i>Adjusted R²=0.9562</i>		<i>Predicted</i>	
				<i>R²=0.7047</i>			
TFC	Model	1434.92	9	159.44	71.60	< 0.0001	significant
	A-Solvent concentration	201.73	1	201.73	90.59	< 0.0001	
	B-Extraction time	700.22	1	700.22	314.44	< 0.0001	
	C- Mixing speed	56.49	1	56.49	25.37	0.0005	
	AB	7.84	1	7.84	3.52	0.0901	
	AC	324.23	1	324.23	145.60	< 0.0001	
	BC	10.41	1	10.41	4.67	0.0560	



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	A ²	80.11	1	80.11	35.98	0.0001	
	B ²	9.89	1	9.89	4.44	0.0613	
	C ²	3.84	1	3.84	1.72	0.2187	
	Residual	22.27	10	2.23			
	Lack of Fit	11.53	5	2.31	1.07	0.4699	not significant
	Pure Error	10.74	5	2.15			
	Cor Total	1457.19	19				
		<i>C.V.: %3.31</i>		<i>R²=0.9847</i>	<i>Adjusted R²=0.9710</i>	<i>Predicted R²=0.9229</i>	
AA-DPPH	Model	158.26	9	17.58	130.51	< 0.0001	significant
	A-Solvent concentration	112.51	1	112.51	834.97	< 0.0001	
	B- Extraction time	0.9462	1	0.9462	7.02	0.0243	
	C- Mixing speed	0.1758	1	0.1758	1.30	0.2799	
	AB	1.27	1	1.27	9.45	0.0118	
	AC	3.72	1	3.72	27.64	0.0004	
	BC	2.64	1	2.64	19.56	0.0013	
	A ²	23.86	1	23.86	177.08	< 0.0001	
	B ²	0.2599	1	0.2599	1.93	0.1951	
	C ²	0.0235	1	0.0235	0.1743	0.6852	
	Residual	1.35	10	0.1347			
	Lack of Fit	0.9835	5	0.1967	2.70	0.1496	not significant
	Pure Error	0.3640	5	0.0728			
	Cor Total	159.61	19				
		<i>C.V.: 4.01%</i>		<i>R²=0.9916</i>	<i>Adjusted R²=0.9840</i>	<i>Predicted R²=0.8836</i>	

The ANOVA test provides model fit verification based on the most popular statistical tools such as F-value, P-value, lack of fit value, coefficients of determination (R^2 , adjusted R^2 and predicted R^2), and coefficient of variation (CV). P values indicate that the quadratic models constructed for TPC, TFC and DPPH are both statistically significant ($P < 0.05$) and consistent with experimental findings.

Considering the HAE process parameters for TPC yield, solvent concentration was the most influential parameter ($P < 0.05$), followed by extraction time and mixing speed. The effects of solvent concentration were also statistically significant ($P < 0.05$). As for TFM, ethanol concentration, extraction time and second order ethanol concentration were the most important parameters ($P < 0.0001$), followed by mixing speed and extraction time statistically ($P < 0.05$). The interaction of ethanol concentration and mixing speed were also followed statistically ($P < 0.05$). In addition, the interaction of ethanol concentration and mixing speed was also statistically ($P < 0.05$) effective in the recovery of TFC from HAE and *Teucrium chamardrys* leaf extraction.

Considering the HAE process parameters for AA-DPPH yield, solvent concentration and secondary ethanol concentration were the most important parameters ($P < 0.05$).

Model fitting can be evaluated with lack of fit, coefficient of variance (C.V.), R^2 , and adjusted R^2 values. As seen in Table 3, R^2 values for all the systems are close to 1, while adjusted R^2 values are in good agreement with R^2 . Additionally, non-significant lack of fit is desirable since the model is wanted to fit.

Moreover, C.V. values for the 3 systems are calculated less than 10%, which indicates the reliability of the experimental data.

In order to understand how independent factors, affect the system, three-dimensional graphics were also drawn with the program (Figure 3.2-3.4).

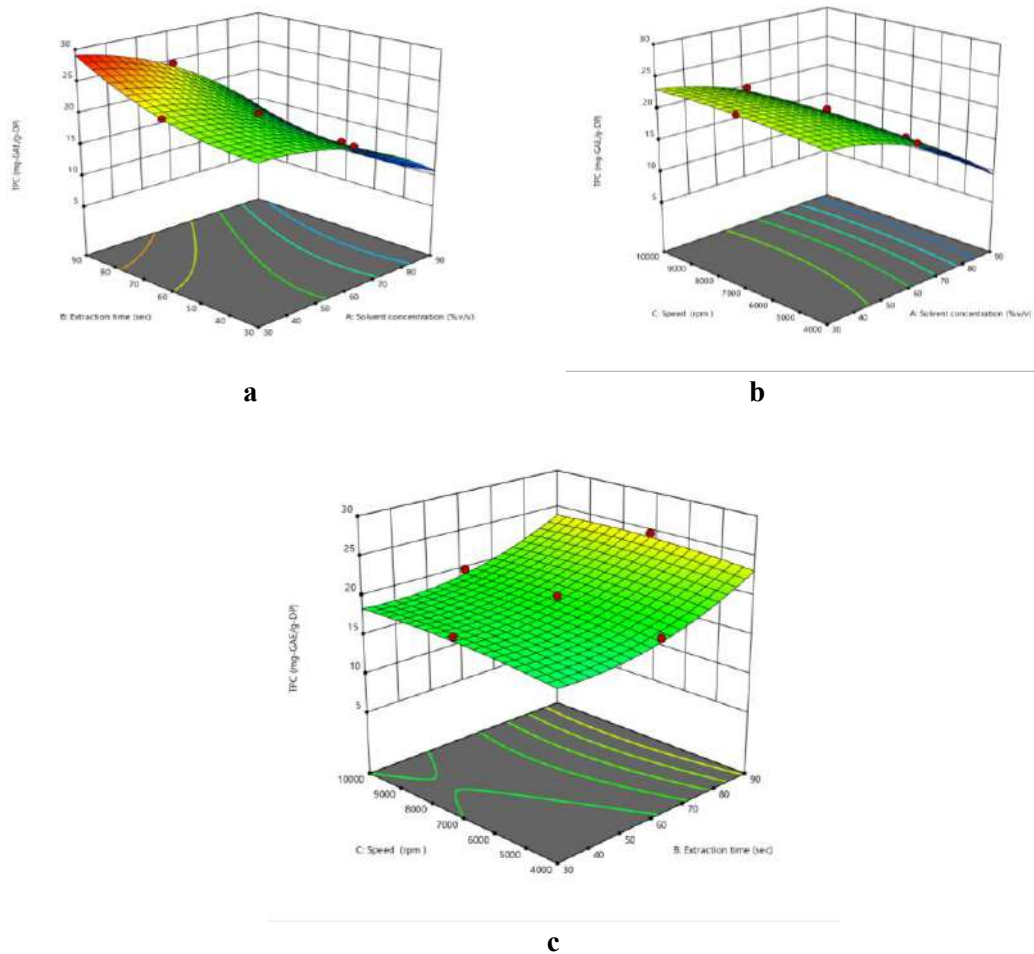


Figure 0.2: The response surface plot for the obtained TPC of the applied HAE method; (a) as a function of solvent concentration over time (speed = 7000 rpm); (b) solvent concentration as a function of rate (time = 60; (c) as a function of the acceleration time (solvent concentration = 60%).

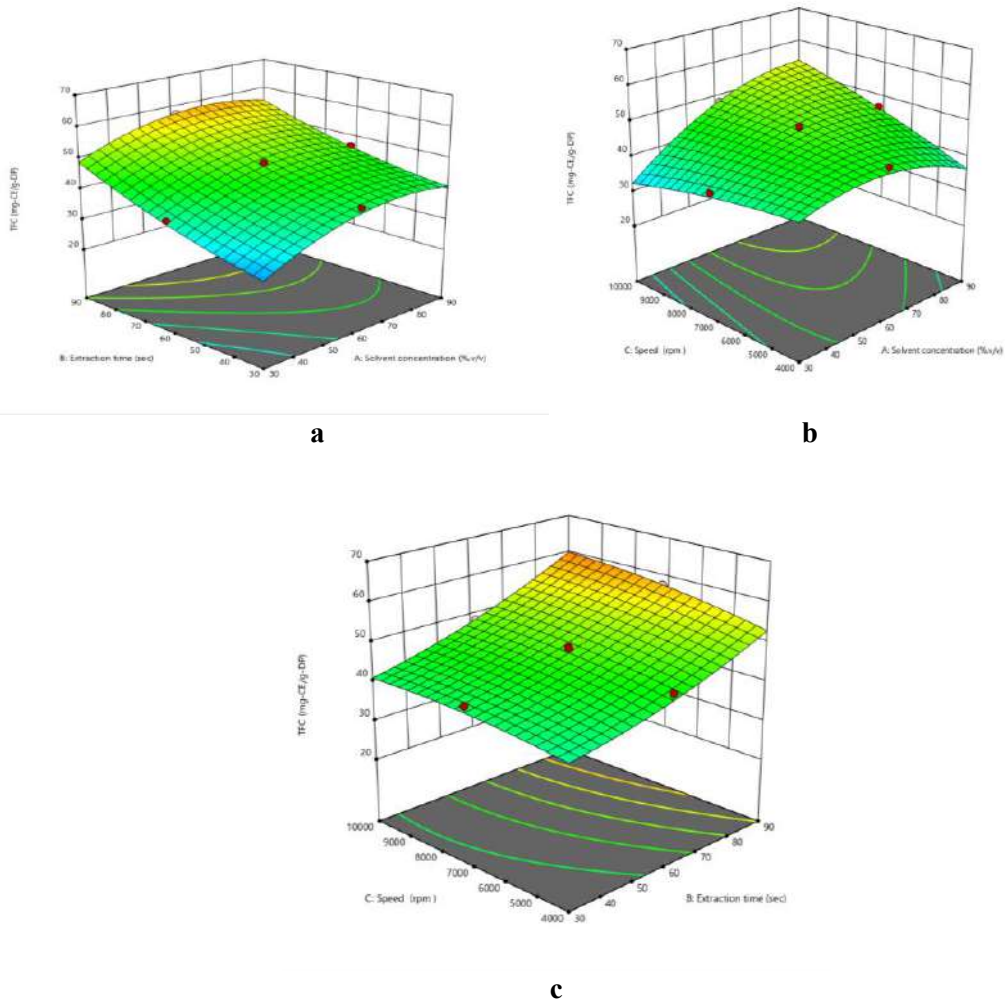


Figure 0.3: The response surface plot for the obtained TFC of the applied HAE method; (a) as a function of solvent concentration over time (speed = 7000 rpm); (b) solvent concentration as a function of rate (time = 60; (c) as a function of the acceleration time (solvent concentration = 60%).

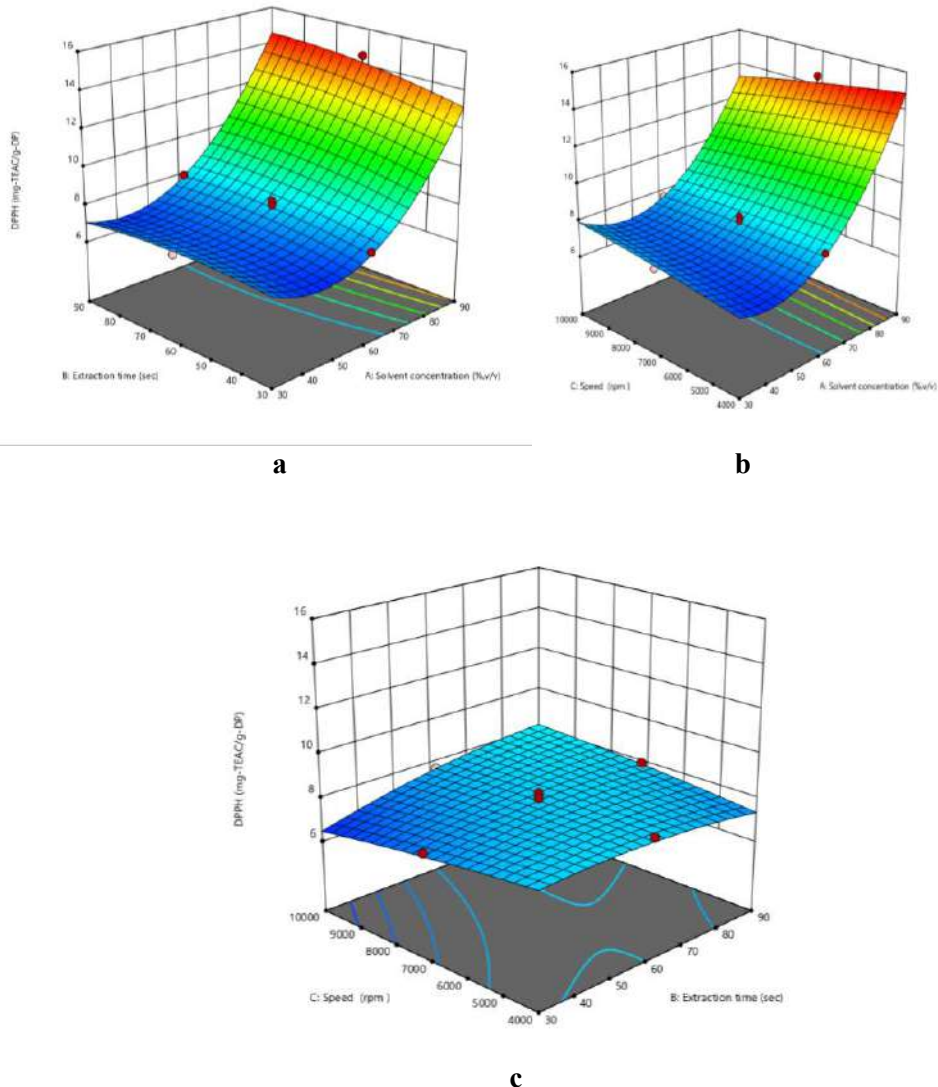


Figure 0.4: The response surface plot for the obtained AA-DPPH of the applied HAE method; (a) as a function of solvent concentration over time (speed = 7000 rpm); (b) solvent concentration as a function of rate (time = 60; (c) as a function of the acceleration time (solvent concentration = 60%).

