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The 9th International Mediterranean Symposium on Medicinal and Aromatic Plants

03-05 May 2023 / Ankara - TURKIYE

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PROCEEDINGS BOOK

ABSTRACTS & FULL PAPERS

May 03rd – 05th, 2023

Ankara – Türkiye

ISBN: 978-625-00-1361-8 (PDF)

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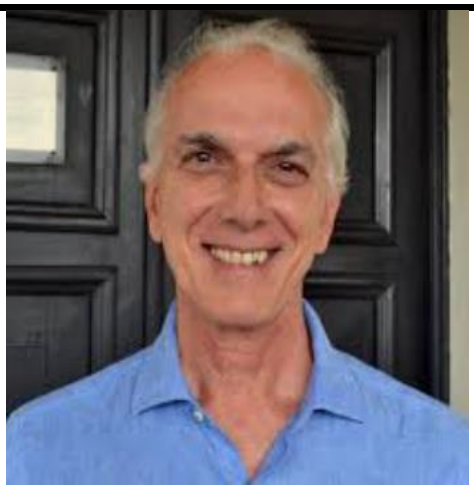

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Dear Colleagues,

Having a respected scientific board and organizing committee members from all over the world, MESMAP Symposium series started in 2013. The first Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP-2013) was held on April 17–20, 2013 in Gazimagosa (Famagusta), Turkish Republic of Northern Cyprus (TRNC), which was organized by the Faculty of Pharmacy, Eastern Mediterranean University (EMU), in joint with AMAPMED (Association of Medicinal and Aromatic Plants of the Mediterranean). MESMAP-2 Symposium was held on April 22–25, 2015, in Antalya – Türkiye, which was organized by academicians from Gazi University (Türkiye), Gaziantep University (Türkiye), Kilis 7 Aralik University (Türkiye), Yüzüncü Yıl University (Türkiye), Association of Pharmaceutical Teachers of India (APTI – INDIA) joint with AMAPMED (Association of Medicinal and Aromatic Plants of the Mediterranean). INDUSTRIAL CROPS AND PRODUCTS JOURNAL with a high impact factor from the Elsevier Group, published a special issue covering some of the full papers selected after scientific evaluation. MESMAP-3 Symposium, which was held on April 13–16, 2017 in Girne (Kryneia) in the Turkish Republic of Northern Cyprus (TRNC), was the third event of the MESMAP symposium series on Medicinal and Aromatic Plants. After scientific evaluation, selected full papers were published in the Indian Journal of Pharmaceutical Education and Research (IJPER), indexed by THOMSON REUTERS. MESMAP-4 Symposium, which was held on April 18–22, 2018 in Sherwood Breezes Resort Hotel Antalya, Türkiye, was the fourth event of the MESMAP Symposium Series on Medicinal and Aromatic Plants. Then, the fifth one was the MESMAP-5 symposium, which was organized as a joint meeting with ISPBS-5 at Cappadocia on April 24-28, 2019. After scientific evaluation, selected full papers from the MESMAP-5 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. Afterwards, the MESMAP-6 Symposium was organized on October 15–17, 2021, and this symposium was supported by the TÜBTAK 2223-B National Scientific Meetings Grant Program. After scientific evaluation, selected full papers from the MESMAP-6 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. Then, MESMAP-7 was organized during November 18–20, 2022, and hosted by Dokuz Eylul University and Torbalı (Izmir) Chamber of Commerce, Türkiye. Last year, MESMAP-8 was organized during October 20–22, 2022, in Izmir–Türkiye. After scientific evaluation, selected full papers from the MESMAP-8 Symposium were published in Molecules and the Brazilian Journal of Pharmacognosy, indexed with THOMSON REUTERS. Furthermore, MESMAP-8 was supported by the TÜBTAK 2223-B National Scientific Meetings Grant Program. After eight successful series of MESMAP symposiums with the participation of prominent keynote and invited speakers, worldwide scientists, and young researchers, MESMAP-9, hosted by Gazi University Pharmacy Faculty in Ankara-Türkiye, was the ninth series of the meeting, and you can find the abstracts in this ABSTRACTS &PROCEEDINGS BOOK. We would like to encourage the participants of MESMAP-9 to submit the full papers to the special issue journal 'Phytochemistry Letters', and other contracted journals, including 'Annals of Phytomedicine', 'International Journal of Agriculture, Environment, and Food Sciences," and 'Current Perspectives on Medicinal and Aromatic Plants (CUPMAP). We would like to acknowledge the support of the Turkish General Directorate of Forestry, TURKISH AIRLINES, Gazi University, Gaziantep University, Khon Kaen University, Kumamoto University, 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, AKS Cosmetics, Laber Cosmetics Company, Pharmateks Medicine and Chemistry, Hünnap Herbal Products, BASEM Lavanta, ZAR BORJ Saffron and NS Herbals Company, and all the other supporters. The organizing committee hopes that the participants of the MESMAP-9 Symposium will have an amazing experience and unforgettable memories to take back to their homes. We would like to thank all our participants from almost all parts of the world for their valuable participation and scientific contribution to MESMAP-9. We are planning to organize the 10th series of MESMAP meetings in spring 2024, and it will be a great honor to see you again at the MESMAP-10 symposium.

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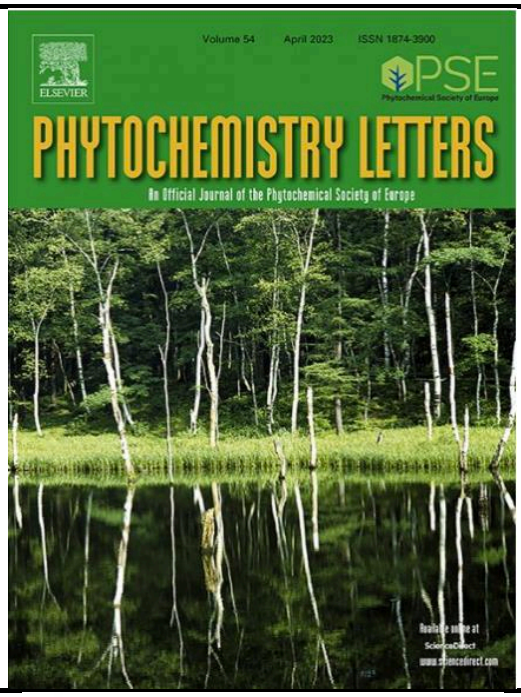

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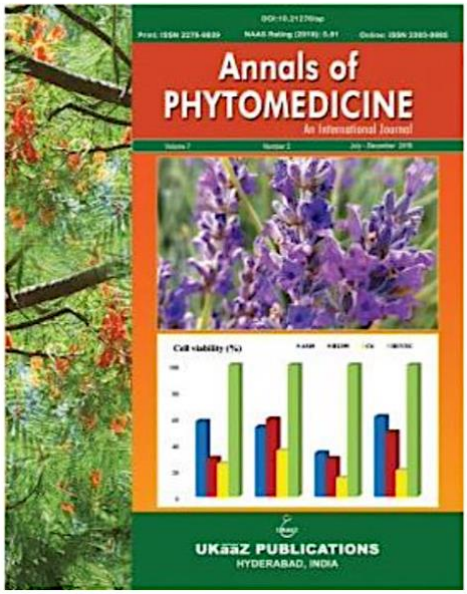

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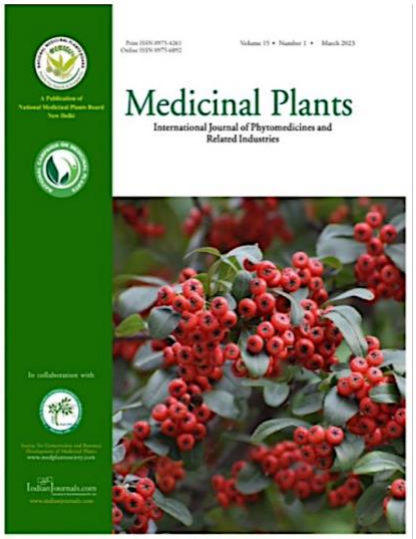