The effects of the independent variables on the responses can be explained with three-dimensional response surface plots created from the model equations. As shown in Figure 3.2-3.4, the effects of independent HAE variables on TPC, TFC, and AA-DPPH, when one of the independent factors is constant, the color of the field changing from blue to red indicates increased response.

As shown in Figure 3.2-3.4, the mixing speed and extraction time have a positive effect on the systems. Therefore, TPC, TFC and AA-DPPH slightly increased with increase in mixing speed (rpm) and extraction time (sec).

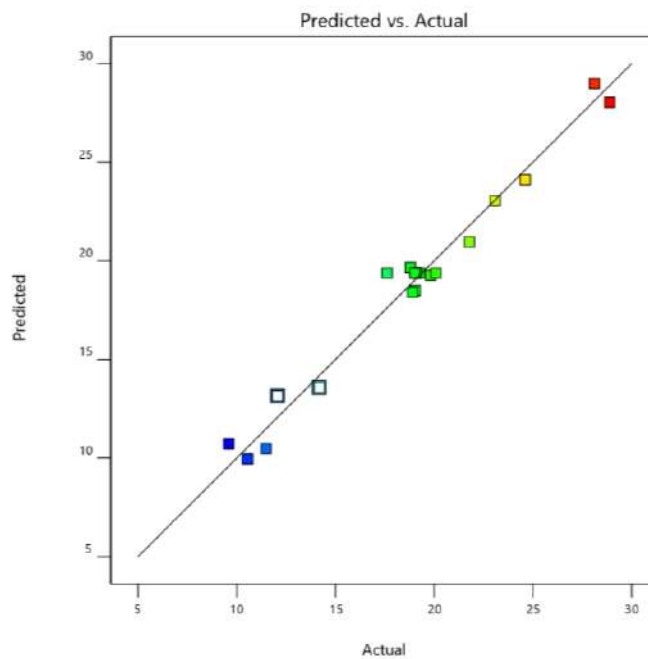
Also, as shown in Figure 3.2.a, the amount of TPC decreased with increasing ethanol concentration. In addition, TFM slightly increased with increase in ethanol concentration, as shown in Figure 3.3.a. on the other hand, the amount of AA-DDPH significantly increased with increase in ethanol concentration, as shown in Figure 3.4.a.

3.3. Optimization results

The optimum conditions for obtaining the highest TPC, TFC and AA-DPPH efficiencies are given in Table 3.3. Optimal conditions for obtaining maximum TPC, TFC and AA-DDPH (19.365 mg-GAE/g-DP TPC, 59.780 mg-CE/g-DP TFC and 10.727 mg-TEAC/g-DP AA-DPPH) were 90 sec extraction time, 7800 rpm stirring speed and 76% (v/v) ethanol solution. determined. Validation experiments were also performed under software conditions. The difference (< 2%) between the actual and predicted values confirms that the quadratic models are accepted. In fact, the adequacy of the model can also be evaluated by calculating the correlation between actual and predicted quantity as show in figure 3.5.

Table 0.3: Confirmation results of optimum conditions for HAE.

Optimum Extraction Conditions			respons	Actual	Predicted	error
A (%v/v)	B (rpm)	C (sn)				
76	7800	90	TPM	19.371	18.70	<2
			TFM	59.779	57.978	
			DPPH	10.727	8.859	



a

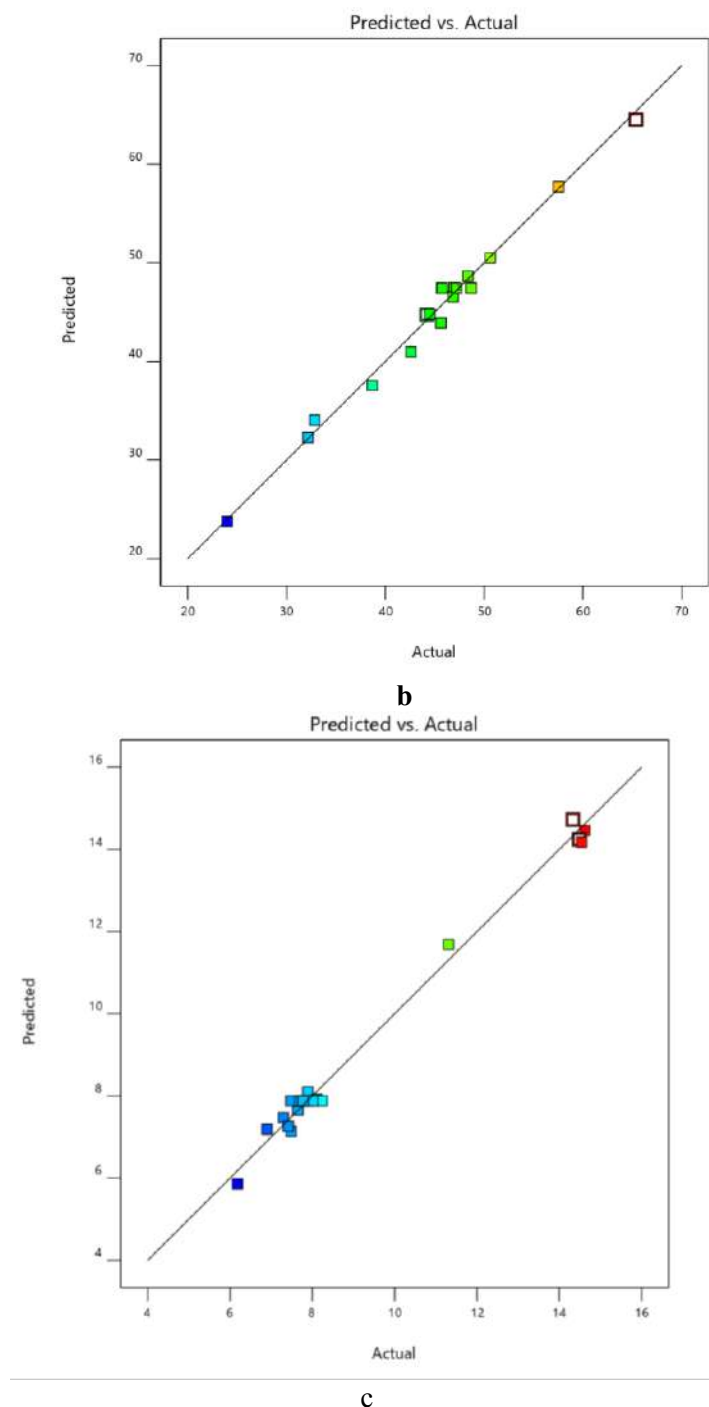


Figure 0.5: Actual versus predicted findings for TPC, TFC and AA-DPPH (a) TPC; (b) TFC; (c) AA-DPPH.

3.4.HPLC analysis

Individual phenolic compound (4-hydroxycinnamic acid) identification was completed by high-performance liquid chromatography (HPLC). Agilent 1260 chromatographic equipment (Agilent, Waldbronn, Germany) consisting of a quaternary pump, a degasser, a manual injector, and a diode-array

detector (DAD) was used. An Agilent Eclipse Plus C18 RRHD 18 column (3.0mm × 50.0mm id, 1.8mm particle size) was employed for the analysis of 4-hydroxycinnamic acid. Column temperature was 40 °C, flow rate was 2mL min⁻¹ and the injection volume was 20mL. Quantity of 4-hydroxycinnamic acid was estimated for all extract samples by peak areas and standard curves of corresponding standards as shown in table 3.4. whereas the amount of 4-hydroxycinnamic acid changed from 0.170236 mg/g-DP to 3.29472 mg/g-DP.

Tabela 0.4: Experimental results of the obtained HPLC capacity of the HAE method applied to *Teucrium chamaedrys* leaves *.

Run	A: Solvent concentration n (%, v/v)	B: Extraction time (sec)	C: Mixing speed (rpm)	HPLC (mg /g DP)
1	30	30	4000	1.896± 0.003
2	60	60	7000	2.169± 0.005
3	30	60	7000	2.011± 0.002
4	90	30	10000	0.755± 0.004
5	60	60	7000	2.080± 0.001
6	60	60	7000	2.173± 0.006
7	90	90	4000	1.009± 0.001
8	90	60	7000	0.917± 0.006
9	30	90	10000	2.087± 0.004
10	60	90	7000	4.146± 0.004
11	90	90	10000	1.410± 0.007
12	90	30	4000	0.170± 0.001
13	30	30	10000	0.912± 0.006
14	30	90	4000	2.359± 0.005
15	60	60	7000	2.006± 0.001
16	60	60	4000	0.856 ± 0.003
17	60	60	7000	2.053±0.001
18	60	60	10000	0.742±0.002
19	60	60	7000	2.340±0.005
20	60	30	7000	3.295±0.001

*Data are given as the arithmetic mean of 3 replicates.

4. Conclusion

Teucrium chamaedrys leaves were extracted by an alternative green method. Samples were found to be rich in phenolic and Flavonoid contents. The optimization study has suggested that 7800 rpm of mixing speed, 90 s of extraction time and 76% EtOH are optimum conditions to obtain maximum TPC (19.3715 mg-GAE/g-DP), TFC (59.779 mg-CE/g- DP) and DPPH (10.727 mg-TEAC/g-DP) from *Teucrium chamaedrys* leaves. The linear, square and interaction coefficients of all the parameters were found statistically significant.

hence this result *Teucrium chamaedrys* has shown to be a powerful source of bioactive ingredients depending on the in vitro analyses.



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FULL TEXT - ORAL PRESENTATION

GREEN EXTRACTION OF PHENOLIC COMPOUNDS FROM OLIVE LEAF BY MEANS OF DEEP EUTECTIC SOLVENTS: PRINCIPAL COMPONENT ANALYSIS AND OPTIMIZATION

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Abstract

Olive leaf extract has been recommended as natural additive for drug and food industries because of its rich phytochemical content [1]. Therefore, it is very important to evaluate this valuable component with green methods. From this point of view, deep eutectic solvents (DESs) have been utilized as alternative green solvent. This third type solvents can easily synthesis by mixing at least two components (hydrogen bond donor and hydrogen bond acceptor) [2].

The aim of present research is to suggest a DES-based microwave assisted extraction of phenolic substances from olive leaves by application of chemometric study. 15 individual citric acid-ethylene glycol DESs were synthesised with three molar ratios (1:2, 1:3, 1:4). Additionally, effect of different water addition level in DES (10, 30, 50, 70 and 90%) was investigated, and evaluated by principal component analysis (PCA). It was found that the best DES combination was obtained with 1:2 molar ratio and 70% water addition. Once the best combination was chosen, response surface method (RSM) was applied as statistical experimental design approach through Box-Behnken design. The effect of independent parameters was selected as microwave power, extraction time and solid/solvent ratio with three levels (300-500 W, 40-60 s and 0.2g/20 mL-0.2/40mL). The greatest phenolic (TPC), antioxidant activity and oleuropein yields of olive leaf extract were calculated with optimization study. Furthermore, analysis of variance (ANOVA) test was used to define the impacts of process parameters, their interactions and second powers by means of Design-Expert version 12 (Stat-Ease, Minneapolis, MN, USA) software trial. ANOVA also provided analysis of the model fitting indicators

Key Words: Phenolic compounds, oleuropein, microwave assisted extraction, PCA, Box-Behnken-RSM.

1. Introduction

Polyphenols are compounds with a benzene ring with high antioxidant properties. These compounds are of great importance for human health due to their antioxidant properties. Polyphenols are secondary metabolites found in parts of plants such as flowers, leaves, fruits, stems and roots, and they are examined in four classes as flavonoids, phenolic acids, stilbenes and lignans [3–5]. Spices and aromatic plants, which have been used as additives to increase the smell and taste of foods for years, are gaining importance. Therefore, the evaluation of various plant leaves and parts that come out as waste has become interesting both in terms of ecology and human health.

The leaves of the olive tree have been used in many cultures to treat various diseases. It is known that tea obtained from olive leaves in traditional medicine has therapeutic effects against diseases such as cough, sore throat, fever and cystitis, and the leaf is also used for dermatological diseases [6–8]. Today, various supplements containing olive leaves are sold in various forms (liquid, tablet, powder, etc.) with claims such as diabetes, high blood pressure, cardiovascular diseases, colds, and supporting the immune system. The main antioxidant compounds found in olive leaves are; oleuropein, hydroxytyrosol, rutin,

verbacoside, catechin, luteolin-7-glucoside and apigenin-7-glucoside. Oleuropein is a phenolic compound that has been the subject of scientific studies for years [9].

In the phase diagram, during the mixing of two or more compounds, a mixture with a lower value than the melting points of the compounds forming this mixture can be formed. The point where this mixture occurs is called the eutectic point and the resulting mixture is called the deep eutectic solvent. The deep eutectic solvent formulation generally consists of binary mixtures [10, 11]. One of the components is a hydrogen bond acceptor (HBA) and the other is a hydrogen bond donor (HBD). The main reasons for using deep eutectic solvents in the extraction process are their high solubility, adjustable physicochemical properties such as viscosity and polarity [12].

The present work focuses on the investigation of molar ratio variation of DESs and effect of water level addition by principal component analysis (PCA), firstly. Additionally, it was aimed to explore the effect of microwave power, extraction time and solvent volume in microwave assisted extraction on the extraction efficiency. Extraction process conditions were optimized and modelled by means of Box-Behnken design of response surface method (Box-Behnken-RSM).