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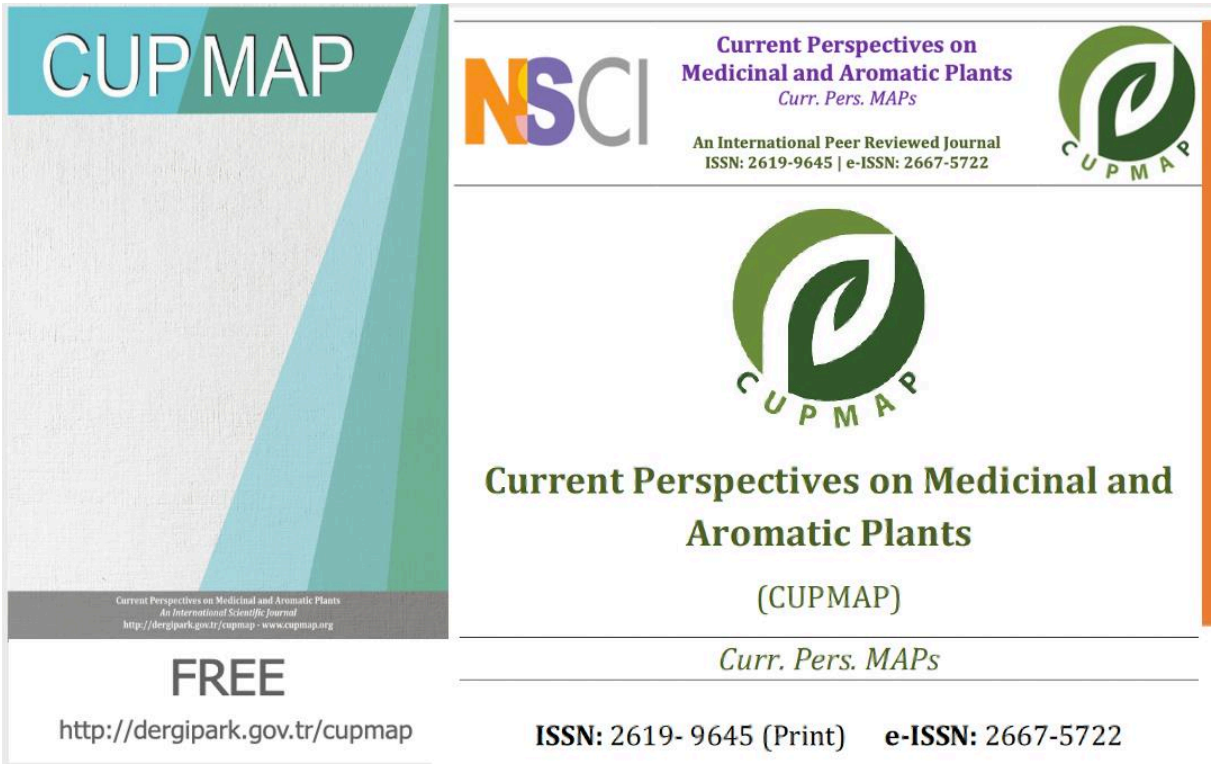

CUPMAP is an open access, peer-reviewed and refereed international journal published by MESMAP scientific group. The main objective of the CUPMAP is to provide an intellectual outlook on the scientific researches on Medicinal and Aromatic Plants. CUPMAP have distinguished goals to promote interdisciplinary scientific studies in which results could easily be used in industrial production on MAPs. This international scientific journal publishes research papers related to Medicinal and Aromatic Plants in the fields of science and technology such as Biology, Molecular Biology and Genetics, Chemistry, Agriculture, Biochemistry, Botany, Ethnobotany, Environmental Science Forestry, Horticulture, Health Care & Public Health, Nutrition and Food Science, Pharmaceutical Sciences, and so on. CUPMAP publishes original research papers, applied studies, and review articles in MAPs science and technology. Special Issues devoted to important topics in the MAPs science and technology could also be published. CUPMAP Journal publishes Biannually (on June and December) in both print and on-line versions. The publication language of the journal is English. The Journal of CUPMAP welcomes article (research or review) submissions and does not charge any article submission or processing charges. CUPMAP invites papers for **Volume 6 Issue 1**, which is scheduled for release in June 2023. Deadline for submission is June 02, 2023.

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MESMAP-9 Sempozyumunda toplam 116 bildiri sunulmuştur, bunlardan 25 tanesi poster sunumu şeklinde olup; sunulan sözlü bildirilerin %55'lik kısmı yabancı katılımcılar tarafından sunulmuştur. Sempozyuma yaklaşık 30 farklı ülkeden bilim insanı katılım sağlamıştır. Katılım Sağlayan Ülkeler: Austria, Serbia, Italy, South Africa, Bulgaria, Greece, USA, India, Poland, Saudi Arabia, Ethiopia, Romania, Ukraine, Slovakia, Thailand, Israel, Algeria, Brazil, Albania, Morocco, Tunisia, Iran, Vietnam, Georgia, Nepal, Uzbekistan, Kazakhstan, Azerbaijan, Turkish Republic of Northern Cyprus, and Türkiye.

Sempozyuma katılım sağlayan katılımcılar ve sunum başlıkları 'MESMAP-9 Abstracts & Proceedings Book' kitabının içindekiler kısmında sunulmuştur. MESMAP-9 Sempozyumu aşağıda yer alan YÖK Akademik Teşvik ve Yükselme kriterlerini sağlamaktadır.

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

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

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KEYNOTE SPEAKER

Austrian Drug Screening Institute- Bridging Basic Research and Industry Innovative methods for evaluation of efficacy, safety and quality of raw materials and final products in the phyto-area

Prof. Dr. Günther K. Bonn

ADSI - Austrian Drug Screening Institute, University of Innsbruck, Innrain 66a, 6020 Innsbruck, Austria
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Innovative approaches in analytical chemistry and cell-biology have become increasingly imperative for natural product research, e.g., the latest developments in chromatography enable research in otherwise inaccessible areas of natural product isolation. Since plant extracts consist of several hundred to thousands of diverse compounds in very different concentrations, the use of novel enrichment and purification methods based on advanced solid-phase extraction techniques in combination with high-resolution chromatographic separation and mass spectrometric detection is of utmost importance for the establishment of reliable phytochemical extract profiling besides the accurate quantification of specific plant metabolites. Significant progress is accomplished in developing new stationary phases that can be personalized to a particular application, thus offering endless possibilities for optimizing selectivity. Additionally, combining separation science with spectroscopy allows for merging various technologies in phyto-pharmacy and phyto-cosmetics. Near- and mid-infrared (IR) spectroscopy enables swift and non-invasive qualitative and quantitative analyses of raw plants and extracts. All of these approaches offer advanced strategies not only for R&D but also for quality control in phyto-analysis. Alongside analytical studies to identify and quantify profiles or specific compounds, comprehensive biological studies can be used to identify biological activities and perform the necessary safety assessment.

Longan fruit concentrates were obtained through milling, pressing, heating/cooling, and subsequent vacuum distillation at elevated temperatures and were qualitatively and quantitatively analyzed through UHPLC-qTOF/MS. In addition, in vitro antioxidant assays, anti-enzyme assays, two-dimensional HaCaT-based in vitro assays, and assays in the three-dimensional reconstructed human epidermis (3D-RHE) were performed.

The comprehensive investigations demonstrated high antioxidant potential, a significant reduction in collagenase activity, and dose-dependent skin whitening effects. Skin-soothing effects were demonstrated on 2D-HaCaT and 3D-RHE models. The in vitro skin irritation and corrosion tests performed according to OECD guidelines and the patch test performed according to ICDRG guidelines also confirmed excellent skin tolerance.