2. Material and Methods

2.1. Materials

Olive leaf samples were supplied from Agean (Ayvalık, Turkey) at harvesting time for oil production. Leaf samples were dried and were ground (Moulinex Super Blender Grinder, LM209041).

Compounds for DES preparation including citric acid and ethylene glycol were obtained from Merck (Darmstadt, Germany).

Folin-Ciocalteu reagent, sodium carbonate ($\geq 99.0\%$), (+)-catechin, hydrochloric acid, sodium hydroxide, 2,2-diphenyl-1-picrylhydrazil (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and gallic acid monohydrate ($\geq 98.0\%$) were from Sigma-Aldrich (St. Louis, MO, USA), ethanol ($\geq 99.8\%$), methanol ($\geq 99.9\%$), aluminum chloride, sodium nitrite, and potassium persulfate were supplied from Merck (Darmstadt, Germany).

2.2. Preparation of DESs

Deep eutectic solvents are prepared by combining a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD). The relevant were mixed along with heating at 80°C by a magnetic stirrer (Model MSH-20D, DAIHAN Scientific Co. Ltd., Korea).

Citric acid as HBA and ethylene glycol as HBD were matched in different molar ratios. (Table 1). Additionally, the effect of different water level addition into the DESs was investigated.

Table 1. List of DESs initially tested for extraction.

No	HBA	HBD	Molar Ratio	Water Level (%)
1			1:2	10
2			1:2	30
3			1:2	50
4			1:2	70
5			1:2	90
6			1:3	10
7			1:3	30
8	Citric Acid	Ethylene Glycol	1:3	50
9			1:3	70
10			1:3	90
11			1:4	10
12			1:4	30
13			1:4	50
14			1:4	70
15			1:4	90

2.3. Microwave Assisted Extraction

Microwave assisted extraction equipment was used for the extraction of olive leaf samples (NEOS-GR, Milestone Srl, Italy). Firstly, 0.2 g of olive leaf and 20 mL DES were added to bottom flask in order to identify the most efficient DES combination. Extraction process were operated at 400 Watt (W) and 60 second (s) conditions. After the determination of best DES combination, the extraction conditions was constructed according to the independent parameters with their 3 levels. Power (300–500 W), extraction time (40–80 s), and solvent volume (20-40 mL) were the independent process parameters (X_1 , X_2 , and X_3).

2.4. Determination of total polyphenol content

Total polyphenol content (TPC) was specified by UV spectrophotometry based on the Folin-Ciocalteu method. UV-spectrophotometer (PG Instruments, T60/Leicestershire, England, UK) was set to 765 nm to measure absorbance values. Calculation was made using the calibration curve drawn with gallic acid. The results were expressed as equivalent of mg of gallic acid per g of dried leaf (mg-GAE/g-DL).

2.5. Determination of antioxidant activity

Antioxidant activity of samples were determined in compliance with DPPH assay [13]. 100 μ L of samples were diluted by 600 μ L 80% methanolic solution of DPPH radical. The reading absorbance was 517 nm. Trolox solution was utilized to create a standart curve. Results were expressed as mg trolox equivalent (TAEC) antioxidant capacity per gram of dried leaf.

2.6. HPLC Analysis

Oleuropein analysis was achieved with high-performance liquid chromatography (HPLC) using an through Agilent 1260 (Agilent, Waldbronn, Germany) system. Conditions of HPLC were reported in detail in our previos studies.

2.7. Principal Component Analysis

TPC, DPPH and HPLC results of DESs extracts were evaluated with principal component analysis (PCA). This method allows classification and size reduction of complex data sets. Water levels of DESs were investigated and categorized. Their properties such as TPC, DPPH were used to appraise their relationship statically using PCA. JMP® software was used in order to employ PCA.

2.8. Experimental Design

Box-Behnken design (BBD) was completed with three process parameters in order to model extraction process (Table 2.) Three-dimensional (3D) graphics were constituted to illustrate the independent variables. In this study, Design-Expert (Stat-Ease, Minneapolis, MN, USA) software version 12.012 was used.

Table 2. Process parameters with levels in Box-Behnken-RSM

	Independent Variables	Level		
		Low	Medium	High
X_1	Microwave Power (Watt)	300	400	500
X_2	Extraction Time (s)	40	60	80
X_3	Solvent Volume (mL)	20	30	40

3. Results and Discussion

3.1. Evaluation of DESs Performances and Chemometric Analysis

Citric acid-ethylene glycol DESs were designed with 15 different water content and three molar ratios (1:2, 1:3, 1:4) in this study. As seen in Figure 1, 15 different DESs and pure water compared for extraction efficiency with regard to TPC, AA and oleuropein content. It was examined to find the optimum point since the water content in DESs greatly affects the extraction efficiency. As can be seen from the Figure 1, it is seen that the extraction efficiency increases as the water content in the streams increases up to a certain point. The highest TPC, AA and oleuropein contents were observed in the deep eutectic solvent prepared with 1:2 molar ratios. Moreover, the superiority of the prepared DESs to pure water can be seen from the results. It has been observed that the DES with 70% water content and 1:2 molar ratio is four times more effective than pure water in terms of TPC. Considering these results, the rest of the study was continued with the specified solvent.

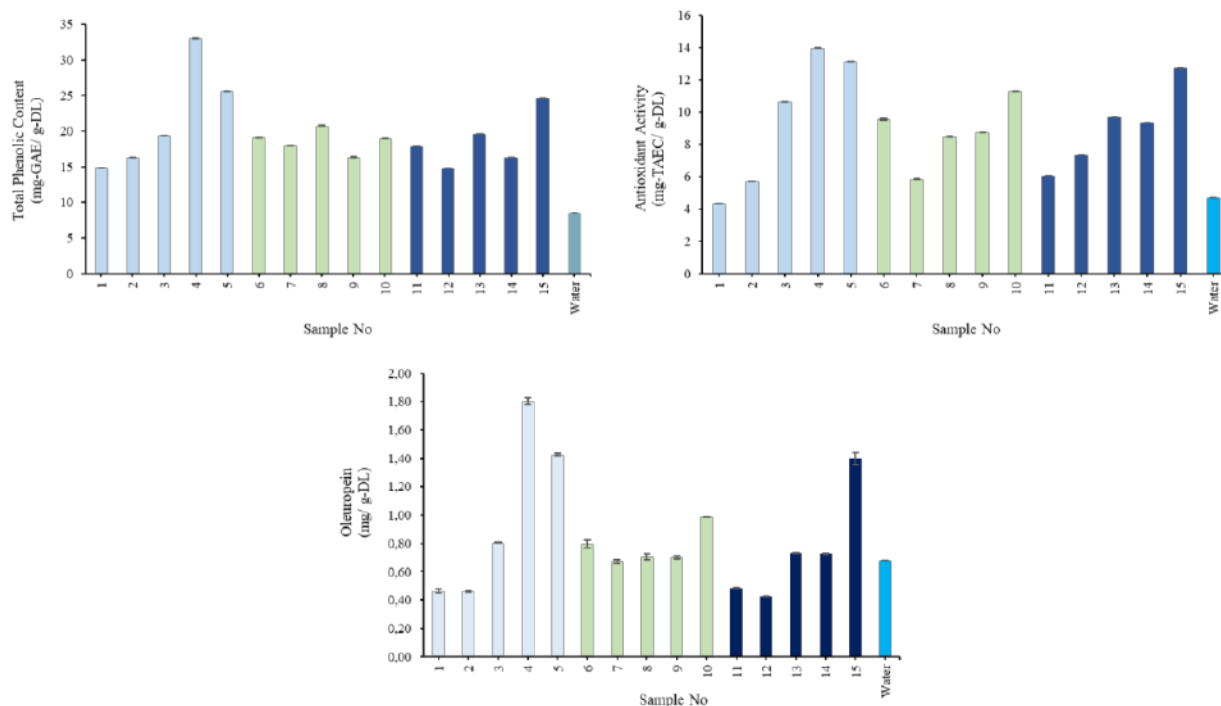


Figure 1. Extraction yields of different solvents for total phenolic content, antioxidant activity and oleuropein levels.*

*Error bars indicate the SD (n = 3).

3.2. Chemometric Analysis

Chemometric study has been performed by principal component analysis. The score plot of solvents were presented in the Figure 2. The contribution of each parameter to two factors and the distribution of varieties were calculated as 90.2% for the first component and 5.67% for the second component. The amount of total phenolic compounds had a positive, high and significant correlation with PC1. In general, solvents with the same water content showed a similar distribution. Based on the chemometric analysis, water levels has a significant affect regardless of the molar ratio of DES system.

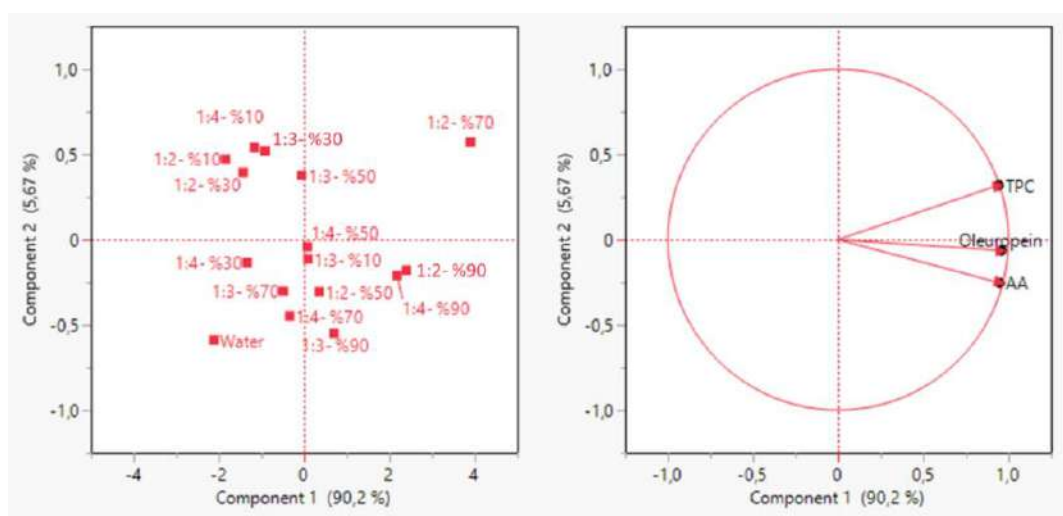


Figure 2. Score plot of PCA for the analysis of total phenolic content, antioxidant activity and oleuropein level of olive leaf extract.

3.3. Effects of independent parameters on MAE

Experimental plan of present study and the results of TPC and antioxidant activity were given in the Table 3. 17 different experimental study were designed by the software (Design-Expert) with 5 center pointes (400 Watt microwave power, 60 s extraction time, 30 mL solvent volume). 3D (three dimensional) surface plots are created in order to illustrate to more distinctly the interaction between variables.

Table 3. Experimental results formed by Box-Behnken-RSM*

	Independent Variables			TPC	Antioxidant Activity
	X ₁	X ₂	X ₃	(mg-GAE/ g-DL)	(mg-TAEC/ g-DL)
1	500	60	20	23.53±0.001	25.76±0.003
2	400	60	30	18.25±0.002	22.93±0.001
3	500	80	30	26.17±0.001	26.77±0.002
4	300	60	20	24.03±0.003	25.97±0.002
5	400	60	30	17.50±0.005	23.08±0.004
6	400	40	40	14.99±0.001	20.73±0.001
7	400	60	30	18.46±0.002	23.12±0.005
8	400	60	30	18.03±0.001	21.76±0.004
9	400	40	20	20.37±0.003	25.22±0.001
10	300	60	40	17.10±0.001	20.10±0.003
11	300	40	30	19.55±0.001	25.42±0.001
12	400	80	40	17.61±0.004	18.15±0.002
13	400	80	20	25.37±0.003	27.75±0.001
14	500	60	40	18.43±0.001	17.76±0.001
15	500	40	30	21.03±0.002	21.23±0.003
16	400	60	30	18.78±0.001	22.44±0.004
17	300	80	30	26.54±0.001	23.03±0.003

* Each value is the mean±standard deviation of three replicate analyses.

Figure 3 demonstrates the effects of independent variables on the MAE of TPC from olive leaf. As seen in the figures the total amount of phenolic substances in olive leaf under different MAE conditions varies between 14.99 and 26.54 mg-GAE per gram of dried leaf (Table 3).

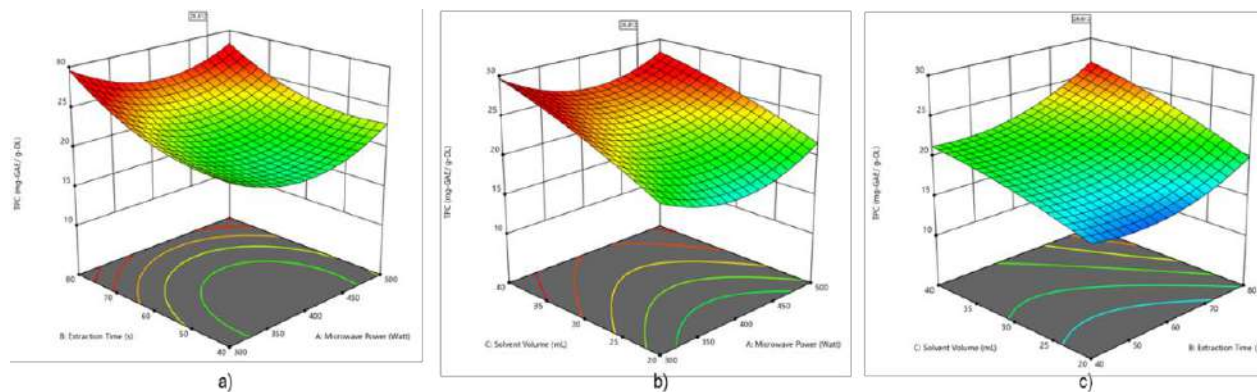


Figure 3. Response surface plots showing the impacts of independent variables including (a) extraction time (s) and microwave power (watt); (b) solvent volume (mL) and microwave power (watt); (c) solvent volume (mL) and extraction time (s) on the total phenolic content (mg-GAE/g-DL).