The results obtained by these highly sophisticated analytical and biological techniques demonstrate the highly effective anti-aging properties of longan fruit concentrate. Combined with the safety assessment, these positive effects offer high potential in phytocosmetic formulations.

Keywords: Phytocosmetics, anti-aging, 3D-RHE, UHPLC-qTOF/MS, Dimocarpus Longan

Literature:
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KEYNOTE SPEAKER

PLANT BIOACTIVE COMPOUNDS FOR TREATMENT OF CANCER: MYTH OR CHANCE?

Zora Dajić Stevanović[1], Sanja Mijtović[2], Danijela Maksimović-Ivanić[2]

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[2]Department of Immunology, Institute for Biological Research "Siniša Stanković" - National Institute of the Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, R. of Serbia

Cancer is commonly treated using conventional treatments such as radiotherapy, chemotherapy, and surgical procedures. Nevertheless, standard therapies cause severe side effects and often lead to multidrug resistance in cancer cells. Natural products, primarily the plant secondary metabolites, are known as efficient bioactive compounds used in the prevention and treatment of different disorders and diseases, including cancer. There are many ethnobotanical reports highlighting the use of various plant drugs that exhibit anti-inflammatory, immune modulatory, and consequent anti-cancer properties. More than 3000 plant species and over 10,000 secondary plant compounds were identified and researched for cancer treatment, including a range of alkaloids, flavonoids, lignans, condensed tannins, terpenoids, saponins, shikonins, stilbenes, and others. Furthermore, phytochemicals could increase the efficiency of standard anticancer drugs and mitigate their adverse effects. This review looks at the most researched bioactive plant compounds that are able to carry out various anticancer mechanisms, such as inhibition of cancer cell activating factors and inflammatory mediators; up-regulation of antioxidant enzymes, p52, and other DNA repair systems; apoptosis, autophagy; reduction of the angiogenesis and metastasis ability of cancer cells; sensitization of multi-drug resistant cancer cells; and epigenetic regulation [1]. The main results of our recent extensive in vitro and in vivo studies on the antitumor properties of selected Balkan herbs will be presented, with particular emphasis on the effects of the ethanolic extract of *Alchemilla vulgaris* agg. in mice and human lung, breast, colon, and melanoma cancers [2]. The associated anti-cancer mechanisms were attributed to the blockade of cell division, caspase-dependent apoptosis, and autophagic cell death by downregulation of the PI3K/Akt and MAPK kinase pathways [3]. Finally, in-depth single compound effects in mechanistic studies versus holistic and integrative concepts addressing the cancer microenvironment and complex interactions with the human microbiome, as well as the need for an individual therapy approach, should be considered.

Keywords: plant metabolites and extracts, cytotoxicity, anticancer mechanisms, Balkan herbs

Acknowledgment: Research activities were realized through bilateral strategic research project "Biological activity of the fractions and isolated compounds from selected Balkan medicinal plants, No. 06/2018, funded by the Ministry of Science of R. of Serbia and R. of China.