Figure 4 shows the effects of parameters on MAE of antioxidant activity from olive leaf. It is seen that the antioxidant activity levels of the microwave-treated samples varied from 17.76 to 27.75 mg-TAEC/g-DL based on the measurements of DPPH assay.

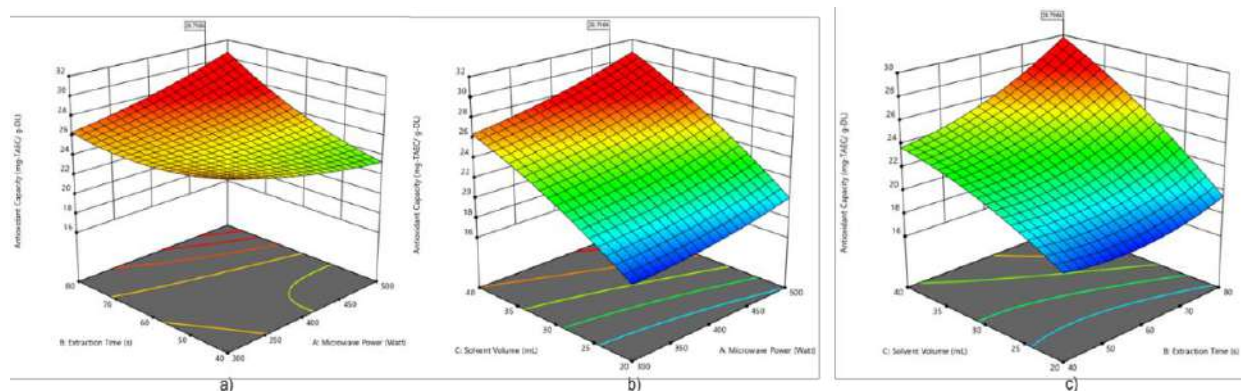


Figure 4. Response surface plots showing the impacts of independent variables including (a) extraction time (s) and microwave power (watt); (b) solvent volume (mL) and microwave power (watt); (c) solvent volume (mL) and extraction time (s) on the antioxidant activity (mg-TAEC/g-DL).

Considering the Figure 3 and Figure 4 solvent volume increase in the extraction of olive leaf by means of MAE method had positive effect both TPC and antioxidant activity. It might be explain by the solubility saturation of the extracted compounds. Similarly, as is seen from the figures that the extraction efficiency increases as the extraction time increases. Microwave power is a slightly effective factor in terms of extraction efficiency. In particular, it was observed that the level of antioxidant activity was slightly increased.

3.4. Box-Behnken-RSM

ANOVA table was created while microwave assisted extraction from olive leaf by means of DES. ANOVA test results were demonstrated in the Table 4 for TPC and AA levels of olive leaf extracts. Second order polynomial equations produced for both answer values were found to be statistically significant ($p < 0.0001$). The equations are given by Equations (1) and (2):

$$\begin{aligned} \text{TPC} & 18.20 + 0.2423 X_1 + 2.47 X_2 + 3.15 X_3 - 0.4612 X_1X_2 - 0.4583 X_1X_3 + & (1) \\ (\text{mg-GAE/ g-DL}) & 0.5950 X_2X_3 + 3.16 X_1^2 + 1.96 X_2^2 - 0.5825 X_3^2 \\ \text{AA} & 22.67 - 0.3737 X_1 + 0.3871 X_2 + 3.5 X_3 + 1.98 X_1X_2 + 0.5338 X_1X_3 + 1.28 & (2) \\ (\text{mg-TAEC/ g-DL}) & X_2X_3 + 0.4396 X_1^2 + 1.01 X_2^2 - 0.7096 X_3^2 \end{aligned}$$

Solvent volume of the extraction process was found to be significant for both response parameters ($p < 0.05$). Extraction time was found to be statistically significant just for TPC. Additionally, the second-order forces of the microwave power and extraction time were found significant for TPC. The interaction between microwave power and extraction time were found significant AA ($p > 0.05$). Moreover, the lack of fit value for both answer values was estimated to be higher than 0.05, and this situation supports that the models used are suitable for the data. It can be said that there is a satisfactory relationship between the experimental data and the estimated data for both systems (TPC and AA) based on the ANOVA findings.

Table 4. Analysis of variance findings on encapsulation efficiency values of microcapsules in terms of TPC and antioxidant activity

TPC	Sum of Squares	df	Mean Square	F-value	p-value	
Model	192.98	9	21.44	41.35	< 0.0001	Significant
X ₁	0.4696	1	0.4696	0.9057	0.3730	
X ₂	48.78	1	48.78	94.07	< 0.0001	
X ₃	79.17	1	79.17	152.67	< 0.0001	
X ₁ X ₂	0.8510	1	0.8510	1.64	0.2410	
X ₁ X ₃	0.8403	1	0.8403	1.62	0.2437	
X ₂ X ₃	1.42	1	1.42	2.73	0.1424	
X ₁ ²	41.92	1	41.92	80.85	< 0.0001	
X ₂ ²	16.24	1	16.24	31.31	0.0008	
X ₃ ²	1.43	1	1.43	2.75	0.1409	
Residual	3.63	7	0.5186			
Lack of Fit	2.71	3	0.9029	3.92	0.1100	Not significant
Pure Error	0.9213	4	0.2303			
Core Total	196.61	16				
<i>C.V.: 3.54%</i>		<i>R²=0.9815</i>		<i>Adjusted</i>		
<i>R²=0.9578</i>						

AA	Sum of Squares	df	Mean Square	F-value	p-value	
Model	130.42	9	14.49	32.15	< 0.0001	Significant
X ₁	1.12	1	1.12	2.48	0.1594	
X ₂	1.20	1	1.20	2.66	0.1470	
X ₃	97.74	1	97.74	216.85	< 0.0001	
X ₁ X ₂	15.72	1	15.72	34.87	0.0006	
X ₁ X ₃	1.14	1	1.14	2.53	0.1558	
X ₂ X ₃	6.54	1	6.54	14.51	0.0066	
X ₁ ²	0.8136	1	0.8136	1.80	0.2210	
X ₂ ²	4.27	1	4.27	9.47	0.0179	
X ₃ ²	2.12	1	2.12	4.70	0.0667	
Residual	3.16	7	0.4507			
Lack of Fit	1.83	3	0.6116	1.85	0.2782	Not significant
Pure Error	1.32	4	0.3301			
Core Total	133.58	16				
C.V.:2.92 %			<i>R</i> ² =0.9764			<i>Adjusted</i>
<i>R</i> ² =0.9460						

3.5. Optimization and Verification

A validation study of the optimum conditions predicted by the relevant software was carried out. Optimum MAE conditions for TPC and AA to gain maximum extraction efficiency with verification results were presented in Table 5. Considering the small and acceptable error rates, the proposed conditions were found to be acceptable.

Table 5. Verification results of the optimum conditions for the microwave assisted extraction of sour cherry peels

Optimum Extraction Conditions			Parameter	Predicted	Experimental	Error
X ₁ (Watt)	X ₂ (s)	X ₃ (mL)				
500	80	35	TPC	26.73	25.81±0.001	3.56
			AA	28.12	27.56±0.003	2.03

* Data are given as the mean (n=3) ± standard deviation

4. Conclusion

The present research has investigated the various DESs combinations in microwave assisted extraction of phenolic compounds from olive leaf. The study has suggested that citric acid-ethylene glycol mixture synthesised at the molar ratio of 1:2 and 70% water level. Statistical experimental design study was applied after the selection of the best DES. The relevant software suggest that 500 W microwave power, 80 s extraction time and 35 mL solvent volume as optimum process parameters to achieve TPC (26.73 mg-GAE/g-DL) and AA (28.12 mg-TEAC/g-DL) from olive leaf. As a result, it was observed that the



experimental data and the estimated data were compatible with each other, when statistical indicators were taken into account such as p-value, coefficients of determination and root mean square error.

Conflict of Interest

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

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FULL TEXT - ORAL PRESENTATION

INVESTIGATION OF PHYSIOCHEMICAL PROPERTIES, POLLEN GERMINATION, VIABILITY AND TUBE LENGTHS OF *Colchicum kurdicum* (BORNM.) STEF. AND *Colchicum szovitsii* FISCH. ET MEY. GROWN IN VAN REGION

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Abstract

The geophytes plants constitute an important part of the floristic richness in our country. Bulbous plants or flower bulbs are the other synonyms for this plant family. The geophytes with a 35 percent endemism rate have more than 4.300 plants worldwide and 1.060 plants in our country. The most important geophytes belonged to Liliaceae family. *Colchicum* sp. is one of the most important genera especially in our country, which is represented by 34 species, three variants and 16 species are endemic.

In this study, pollen height, pollen width, pollen number, pollen viability percentages with viability tests, pollen germination percentage, pollen germination tube length, nitrogen balance index (NBI), chlorophyll, flavonol and anthocyanin amounts were determined in *Colchicum szovitsii* and *Colchicum kurdicum* species collected from the flora in Van province and were compared with each other. The results showed that pollen width, height and number were 25.53µm, 45.98µm and 10 in *Colchicum szovitsii* species, while it was determined as 30.33µm, 59.27µm and 28.0 in *Colchicum kurdicum* species respectively. For determination of pollen viability percentage, the IKI test (iodized potassium iodide), Safranin and TTC tests (2,3,5 triphenyl tetrazolium chloride) were applied. Pollen viability rates in *Colchicum szovitsii* and *Colchicum kurdicum* species were 77.77% and 87.55% in IKI test, 84.90% and 88.72% in Safranin test, and 72.13% and 77.65% in TTC test, respectively. Pollen germination rate and pollen germination tube length were also 36.61-88.42µm in *Colchicum szovitsii* and 64.99%-131.66µm in *Colchicum kurdicum* species. The NBI, chlorophyll, flavonol and anthocyanin values were determined as 36.2 dualex index, 62.0 dualex index, 1.89 dualex index and 0.01 dualex index in *Colchicum szovitsii* species, while 39.36 dualex index, 61.3 dualex index, 1.62 dualex index and 0.02 dualex index in *Colchicum kurdicum* species.

Key Words: Chlorophyll, Geophyte, Pollen morphology, Pollen vitality.



1. Introduction

Bitter crocus is herbaceous and cormous perennial plant belonged to Liliaceae family. Recently, 99 species were accepted which are naturally distributed worldwide and considered as the main gene center for flora of Turkey with 49 species (Kaya, 2011). There are 50 bitter crocus species naturally distributed in Turkey which 22 species are endemic (Güner et al., 2002). *Colchicum* species are very dangerous plants for human and animal health because they contain poisonous alkaloids (Yaldız et al., 2010). The most important alkaloid in bitter crocus species is colchicine (colchicine, C₂₂H₂₂NO₆), which was first isolated in 1820. Today, it plays an important role in treatment of several diseases in the world, alone or in combination with other different drugs (Toplan et al., 2016). Bitter crocus species contain flavonoids, phenolic acids, tannins and fatty acids as well as alkaloids (Evans, 2002). Its species are classified in two main groups' base on their flowering time in autumn and in spring. Spring-flowering types had different names in different regions such as Aliöksüz, Öksüzali, Öksüzdoğan, Snow flower in Anatolia (Kaya, 2011).

Turkey is considered as important natural distribution area of bitter crocus species. The bitter crocuses are used in the treatment of several diseases all over the world on different species. The cultivation of rare plant species which are needed by humans in cultural environments is very important (Uysal and Kaya, 2019).

The healthy development of pollen, which is the male sex cell in plants, as well as its high viability and germination abilities are of great importance for fertilization. In addition to these features, which are considered as pollen quality parameters, the numbers of pollen produced in flowers are also important. Also, the amount of normally developed pollen in terms of morphology has great importance (Eti, 1990; Normand et al., 2002; Abacı and Asma, 2014). So, determination of pollen morphology, as well as the viability and germination abilities of the pollen have important for both cultivated and breeding studies (Adıgüzel et al., 2018).

In this study, the pollen germination, vitality, pollen tube lengths and physicochemical properties of *Colchicum szovitsii* and *Colchicum kurdicum* species which grow naturally in the Van region were studied.

2. Material and Methods

Samples collected from the flora of Van province was compared morpho-anatomically which consisted of *Colchicum szovitsii* and *Colchicum kurdicum* species.

Species information: *Colchicum kurdicum* - B9 Van: Bahçesaray, between Van and Bahçesaray, Karapet Pass, 38° 09' 13" N, 42° 54' 15" E, wet areas with snow, 3015 m, 15.07.2020; *Colchicum szovitsii* – B9: Bitlis: Tatvan, between Van and Tatvan, south of Alacabuk village, 38° 22' 17".

The flower and leaf samples were transferred to the Physiology and Cytology Laboratories of the Field Crops Department for further analysis.

A Leica DM500 light microscope (equipped with Leica ICC50 HD camera and Leica LAS EZ (version 3.0) software) was used for pollen counting and measurements. The number of pollen per area was determined as the number per mm².

Number of Produced Pollen per Flower (NPP): One day before anthesis, ten flower buds from each species were randomly collected for counting the number of produced pollen grain flower-1. The flower buds were divided into two groups and each of five flower buds were incubated in glass bottle at the room temperature for 24 hours until opening. The pollens were kept in micro tubes (5 ml) and homogenized with 2 ml of distilled water for each group of buds. Total NPP grains were counted by Leica light microscope (Eti, 1991).

Pollen viability tests: TTC (2, 3, 5-triphenly tetrazolium chloride), IKI (iodine+potassium iodide) and safranin stain tests were used for determination pollen viability. Pollen grains were treated by 1%TTC solution and studied after two hours. The grains of pollen were counted to determine viability after a

couple of minutes in the IKI medium (1g KI and 0.5g I dissolved in 100ml distilled water). The pollen viability was obtained one hour following treating by safranin medium [(1g safranin dissolved in 95% alcohol (40 ml) and the final volume was made up to 100 ml. One part safranin was mixed with two parts glycerol and one part distilled water (1:2:1)]. To determine viability, three hundred pollen grains of each replicate from four different areas were counted under a light microscope. In this study, pollen morphological homogeneity was also investigated (Eti, 1990).

Germination of Pollen (PG): The pollen grains were incubated on germination culture medium including 15% (w/v) sucrose and solidified with 1% (w/v) agar. Pollen grains were spread uniformly on the germination medium in 6 cm diameter Petri dishes. They were incubated for 12 hours; at 25±1 °C. Germinated pollen grains were counted when the pollen tube length exceeded twice the pollen diameter. Two random microscopic fields were counted in a Petri dish and two Petri dish were counted per species. In addition, the lengths of pollen tubes in each replication in six areas of agar-plate medium were measured using an ocular micrometer on a Leica light microscope at the end of the 24 h incubation period (Sharafi ve Bahmani 2011).

Nitrogen balance index (NBI), chlorophyll (Chl), flavonol (Flav) and anthocyanin (Anth) contents in the leaves of the plants were measured in real time and non-destructively on the leaf by Dualex scientific+ (FORCE-A, France) device.

3. Results and Discussion

Pollen width, length and number of *Colchicum* species showed significant differences (Table 1). Pollen width, length and number in *C. kurdicum* were detected 45.98 µm, 25.53 µm and 10, while, in *C. szovitsii* species were 59.27 µm, 30.33 µm and 28, respectively. Pollen of Liliales is unusually diverse, including a wide range of aperture types and a variety of surface sculpturing patterns and wall stratification (Rudall et al. 2000). It is mostly ellipsoidal in shape, with dimensions of (16.6–)47.7(–114.8) ×(10.3–)26.0(–78.6) mm, or circular to oval, with a diameter of (10.0–)28.7(–47.5) mm. It is mostly ellipsoidal in shape, with dimensions of (16.6–)47.7(–114.8) × (10.3–)26.0(–78.6) mm, or circular to oval, with a diameter of (10.0–)28.7(–47.5) mm (Furness et al., 2015). It was determined that the results obtained from our study were similar to reported previous results

Table 1. Pollen width, length and number of *C. kurdicum* and *C. szovitsii* species.

	Pollen width (µm)	Pollen length (µm)	Pollen number
<i>C. kurdicum</i>	45.98±3.29	25.53±5.57	10±1.00
<i>C. szovitsii</i>	59.27±1.81	30.33±2.67	28±2.64

The IKI, Safranin and TTC viability tests results showed that pollen viability rates of *C. szovitsii* species were higher than *C. kurdicum* species (Table 2). The viability rate obtained by the IKI test was 77.59% in *C. kurdicum* species and 87.55% in *C. szovitsii* species, respectively. The viability rate in the safranin test was determined as 84.90 and 88.72 in *C. kurdicum* and *C. szovitsii* species, respectively. In the TTC test, the viability rates were determined as 72.13% in *C. kurdicum* species and 77.65% in *C. szovitsii* species. In the TTC test, lower rates of pollen viability were determined compared with other two test methods. It was observed that the best pollen viability could be obtained in the Safranin test. In a study on the viability test performed on roses, it was revealed that the lowest viability rate was measured in the TTC test, and the highest rate was in the Safranin test (Erbaş et al., 2015) which is similar to our findings.

The viability rates obtained by the agar plate method are presented in Table 2. The results obtained from biological and chemical methods (Safranin, IKI and TTC) were similar. It was determined that the germination of *C. kurdicum* (36.61%) was lower than that of *C. szovitsii* (64.99%). Pollen germination tube length was 131.66 µm in *C. szovitsii* species and 88.42 µm in *C. kurdicum* species. Low pollen germination rate and short pollen tube are the main factors affecting seed setting rate and

plant productivity. For this reason, it is necessary to know the vitality status of plant pollen for breeding programs (Abacı and Asma, 2014).

Table 2. Biological and chemical viability tests in *C. kurdicum* and *C. szovitsii* species.

	IKI (%)	Safranin (%)	TTC (%)	Pollen germination percentage (%)	Pollen germination tube length (µm)
<i>C. kurdicum</i>	77.59±2.50	84.90±2.56	72.13±2.57	36.61±7.29	88.42±13.78
<i>C. szovitsii</i>	87.55±3.08	88.72±6.54	77.65±3.39	64.99±6.01	131.66±7.76

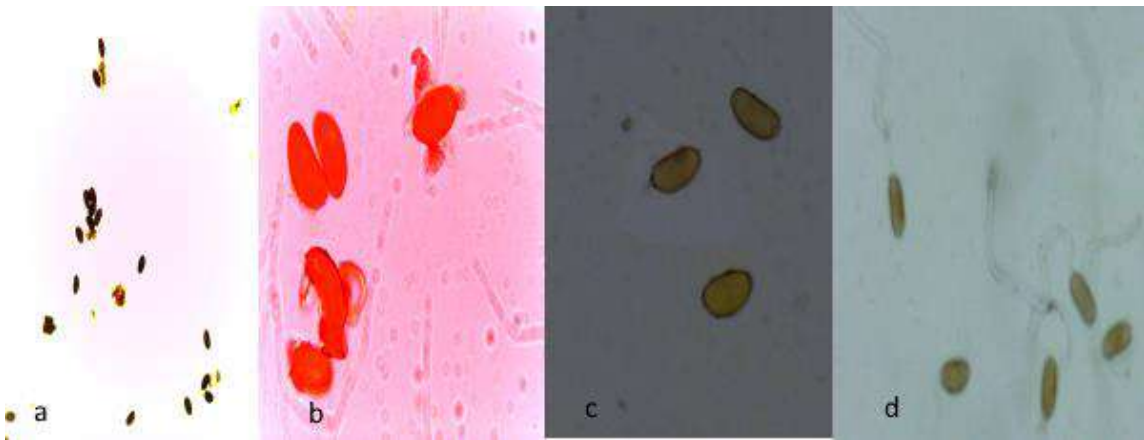


Figure 1. *C. kurdicum* species a. IKI b. Safranin c. TTC d. Pollen germination tube.

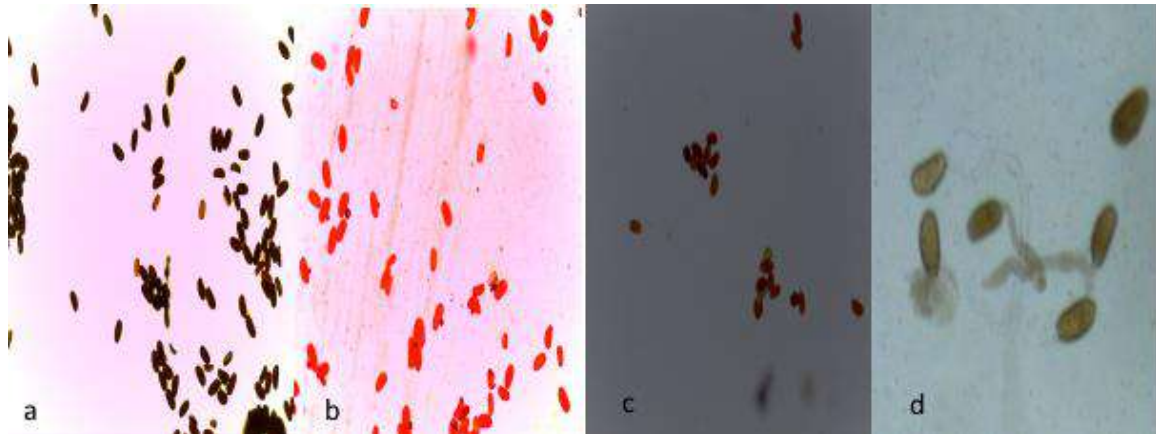


Figure 2. *C. szovitsii* species a. IKI b. Safranin c. TTC d. Pollen germination tube

Nitrogen balance index is accepted as an important parameter on the growth and development of plants. NBI values are a method used to obtain accurate information about yield and quality in plant production (Oral et al., 2021). According to the data obtained using the Dualex Scientific+ device, the amount of NBI was determined as 39.36 dualex index in *C. szovitsii* species and 36.2 dualex index in *C. kurdicum* species. Chlorophyll value, which is the effective parameter in photosynthesis, was higher in *C. kurdicum* (62.0 dualex index) than *C. szovitsii* (61.3 dualex index). Flavonols have also important roles on flowering in plants. During pollination, they produce red-blue or yellow pigments that serve to attract insect and bee populations (Birman, 2012). In this study, flavonol values were determined as 1.62 dualex index and 1.89 dualex index in *C. szovitsii* and *C. kurdicum* species, respectively.



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Anthocyanins are water-soluble compounds of various colors, just like flavonols. They are found in the cavities within the epidermal and mesophyll tissue in the plant. It creates a secondary defense mechanism in plants by providing color change in various organs against abiotic factors such as drought stress (Aztekin and Kasim, 2016). It was determined that the amount of anthocyanin was higher in *C. szovitsii* (0.026 dualex index) species than in *C. kurdicum* (0.016 dualex index).

Table 3. physiological values of *C. kurdicum* and *C. szovitsii* species.

	NBI (Duaalex index)	Klorofil (Duaalex index)	Flavonoid (Duaalex index)	Anthosiyenin (Duaalex index)
<i>C. kurdicum</i>	36.2±3.61	62±4.65	1.89±0.03	0.016±0.005
<i>C. szovitsii</i>	39.36±3.17	61.3±3.25	1.62±0.13	0.026±0.01

4. Conclusion

Chemical viability tests (safranin, IKI and TTC), biological viability tests (agar plate), germination tests and some physiological parameters were investigated in the pollen of *Colchicum* species during the flowering period. There were significant differences in pollen viability rates among different chemical and biological viability tests and methods. Among the chemical tests, the highest viability rate was obtained in the safranin test and the lowest viability rate was obtained in the TTC test. It was determined that NBI and anthocyanin amounts were high in *Colchicum szovitsii* species, and chlorophyll and flavonol levels were high in *Colchicum kurdicum* species. As a result of the study, it was determined that *Colchicum szovitsii* species had higher values in terms of the studied parameters.

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FULL TEXT - ORAL PRESENTATION

**MICROENCAPSULATION OF CLARY SAGE (*SALVIA OFFICINALIS*
L.) ESSENTIAL OIL BY COMPLEX COACERVATION:
MICROCAPSULE CHARACTERIZATION AND ENCAPSULATION
EFFICIENCY**

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Abstract

Clary sage essential oil is obtained from *Salvia sclarea* L. (family Lamiaceae). The main components of clary sage oil are linalool and linalyl acetate [1]. It has anti-oxidant, anti-stress, antimicrobial, anticancer, anti-tumor, cytotoxic, antidepressant, and anti-inflammatory effects [2-3]. However, it is chemically unstable and susceptible to environmental degradation. In order to overcome the disadvantages and increase its application in foods, perfumery, and aromatherapy, microencapsulation technology is one of the most promising ways. Microencapsulation is a means of applying relatively thin coating materials to core materials such as very tiny droplets or particles of liquid or solid materials [4]. This study aimed to investigate the microencapsulation of clary sage essential oil by a complex coacervation method, using gelatin and gum Arabic as the coating material. In the synthesis process, clary sage oil was emulsified in a polymeric solution by ultrasonic treatment for determined times (5, 10, 15, and 20 minutes). The effect of ultrasonication time on the encapsulation efficiency of clary sage oil through the complex coacervation process was investigated. The optimal time of ultrasonic treatment for the complex coacervation between gelatin and gum Arabic was observed to be 20 minutes. The sage oil microcapsules showed excellent encapsulation ability as indicated by high total oil and low surface oil. The morphology and chemical structure of microcapsules was investigated with a Field Emission Scanning Electron Microscopy (FESEM) and a Fourier Transform Infrared Spectrometer (FT-IR). The results confirmed that clary sage oil was successfully encapsulated with gelatin and gum Arabic via complex coacervation method.

Key Words: Microencapsulation, ultrasonic treatment, clary sage essential oil, complex coacervation

1. Introduction

Essential oils are complex liquid mixtures containing compounds with different organic functions, such as hydrocarbons, alcohols, aldehydes, esters, and phenols. They are extracted from different parts of the aromatic plants, such as flowers, leaves, seeds, and fruits [1]. They have superior biological activity properties such as antibacterial, cytotoxic, antifungal, anticancer, antimutagenic, antidiabetic, antiviral, and antioxidant properties [2]. Because of these properties, essential oils can be used in the cosmetic, pharmaceutical, food, perfumery, and agricultural industries [3]. However, most components of essential oils are sensitive to light, moisture, oxygen, and high temperature, limiting their use in practical application. Therefore, encapsulation is one of the most effective techniques to protect them against degradation and evaporation. In this technique, bioactive compounds as core or active agents are surrounded by wall materials [4,5]. Several methods for encapsulation have been investigated including spray chilling [6], complex coacervation [7], liposome formation [8], spray-drying [9],



emulsification/internal gelation [10]. Among these techniques, complex coacervation is the most favored process due to providing a higher encapsulation efficiency and higher thermal stability compared to other techniques [11]. In this technique, the ionic interaction between two oppositely charged biopolymers leads to the formation of coacervates and phase separation. In most studies, the complex coacervation process has been used to encapsulate essential oils such as roasted coffee [12], Sichuan pepper [13], sacha inchi [14], mustard (*Sinapis alba*) seed [15], oregano (*Origanum vulgare*) [16], thyme, clove, cinnamon and black pepper [17] essential oils.