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KEYNOTE SPEAKER

OXYPRENYLATED PHYTOCHEMICALS: NEW ACQUISITIONS AND FINDINGS OVER THE LAST FIVE YEARS

Prof. Dr. Francesco Epifano

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Oxyprenylated secondary metabolites from plants, fungi, and microbial sources have greatly attracted the attention of researchers during the last years and they emerged as biologically active natural compounds with a great potential for the next future. The first findings about this class of rare phytochemicals dates back the very beginning of the new millennium and were mainly focused on the establishment of new synthetic schemes and to the characterization of their biological activity in in vitro and animal models. Thus, oxyprenylated phytochemicals were seen to be effective dietary feeding active cancer chemopreventive, anti-bacterial, anti-protozoal, anti-fungal, anti-inflammatory, neuroprotective, and anti-oxidant agents. Such acquisitions greatly enhanced their preventive and therapeutic potential and prmopted efforts made during the last five years towards three main directions: a) characterization of oxyprenylated secondary metabolites as novel and additional components of the phytochemical pool of food, healthy, and medicinal plants, b) set up of new, more efficient, and chemically "greener" extraction methodologies from these same vegetable sources, c) provision of more details about their biomolecular mechanisms of action underlying the in so far observed in vitro and in vivo effects. In this context, *O*-prenyl phytochemicals have been found in plant derived food preparations like edible oils, in green edible vegatables (e.g. lettuce and spinach), in several apiaceous medicinal and healthy plants, in commonly used berries and fruits (e.g. goji and pomegranate). Furthermore, green extraction methodologies (e.g. ionic liquids, subcritical butane, solid phase extraction) have been set up and validated also in terms of qualitative and quantitative analysis. Finally, we have shown how oxyprenylated secondary metabolites can trigger specific cell targets and kick off a series of events that amply justify the experimental results obtained in this context so far. Thus, we have found that cholinesterases, melanogenesis enzyems, FXR, metabolic enzymes (e.g. α-amilase, α-glucosidase, and lipase), PPARγ, mitochondria and related substructures are involved in the overall mechanism by which oxyprenylated secondary metabolites interacts with cells and living organisms in general.

Key Words: Biomolecular mechanism of action, *Citrus* spp., Green extraction, Nutraceutical, Oxyprenylated secondary metabolites, Pharmacological activity

Acknowledgements: Financial support to this research by the University "Gabriele d'Annunzio" of Chieti-Pescara is grateful acknowledged.

KEYNOTE SPEAKER

ALL ABOUT GINSENG

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Abstract

Ginseng (Panax ginseng, C.A. Meyer) is a popular herbal remedy used worldwide for decades as an adaptogenic. The name Panax comes from the Greek words "pan (all)" and "akos (healing)" ginseng means 'man-root' due to the shape of the root [1,2]. There are many publications about the efficacy of ginseng on stress, the central nervous system, the immune system and maintaining well-being states and ageing. Ginseng radix is whole or cut dried root, designated white ginseng, treated with steam and then dried, designated red ginseng, of Panax ginseng C. A. Mey. Ginseng Radix contains mainly saponins, polysaccharides, polyacetylenic alcohols and phenolic compounds. The biological activities of the drug are attributed to its saponins. According to the European Pharmacopoeia monograph, Ginseng radix should contain at least 4% saponins[3].

There are many clinical trials performed on ginseng. Nevertheless, the results from clinical studies can sometimes be conflicting, and the types of ginseng used, their origins, compositions or the extraction procedures and the final product contents cannot be explained sufficiently. It has been used to enhance the regular activity of humans [4]. Therapeutic indication for ginseng is defined in ESCOP monograph as "decreased mental and physical capacities such as weakness, exhaustion, tiredness and loss of concentration, as well as during convalescence" [3].

Key Words: Ginseng, adaptogenic, antiaging

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KEYNOTE SPEAKER

EVIDENCE-BASED ETHNOPHARMACOLOGY – EXAMPLES FROM THE AFRICAN FLORA

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Abstract

Southern Africa harbours an impressive floral diversity and ranks as one of the most biodiverse countries in the world. Interweaved within this botanical tapestry is a cultural heritage characterised by rich indigenous knowledge systems (IKS) which have moulded one of the oldest healing modalities, African Traditional Medicines (ATM). This unique blend of medicinal plant use and IKS has created a unique research opportunity in enthnopharmacology. Over the past 20 years our group has endeavoured to provide a scientific rationale for medicinal plant use through an evidence-based research approach of traditional medicines. Several examples will be presented to demonstrate the challenging yet rewarding workflow to explore the chemistry and biological properties of the ethnomedicinal flora of South Africa. Using various in vitro and in vivo approaches, complemented by analytical methods and multivariate data analysis we aim to contribute to the fundamental research base required to convert these botanical assets into tangible consumer products. The various challenges facing translation research and the standardisation of ATMs will be highlighted.

KEYNOTE SPEAKER

ANTI-PSORIATIC POTENTIAL OF PLANT NATURAL COMPOUNDS

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Psoriasis is a chronic inflammatory immune-mediated disease with skin and joint manifestations for which there is no clear cause at present. The disease has an increasing prevalence, appeared to be the second largest contributor to skin-related impairment and hence affecting ca. 2-3% of the world population. In 2014, psoriasis was recognized as a serious noncommunicable disease by the WHO [1]. It manifests itself with characteristic skin lesions covered with silvery dry scales appearing in preferential places, such as elbows, knees and scalp and is associated with multiple comorbidities and significant negative effect on patients' quality of life [1, 2].

Plants and plant-derived molecules have gained considerable interest as a possible alternatives of current psoriasis treatments due to their multi-target benefits and fewer side effects compared to synthetic drugs [2]. We explored the effect of biotechnologically-produced extracts of devil's claw, and their bioactive principles in interferon (IFN)-γ/interleukin (IL)-17A/IL-22-stimulated HaCaT cells as a model of psoriasis-like inflammation. Changes in key inflammatory signaling pathways related to psoriasis development were detected by reverse transcription polymerase chain reaction and western blotting. Treatment with extracts and selected pure compounds improved psoriasis-related inflammation via suppression of the PI3K/AKT signaling in IFN-γ/IL-17A/IL-22-stimulated HaCaT cells. Our results suggest that some phenylethanoid compounds may exhibit therapeutic potential against psoriasis by regulating keratinocyte differentiation through inhibition of the PI3K/AKT pathway.

Acknowledgements: This research received funding from the Bulgarian National Science Fund (contract number КП-06-Н51/14).

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KEYNOTE SPEAKER

EXPLOITATION OF OLIVE OIL INDUSTRY PRODUCTS AND BY-PRODUCTS FOR PILOT ISOLATION AND SEMI-SYNTHESIS OF PROMISING MEDICINAL AGENTS

Prof. Dr. Alexios-Leandros Skaltsonis

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Olive oil and olive fruits, the main products of Olea europaea and the key ingredient of Mediterranean diet, are characterized by substantial nutritional and health beneficial value. [1] However, despite olive oil's economic and health impact, its industry is associated with environmental problems derived from the vast quantity of by-products, such as vegetation waters, olive cake, olive pulp and olive branches and leaves. [2] The amount of olive leaves produce every year exceed 18 million tons and mostly are used as animal feed, compost production or simply are burned, causing serious environmental damage. In a recent study was found that burning of olive tree branches is a major organic aerosol source in the Mediterranean region. [3] However this material still contains high value-added compounds such as triterpenoids, secoiridois, flavonoids, phenolic alcohols, phenolic acids, lignans which are known as olive polyphenols. All these constituents have a strong antioxidant profile and there is an increased industrial interest for possible nutraceutical and pharmaceutical applications. Our work is focused on finding alternative strategies to manage the residues of olive oil industry following two axis. Firstly the development of liquid/ liquid or solid/liquid extraction followed by partition chromatography techniques for the isolation of these compounds in multi gram scale. Secondly the use of some of these compounds such as oleoside, EDA as starting material for the hemi-synthesis of new analogues and their evaluation as potential antitumor agents.

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KEYNOTE SPEAKER

NEUROPROTECTIVE PROPERTIES OF BRAZILIN

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Until the recent past, the sole exemplar of proteins as infectious agents leading to neurodegenerative disorders remained the prion protein. Since then, the self-seeding mechanism characteristic of the prion protein has also been attributed to other neurodegenerative-disease-associated proteins, including amyloid-β (Aβ), tau, and α-synuclein (α-Syn). In model cell line studies, truncated Aβ, viz. amyloid beta (25–35), has been found to influence cellular homeostasis through its interactions with, and via, the disruption of key housekeeping machinery. Here, we demonstrate that the incubation of human neuroblastoma (SH-SY5Y) cell line with Brazilin ((6aS,11bR)-7,11b-dihydro-6H-indeno[2,1-c]chromene-3,6a,9,10-tetrol) prior to Aβ (25–35)-insult protected the cells from oxidative stress and apoptotic cell death. Furthermore, Brazilin mitigated Aβ-induced alterations in protein disulfide isomerase (PDI) and α-synuclein status, both of which are important biomarkers that report on Parkinson's pathogenesis. The results obtained in this study suggest that the tetrol is neuroprotective and helps resist Aβ-induced cross-pathology and amyloidogenic onset.