Clary sage (*Salvia sclarea*) is an aromatic plant belonging to the Lamiaceae family and native to southern Europe and central Asia [18,19]. Three essential oil compounds of clary sage are the monoterpenes linalool and linalyl acetate, and the diterpene sclareol [20]. It has shown anti-oxidant, anti-stress, antimicrobial, anticancer, anti-tumor, cytotoxic, antidepressant, and anti-inflammatory properties [21,22]. Sage is commonly used in medicine to cure illness such as depression, dementia, obesity, diabetes, lupus, heart disease, cancer [23] and it is also well known as a flavoring and aroma agent in food industry. Besides these applications, it is also used in the phytopharmacy and cosmetic industry [24].

The purpose of this study was to microencapsulate clary sage essential oil using gelatin and gum Arabic as wall materials and to investigate the relationship between the encapsulation efficiency of microcapsules and the ultrasonication treatment time in the complex coacervation process.

2. Material and Methods

2.1. Materials

Clary Sage (*Salvia sclarea* L.) essential oil from Phrau Natural Cosmetics was used as the core material. Gelatin and gum Arabic from Alfasol (Istanbul, Turkey) were used as wall materials. Glutaraldehyde (50 wt. % solution in ethanol, Acros Organics) was used as a cross-linker in the complex coacervation process. Hydrochloric acid (HCl, 37%) and sodium hydroxide (NaOH, reagent grade) were purchased from JT Baker (USA). n-hexane (analytical reagent) and isopropyl alcohol chemicals used to determine the surface oil and total oil contents of the microcapsules were obtained from Merck Company (Germany).

2.2. Preparation of Clary Sage Oil-Loaded Microcapsules

The clary sage oil-loaded microcapsules were prepared by complex coacervation method using 200 mL of gelatin solutions (6%, w/w) and 50 mL of gum Arabic solutions (2.0% w/w). In this method, firstly, the wall material solutions were prepared by dissolving the wall material in distilled water. The pure 5.0 mL of clary sage oil was gradually added to the gelatin solutions under stirring at 1000 rpm for 10 min and the emulsions were then sonicated by an ultrasonicator device (Bandelin, HD4100, Germany) at 20 kHz, 50% amplitude and no pulsation ultrasound regime for determined times (5, 10, 15, and 20 minutes) to make uniform emulsions. Then, gum Arabic solutions were added to the emulsions and stirred by a magnetic stirrer (Isolab Laborgerate GmbH, Germany) for another 30 min. After the steps, the pH value of the emulsions was adjusted to 4.0 by adding 0.5 M of hydrochloric acid solution, slowly stirred for 20 min and gradually lowered the temperature to 10°C in an ice bath. 10 mL of glutaraldehyde solution (50% w/v) were slowly added and stirred for 60 min to crosslink the wall polymers of microcapsules. After the crosslinking process, the obtained microcapsules were separated by centrifugation (Nuve, NF 400, Turkey) for 60 min at 4000 rpm and 25 °C, and dried at 50 °C in an oven (Natural Convection Oven, JSON-100, Republic of Korea). The microcapsules named as CSEOM_5t, CSEOM_10t, CSEOM_15t, and CSEOM_20t corresponding to 5, 10, 15, and 20 minutes of ultrasonication time applied during the synthesis process, respectively, were produced.

2.3. Analysis of Total Oil, Surface Oil and Encapsulation Efficiency

The surface and total clary sage essential oil contents of microcapsules were determined gravimetrically according to the methods of Bajac *et al.* 2022 and with some modifications [25].

To determine the surface oil content of microcapsules, about 1.5 g of dried microcapsules were placed in a screw-capped tube and then 15 mL of hexane was added. The mixture was stirred manually for 2 min at room temperature without microcapsule destruction to extract the surface oil that was not microencapsulated. After extraction, the dispersion was filtered by a Buchner funnel and the microcapsules were washed three times with 20 mL of hexane. Finally, the filtrate was collected and evaporated to dryness. The surface sage oil content was determined gravimetrically by the ratio of surface oil mass to dry microparticle mass.

To determine the total sage oil content of microcapsules, around 0.5 g of microcapsules were mixed with 4 mL of hydrochloric acid (4.0 mol/L) in a sealed container. The mixture was agitated for 30 min at 40°C to disintegrate the structure of microcapsules. Then, 15 mL of a solvent mixture (hexane:isopropanol 3:1, v:v) was added into this mixture and stirred in an automatic shaker at 100 rpm to speed up the extraction of the oil into the solvent phase. After extraction, the dispersion was then centrifuged at 4,000 ×g for 10 min and the upper phase containing the dissolved oil was then separated. The extraction step was repeated as described above and all supernatants were collected, and then evaporated to dryness. The total sage oil content was determined gravimetrically by the ratio of the extracted oil mass to the dry microparticle mass. Measurement of the total oil and surface oil was carried out by triplicate for each sample.

The encapsulation efficiency was calculated via the following equation [25]:

$$\text{Encapsulation Efficiency \%} = \frac{W_{TO} - W_{SO}}{W_{TO}} \times 100$$

where W_{TO} is the weight of total clary sage essential oil in microparticles and W_{SO} is the weight of surface oil.

2.4. Characterization of Clary Sage Oil-Loaded Microcapsules

The morphology of microencapsules was studied by a field emission scanning electron microscopy (FESEM, Gemini Supra 40 VP, Zeiss, Germany). After coating the samples with gold using a sputter coater, the specimen was evaluated with FESEM at an accelerating voltage of 20 kv. The magnification of SEM was recorded at 200x.

Fourier Transform Infrared (FT-IR) spectra in the range of 400–4000 cm^{-1} from the pure clary sage essential oil, gelatin, gum Arabic, and the microcapsules were measured using Thermo Scientific Nicolet (S50FT-IR) spectrometer. These measurements were done at room temperature with 50 scans, at a resolution of 4 cm^{-1} .

3. Results and Discussion

3.1. Total Oil, Surface Oil and Encapsulation Efficiency

The effect of ultrasonication time on the surface oil, total oil, and encapsulation efficiency of clary sage oil-loaded microcapsules was investigated and the results are given in Table 1. Encapsulation efficiency values of the sage oil-loaded microcapsules were obtained in the range of 87.00–97.98%. Among the microcapsules, CSEOM_20t microcapsules have the highest encapsulation efficiency (97.98%), while CSEOM_5t microcapsules present the lowest one (87.00%). The results showed that decreasing the ultrasonication time had a positive effect on the encapsulation of clary sage oil. While

the surface oil of the clary sage oil-loaded microcapsules was decreased from 1.25% to 0.34% by increasing the ultrasonication time from 5 minutes to 20 minutes, the total oil increased from 9.63% to 16.80%.

Table 1. Surface oil, total oil and encapsulation efficiency of clary sage oil-loaded microcapsules.

Sample	Total Oil (%)	Surface Oil (%)	Encapsulation Efficiency (%)
CSEOM_5t	9.63±0.28	1.25±0.12	87.00±1.43
CSEOM_10t	12.04±0.61	0.92±0.17	92.34±1.29
CSEOM_15t	15.03±0.58	0.63±0.11	95.78±0.78
CSEOM_20t	16.80±0.22	0.34±0.15	97.98±0.88

Data represented as Mean± SD, n=3.

3.2. Characterization of Clary Sage Oil-Loaded Microcapsules

SEM images of microcapsules obtained by 20 minutes of ultrasonication time are shown in Figure 1. The SEM image of the microcapsules reveals that the microcapsules are almost spherical and elliptical in shape with micro-sized.

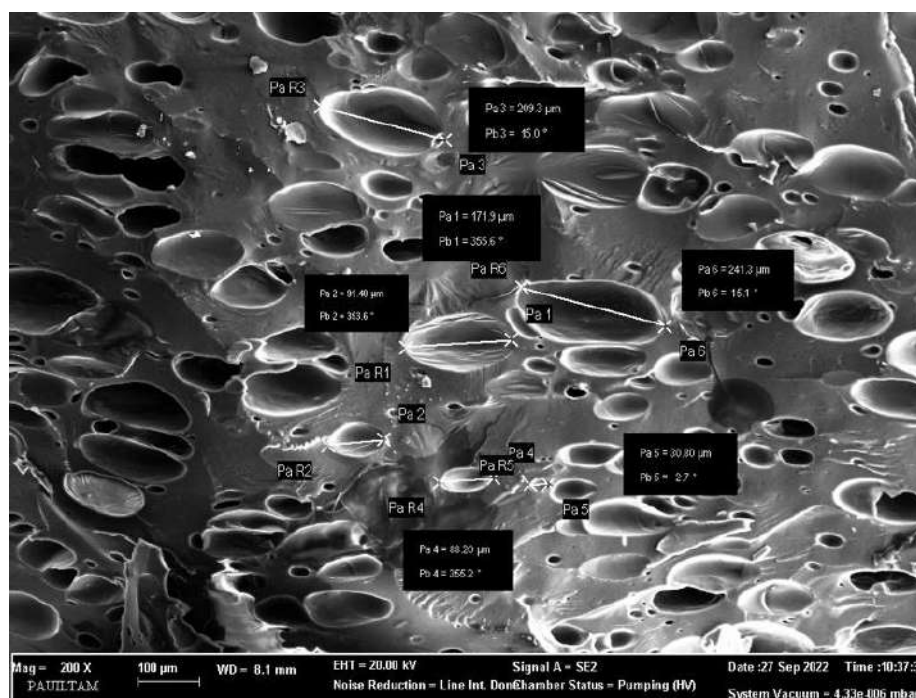


Figure 1. SEM image of clary sage oil-loaded microcapsules

The FTIR was used to confirm the presence of clary sage oil, gelatin and gum Arabic in the microcapsule structure, and to investigate their possible interactions. The obtained FT-IR spectra from pure clary sage essential oil, gelatin, gum Arabic, and the microcapsules are presented in Figure 2.

The FTIR spectrum of clary sage oil displays the characteristic peaks of its main components. In FTIR spectra of sage oil, a characteristic peak positioned at about 1742.6 cm⁻¹ is attributed to camphor [26]. The bands in the regions 2957.8-2872.4 cm⁻¹ are assigned to symmetric, asymmetric C-H stretches (CH₃, CH₂), while the bands in the regions 1455.5-1374.5 cm⁻¹ are related to symmetric, asymmetric bending of CH₃ and CH₂ [26].

In the spectra of microcapsules, the characteristic peaks of clary sage oil are observed unchanged, which is a result of successful incorporation of clary sage oil into the microcapsules. The results showed that chemical stability of sage oil was protected after encapsulation and there was no significant chemical interaction between sage oil and wall materials. It is observed that the broad peak at 3288.7 cm^{-1} and 3346.6 cm^{-1} in the spectrum of the gelatin and gum Arabic, respectively, shifted to 3300.3 cm^{-1} in that of the microcapsule, indicating a result of the crosslinking reaction between the wall materials and glutaraldehyde [27].

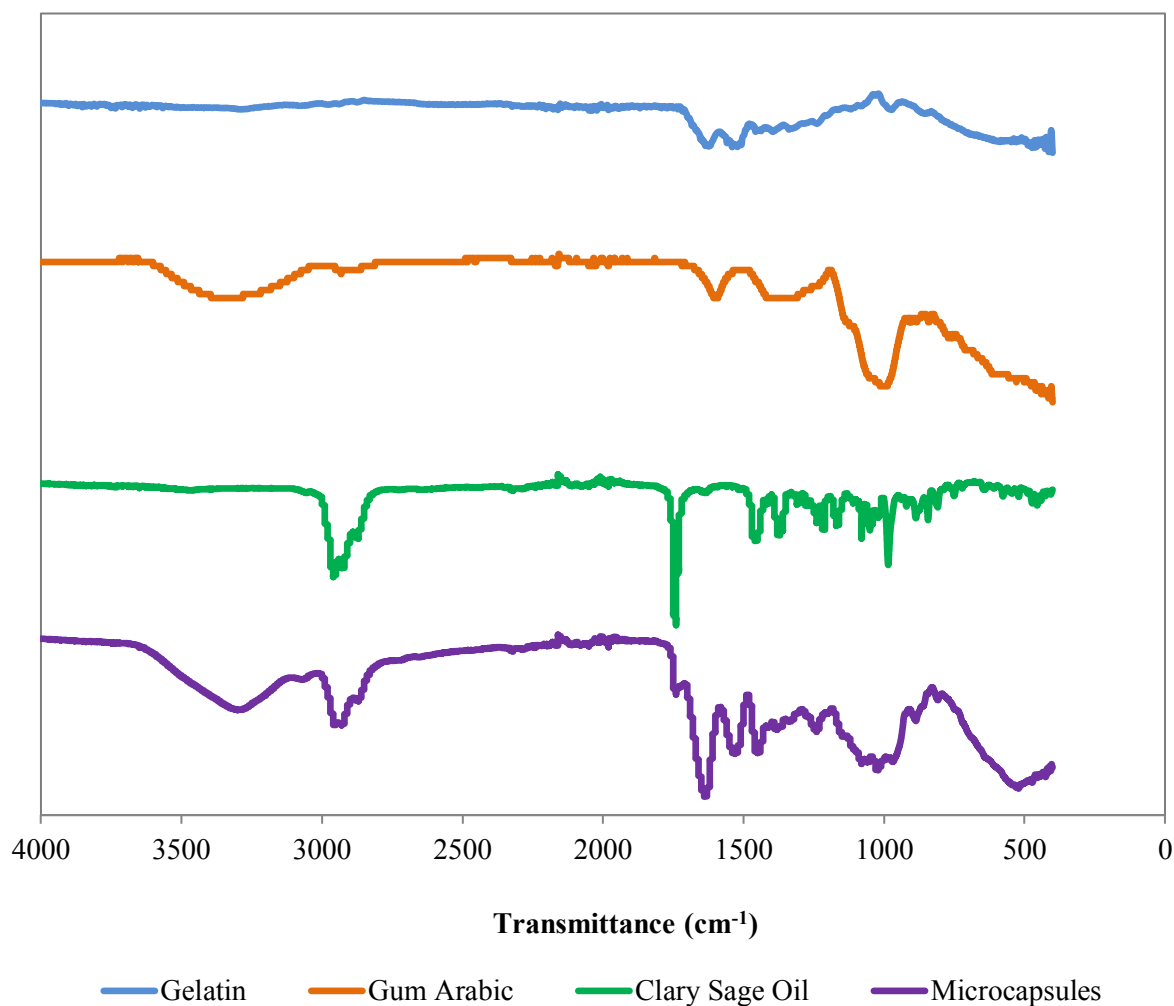


Figure 2. FTIR spectra of pure clary sage essential oil, gelatin, gum Arabic, and the microcapsules.

4. Conclusion

Microencapsulation of clary sage, which could be used in herbal medicine, food and cosmetic products, is known as an efficient process to protect its components from environmental conditions. This study investigated the optimal ultrasonication treatment time for encapsulation efficiency of gelatin-gum Arabic microcapsules containing clary sage oil. Surface oil and total oil varied from 1.25%–0.34% and 9.63%–16.80%, respectively, by increasing the ultrasonication time from 5 minutes to 20 minutes. The results demonstrated that the optimal oil encapsulation efficiency was around 97.98% with



20 minutes of ultrasonication treatment time. The microcapsules were characterized by SEM and FTIR analysis. The characterization results showed that clary sage oil was successfully encapsulated by the complex coacervation method using gelatin and gum Arabic as wall materials.

Conflict of Interest

The author declares any conflict of interest in this research.

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FULL TEXT - ORAL PRESENTATION

REGULATIONS ON MEDICINAL AND AROMATIC PLANTS: FROM
RAW MATERIAL TO FINAL PRODUCTS

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Abstract

The interest and demand for the use of Medicinal and Aromatic Plants (MAPs) and the natural products obtained from them has increased rapidly in recent years. The number of MAPs used in many industries such as food, health, pharmacy, cosmetics, textile etc. has gradually increased nowadays. Although, most of these plants that are used as raw materials in related industries, are collected from nature, some of them are insufficient in terms of quality and demanded in quantity. Most of the MAPs grown in the world and in our country are used as spice and herbal tea, as well as wild fruits and vegetables are valuable food sources and they are among the MAPs grown in agricultural lands in recent years. Good agricultural practices are the basic rule of MAPs cultivation, while monitoring, protection-use-development strategies are followed in wild harvests in terms of protection of plant biological diversity and supply of quality raw materials. In this direction, “Good Collection Practices (GCP)” and “Good Agricultural Practices (GAP)” published by the World Health Organization (WHO) are applied especially by developed countries and other countries are expected to adopt these standards. While the yield and quality of secondary metabolites is the main point for MAPs collected from nature, scientific studies have shown that post-harvest processing plays a very important role in the production of medicinal and aromatic plants. In recent years, remarkable scientific studies have been carried out on post-harvest processing, storage, processing, packaging and marketing. The quality criteria and standards of MAPs in almost all the industries are constantly regulated by well-known published institutions. When these issues are regulated and monitored by the FDA (Food and Drug Administration) in the USA, the responsible authorities in the European Union countries are EMA (European Medicines Agency), EFSA (European Food Safety Authority) and THIE (Tea & Drug Administration Herbal Infusions Europe). As for in Turkey, the Ministry of Agriculture and Forestry is responsible for the wild collection, agricultural production, processing and marketing of MAPs. Both the Ministry of Agriculture-Forestry and Ministry of Health manage regulations and public health of these plants. Moreover, studies on the licensing and inspection of herbal medicines and cosmetic products are carried out by the units of affiliated to the Ministry of Health in Türkiye.

Keywords: MAPs, law and legal issues, GCP, GAP, natural products, herbal medicine

1. Introduction

Medicinal and aromatic plants (MAPs) have been used as ethnobotanic purposes such as food, medicine, herbal tea, spice, dye etc. The number of MAPs used as for these purposes has been estimated to be more than ten thousand all over the world. In every geographic region including country, city, village and tribe found in the world has various useful plants, which have a huge potential for their traditional uses and herbal remedies for various illnesses and diseases. Sometimes, the same plant species could also be used for quite different remedies or purposes in different cultures. On the other hand, their uses for remedies can vary from different cultures. Most of the MAPs used and traded have still been collected from nature all over the world. Some of them having economic value have recently been cultivated under field conditions. Furthermore, few species of MAPs are currently cultivated, compared to the quantity of wild harvested MAPs (FAO, 2011; Paramanya et al., 2021). Nevertheless, production area of cultivated medicinal and aromatic plants have been limited because of handicaps related to domestication and agricultural techniques (Sekeroglu, 2018a; Fierascu et al., 2020). Some of the medicinal and aromatic plants collected from nature and cultivated in Türkiye are figured out in the Figure 1.



Figure 1. Some medicinal and aromatic plants collected from nature and cultivated in Türkiye



2. Advantages and Disadvantages of Wild Harvesting versus Cultivation of Maps

Advantages and disadvantages according to the legal issues of FAO (Food Administrative Organization) are summarized in the Table 1. A summary is given about wild harvesting versus cultivation of medicinal and aromatic plants based on the advantages and disadvantages for plant species and ecosystems (FAO, 2011; Lubbe and Verpoorte, 2011; Sekeroglu, 2018b).

Table 1. Advantages and disadvantages of wild harvesting comparing with cultivation of MAPs

Wild Harvest	Cultivate
<ul style="list-style-type: none"> ➤ it puts wild plant populations in the continuing interest of local people ➤ it provides an incentive to protect and maintain wild populations and their habitats and the genetic diversity of MAP populations ➤ but ... ➤ uncontrolled harvest may lead to the extinction of ecotype and even species ➤ common access to the resource makes it difficult to adhere to quotas and the pre-cautionary principle ➤ in most cases knowledge about the biology of the resource is poor and the annual sustained yields are not known ➤ in most cases resource inventories and accompanying management plans do not exist 	<ul style="list-style-type: none"> ➤ it relieves harvesting pressure on very rare and slow-growing species which are most susceptible to threat ➤ but ... ➤ devaluates wild plant resources and their habitats economically and reduces incentive to conserve ecosystems ➤ narrows genetic diversity of gene pool of the resource because wild relatives of cultivated species become neglected ➤ it may lead to conversion of habitat for cultivation ➤ cultivated species may become invasive and have negative impacts on ecosystem ➤ reintroducing plants can lead to genetic pollution of wild populations

*FAO, 2011

3. Natural Sources and Sustainable Collection of MAPs

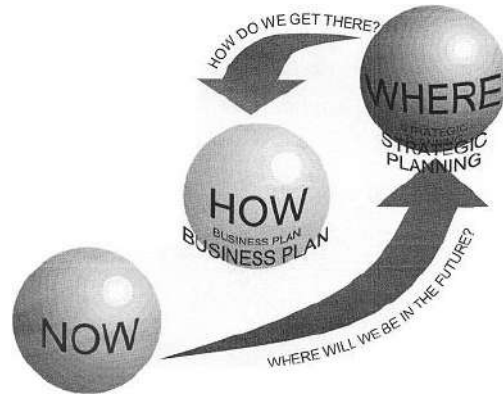
Conservation strategies and legislations of wild MAP species under forests are somewhat being controlled for sustainable collection, but species on the meadows and pastures (Giannenas et al., 2020).

Optimum use of local resources is wealth of the rural populations, and that can be conducted by two main methods including sustainable collection methods and in-situ cultivation. Among

these methods, sustainable collection methods must be obeyed strictly for sustainable development and welfare of next generations in the rural. In-situ cultivation of the most demanded MAP species should be started according to GAP after economic analysis for long term benefits (Franz, 1988; Salgueiro et al., 2010).

Cornerstone of sustainable collection of MAPs:

- ✓ Determination of natural resources
- ✓ Good planning of further steps
- ✓ Optimum use of local resources
- ✓ Rotation in harvest area
- ✓ Novel crops development
- ✓ Spotighting of traditional products
- ✓ Sustainable use of natural resources



Determination of natural resources and conditions:



- ✓ Economically important MAP species in the region
- ✓ Crop design throughout the country
- ✓ Population of them in the certain area
- ✓ Other relative species
- ✓ Gathered and used plant parts
- ✓ Commercial form and value (raw/semi-/processed)
- ✓ Annual collection quantity
- ✓ Threats for wild collection
- ✓ Traditional collection methods
- ✓ Conservation strategies locally used



Market demands related to wild harvested or cultivated MAPs can be vary depending on various reasons. These reasons are given in the Table 2 (Djordjevic, 2017; Sekeroglu, 2018b).

Table 2. Market demands wild harvesting versus cultivation of MAPs

Wild Harvested Plants	Cultivated Plants
<ul style="list-style-type: none"> <input type="checkbox"/> it is cheaper since it does not require infrastructure and investment <input type="checkbox"/> many species are only required in small quantities that do not make cultivation economically viable <input type="checkbox"/> for some plant parts extra-large cultivation areas are required (e.g., Arnica production for flowers) <input type="checkbox"/> successful cultivation techniques do not exist, e.g., for slow growing, habitat specific taxa <input type="checkbox"/> no pesticides are used <input type="checkbox"/> it is often believed that wild plants are more powerful <input type="checkbox"/> but ... <input type="checkbox"/> there is a risk of adulterations <input type="checkbox"/> there is a risk of contaminations through non-hygienic harvest or post-harvest conditions 	<ul style="list-style-type: none"> ❖ it guarantees continuing supply of raw material ❖ it makes reliable botanical identification possible ❖ genotypes can be standardized or improved ❖ quality standards are easy to maintain ❖ controlled post-harvest handling is possible ❖ production volume and price can be agreed for longer periods ❖ resource price is relatively stable over time ❖ certification as organic production is possible ❖ but ... ❖ it is more expensive than wild harvest ❖ it needs substantial investment before and during production

*FAO, 2011

4. Diversity of MAPs and Marketing of Herbal Products based on the Legal Issues

Diversity of the herbal products have amazingly increased in last decade all over the world. Main herbal products having big market are; (Steinhoff, 2003; Arslan et al., 2015; Sekeroglu, 2018a)

- ✓ Herbal medicines
- ✓ Food additives
- ✓ Nutraceuticals
- ✓ Herbal teas
- ✓ Herbal mixtures & pastes
- ✓ Herbal energy drinks
- ✓ Instant fruit and vegetable extracts



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- ✓ Essential and fatty oils for food and cosmetics
- ✓ Essential oils for perfumery and aromatherapy
- ✓ Natural cosmetics and detergents

Related to huge marketing opportunities in herbal products and amazing development in communication, local endemic medicinal plants and traditional herbal formulations have been presented to use of humans on the worldwide. This surprising spread of local MAPs and traditional herbal products could cause some undesired results and serious problems in food security and public health (Thomas et al., 1999; Sekeroglu, 2018b).

Because of huge plant diversity on the planet used as Traditional Herbal Medicine and Herbal Food Additive, governments have crucial problems to establish their own regulations in this issue. USA manages these issues by FDA, some European countries (Belgium, Italy, Sweden, Türkiye, United Kingdom, etc.) have their own plant lists which plants or plants parts are suitable / unsuitable for food and medicinal purposes. They regulate Herbal Products Market in their countries. European Food Safety Authority (EFSA) and European Medicine Agency (EMA) also periodically publish some data and monograph about Medicinal and Aromatic Plants and their herbal products. Comparing these publications, there are a number of inconsistencies among the positive / negative plant lists of the countries. Positive means that useful plants as food and herbal medicine, while negative means that prohibited or toxic plants. Interestingly, the same plant species was given in positive list as food purposes in one country; it was unapproved for food purposes in other country. Thus, traders and industrial bodies dealing with MAPs could have big problems in some countries. Promisingly, every country in Europe like other countries on the worldwide are trying to establish their formal regulations on MAPs and Herbal Medicinal Products for Human Welfare (Arslan et al., 2015; Sekeroglu, 2018a; Sekeroglu, 2018b).

5. Regulations for MAPs and Herbal Products

Governments need regulations for MAPs and their products, because of the reasons as listed below (Saha and Basak, 2020);

- Conservation plant genetic resources
- Public health
- Adulteration problems
- Huge variety of medicinal plant products
- Standardization problems
- Unsuitable manufacturing process
- Uncontrolled marketing

German Commission E, EFSA (European Food Safety Authority), EMA (European Medicine Agency), ESCOP (European Scientific Cooperative on Phytotherapy), THIE (European Herbal Infusions Association) are some of the governmental and non-governmental bodies in Europe for MAPs regulations (Klein et al., 1998; Thomas et al., 1999; EFSA, 2011; Aka et al., 2017).

German Commission E

The German Commission E is a governmental regulatory agency that was established in 1978. It is composed of scientists, toxicologists, physicians, and pharmacists. Evaluated usefulness of 300 herbs, studied literature, clinical studies, case studies, field studies and then prepared monographs (Klein et al., 1998; Blumenthal et al., 2000). The book of complete German commission E monographs including therapeutic guide to herbal medicines is shown in the Figure 2.

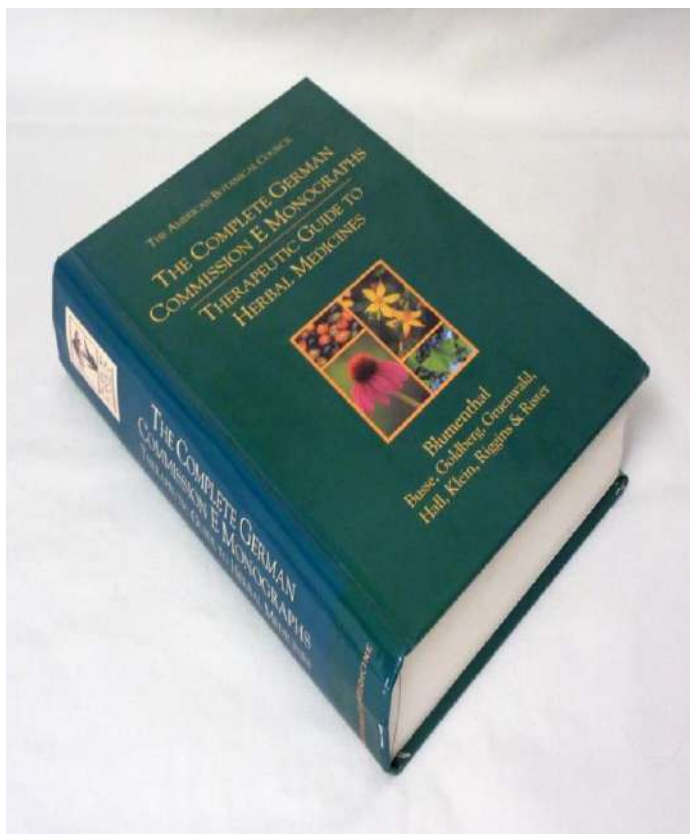


Figure 2. The complete German commission E monographs

The European Food Safety Authority (EFSA)

The European Food Safety Authority (EFSA) is the keystone of European Union (EU) risk assessment regarding food and feed safety. In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides independent scientific advice and

clear communication on existing and emerging risks. The European Food Safety Authority (EFSA) was set up in January 2002, following a series of food crises in the late 1990s, as an independent source of scientific advice and communication on risks associated with the food chain (EFSA, 2011; Authority et al., 2019).



Figure 3. The European Food Safety Authority (EFSA)

The European Medicines Agency (EMA)

The European Medicines Agency (EMA) is a decentralized agency of the European Union, located in London. The Agency is responsible for the scientific evaluation of medicines developed by pharmaceutical companies for use in the European Union (Werner and Merz, 2007).

There are seven Scientific Committees under EMEA as listed below, as well as given in the Figure 4;

- Committee for Medicinal Products for Human Use (CHMP)
- Pharmacovigilance Risk Assessment Committee (PRAC)
- Committee for Medicinal Products for Veterinary Use (CVMP)



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- Committee for Orphan Medicinal Products (COMP)
- Committee on Herbal Medicinal Products (HMPC)
- Pediatric Committee (PDCO)
- Committee for Advanced Therapies (CAT)

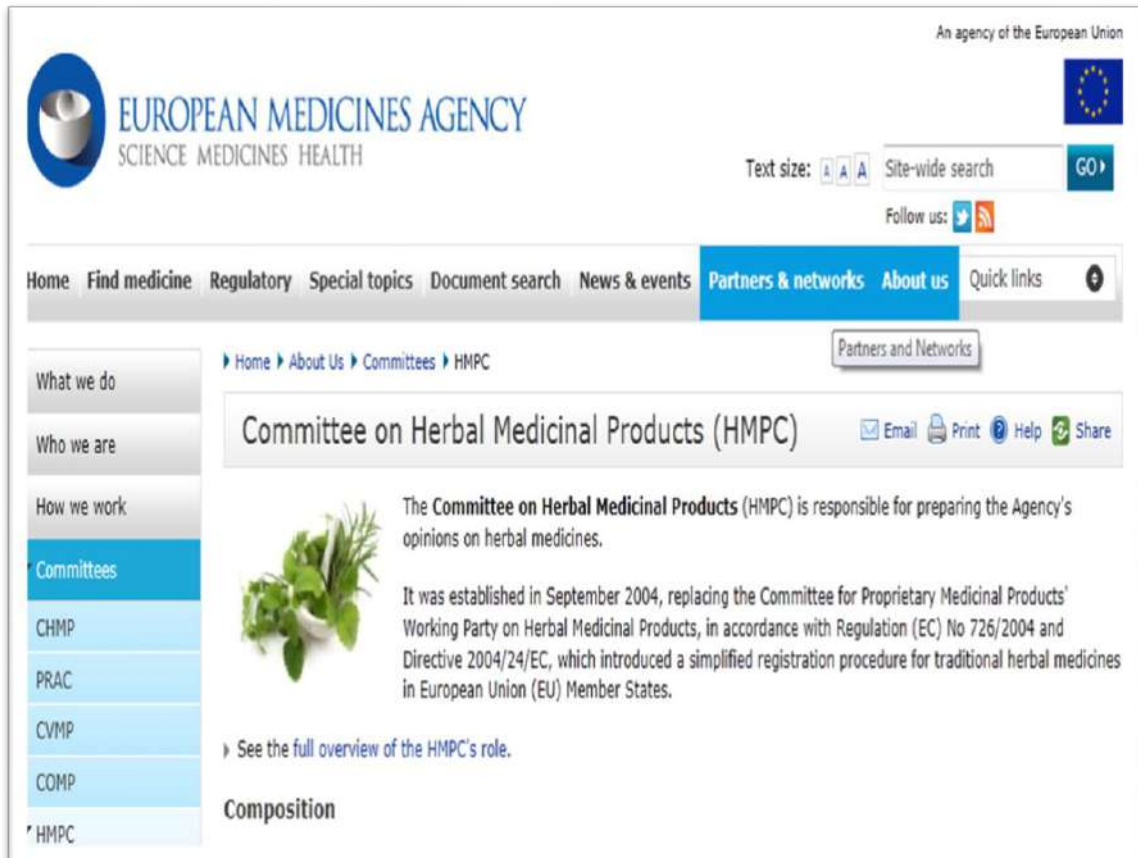


Figure 4. The European Medicines Agency (EMA) and Committee of Herbal Medicine Products (HMPC)

European Scientific Cooperative on Phytotherapy (ESCOP)

ESCOP (European Scientific Cooperative on Phytotherapy) was founded in June 1989 as an umbrella organization representing national phytotherapy or herbal medicine associations across Europe, especially in their discussions with European medicines regulators. Since 1996 it has been based in the United Kingdom as a company limited by guarantee (Monographs, ESCOP, 2003) (Figure 5).



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Figure 5. European Scientific Cooperative on Phytotherapy (ESCOP)

European Tea & Herbal Infusions Association (THIE)

Tea and Herbal Infusions Europe (THIE) is the European association representing the interests of producers and traders of tea (*Camellia sinensis*) and herbal infusions as well as extracts thereof in the EU. The Hamburg-based THIE was founded on 22nd January, 2015 as successor of the European Tea Committee (ETC) and the European Herbal Infusions Association (EHIA) with a long history of more than 60 years. Tea as well as herbal and fruit infusions represent an important cultural heritage and are also part of a modern lifestyle (Figure 6). Diligent selection of natural raw materials, careful processing, and modern production processes, comprehensive quality controls and traditional recipes as well as new blends are the basis for an infinite variety of flavors. The industry steadily improves processes and quality of its products to meet consumer expectations, increasing quality requirements and scientific progress (Rocha et al., 2020).

The following relevant legislation is already in place:

- ✓ Regulation (EU) 2015/2283 on novel foods
- ✓ Regulation (EC) No 1925/2006 on the addition of vitamins and mineral
- ✓ Directive 2004/24/EC on Traditional Herbal Medicinal Products
- ✓ Regulation (EU) 62/2018 List of commodities for which MRLs are set

TEA & HERBAL INFUSIONS EUROPE					Allocation according to Commission Regulation (EU) 2018/62			
English	Latin	German	Allocation		Category	Group	Subgroup	Code number
Agrimony	<i>Agrimonia eupatoria</i> L.	Odermennig	Kraut	Tea, coffee, herbal infusions, cocoa and carobs	herbal infusions from leaves and herbs	strawberry		9632010-002
Alfalfa / Lucerne	<i>Medicago sativa</i> L.	Alfalfa / Lucerne	Kraut	Tea, coffee, herbal infusions, cocoa and carobs	herbal infusions from leaves and herbs	strawberry		9632010-003
Alfalfa	<i>Pimenta dioica</i> (L.) Merr. / <i>Pimenta officinalis</i> Lindl.	Pfeffer	Früchte	spices	fruit spices	alfalfa / pimento		9620510
Almond	<i>Prunus dulcis</i> var. <i>dulcis</i>	Mandel	Blüten	Tea, coffee, herbal infusions, cocoa and carobs	herbal infusions from flowers	rose		9631030-001
Almond	<i>Prunus dulcis</i> var. <i>dulcis</i>	Mandel	Samen	fruits, fresh or frozen; nuts	nuts	almonds		9120010
Alice vera	<i>Alcea barbadensis</i> Mill. / <i>Alcea vera</i> (L.) Turcz.f.	Alice vera	Blutigel	Tea, coffee, herbal infusions, cocoa and carobs	herbal infusions from leaves and herbs	strawberry		9632010-004
Alpine ladies mantle	<i>Alchemilla alpina</i> L.	Silbermantel	Kraut	Tea, coffee, herbal infusions, cocoa and carobs	herbal infusions from leaves and herbs	strawberry		9632010-005
Angelica	<i>Angelica archangelica</i> L.	Engelwurz	Wurzeln	vegetables, fresh or frozen	root and tuber vegetables	celery roots/Hamburg roots		0213070-001
Angelica	<i>Angelica archangelica</i> L.	Engelwurz	Stängel	vegetables, fresh or frozen	leaf vegetables, herbs and edible flowers	celery leaves		0256030-001
Anise	<i>Pimpinella anisum</i> L.	Anis	Früchte	spices	seed spices	anise / aniseed		9610010
Anise	<i>Bixa orellana</i> L.	Annatto	Samen	spices	seed spices	nutmeg		9610090-001
Apple	<i>Malus domestica</i> Borkh.	Apfel	Früchte	fruits, fresh or frozen; nuts	stone fruits	apples		9130010
Apple mint	<i>Mentha suaveolens</i> Ehrh.	Apfelminze	Blätter	vegetables, fresh or frozen	leaf vegetables, herbs and edible flowers	leaf and edible flowers		0256030-001
Apricot	<i>Prunus avium</i> L. / <i>Prunus armeniaca</i> L.	Aprikose	Früchte	fruits, fresh or frozen; nuts	stone fruits	apricots		9140010
Apricot	<i>Prunus avium</i> L. / <i>Prunus armeniaca</i> L.	Aprikose	Samen	fruits, fresh or frozen; nuts	nuts	almonds		9120010-001
Asparagus	<i>Asparagus officinalis</i> L.	Spargel	Sprosse	vegetables, fresh or frozen	stem vegetables	asparagus		0270010
Bamboo	<i>Bambusa vulgaris</i> Schrad. ex Wendl.	Bambus	Sprosse	vegetables, fresh or frozen	stem vegetables	bamboo shoots		9270080
Banana	<i>Musa paradisiaca</i> L.	Banane	Früchte	fruits, fresh or frozen; nuts	miscellaneous fruits with inedible peel, large	bananas		9163020

Figure 6. Tea and Herbal Infusions Europe (THIE)

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6. Conclusions and Future Recommendations

The quality criteria and standards of MAPs used in the food, cosmetics, perfumery and health sectors are constantly regulated, updated and published by well-known institutions and governments. While these studies are carried out, regulated and monitored by the FDA (Food and Drug Administration) in the USA, the responsible authorities in the European Union countries are EMA (European Medicines Agency), EFSA (European Food Safety Authority) and THIE (Tea & Drug Administration Herbal Infusions Europe). In Turkey, the Ministry of Agriculture and Forestry is responsible for the wild collection of MAPs, and the Ministry of Agriculture and Forestry manages agricultural production, processing, marketing and public health of these plants. Studies on the licensing and inspection of herbal medicines and cosmetic products are carried out by the units of affiliated to the Ministry of Health. Joint efforts to develop the medicinal and aromatic plants sector in Türkiye will make our country one of the leading suppliers in the world MAPs market in the future. In conclusion, countries are developing their standardization and regulation strategies for MAPs and their products. In this content, producers and traders in this field should follow the recent developments and reports. Good collection practices (GCP), Good agricultural practices (GAP), good agricultural and collection practices (GACP), good manufacturing practice (GMP), regulations and good laboratory practice (GLP), published scientific ethnobotanical data, and published scientific toxicological data are some of the key points for people about regulations of MAPs from nature to final product for the near future.

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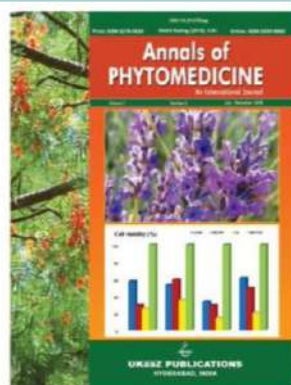
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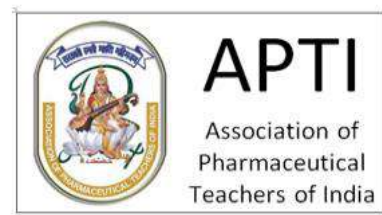
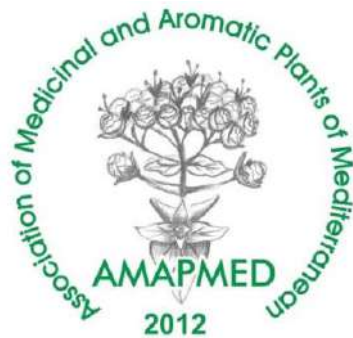
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