Mahesh Narayan, FRSC, is a biophysicist and Professor in the Department of Chemistry and Biochemistry. His current research interests include protein misfolding, neurodegenerative onset, and mechanisms of intervention therein. In addition, he has interests in chemical education and drug design and development and is a proponent of back-of-the-envelope calculations for most problems. He enjoys authorship and co-authorship of ~120 research and review articles, book chapters, and educational works.

KEYNOTE SPEAKER

NATURAL COMPOUNDS OF AERIDES ODORATA LOUR PLAY A VIBRANT ROLE IN HEPATOPROTECTION VIA IMMUNOMODULATORY EFFECTS

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Orchids, also known as mighty miniatures, are generally of extraordinary elegance and ornamental purposes while their therapeutic effects, chemical composition, and pharmacological applications are quite unraveled. We attempted to evaluate the hepatoprotective effects of Aerides odorata, an orchid, in an animal model and the role of immunomodulation in hepatoprotection. The A. odorata methanolic extract (AOE) was tested for acute toxicity using a brine shrimp assay. Gas-chromatography-mass spectrometry (GC-MS/MS), Fourier Transform Infrared Spectroscopy (FTIR), and mass spectrometry (LC-qTOF-MS) were adopted to screen the natural compounds of the tested orchid. Prior to the animal model study, the antioxidant potential of AOE was evaluated in in-vitro methods. The supercoiled pBR322 plasmid was used to investigate the defense of AOE in protecting DNA against oxidative damage caused by H2O2. The hepatoprotective role was assessed in the paracetamol (PCM)-induced hepatic damage model of Wistar albino rats. After the intervention, animal livers were histologically analyzed, and serums were assayed for ACP (acid phosphatase), AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), GGT (gamma-glutamyltransferase), TP (total protein), TB (total bilirubin), LDH (lactate dehydrogenase), antioxidant enzymes such as CAT (catalase), SOD (superoxide dismutase), glutathione peroxidase and LPO (lipid peroxidation). The expression levels of antioxidant genes CAT, SOD, β-actin, PON-1, and PFK-1 were evaluated using the qRT-PCR technique. The study was supported by the anti-ulcerative effects of AOE unfolding its role in changing the ulcer index. The immunomodulatory potential of AOE was measured in Swiss albino mice immunized with 0.5 x 109 cells/mL of Sheep blood cells (SRBC). After 14 days of oral dosing, rats were sacrificed, and their blood was collected for hematological, serological, and immunological assays, and the spleen for histopathological examinations. The results displayed promising antioxidative and DNA damage-protecting effects of AOE which was also found to dose-dependently maximize the liver enzymes (ACP, AST, ALT, ALP, GGP, LDH) and restore the total bilirubin level. Serum total protein and antioxidant enzymes (CAT, SOD, GSH) were noticed to be elevated. The PCM-treated liver tissues were significantly improved in the histological assay. Additionally, the mRNA expression for antioxidant genes CAT, SOD-2, β-actin, PON-1, and PFK-1 was multifold-upregulated. The AOE perfectly protected the rats from ethanol-induced gastric mucosal injury decreasing gastric erosion in all experimental groups. Furthermore, AOE led to a significant increase in pH and mucus weight; a decrease in gastric volume, stomach length, and stomach weight. The experimental animals' total leukocyte count, neutrophil, lymphocyte percentage, hemoglobin, delayed hypersensitivity reaction (DHT), and phagocytic index of animals administered with 100 mg/kg bw were found to be increased in the immune response assay. Among the identified natural compounds by GC-MS/MS and LC-qTOF-MS, eighteen were selected to interact with receptor proteins PDB IDs 1ILG, 1N3U, 1NFI, 1VKX, 2JOD, 7API, 317H via docking study and checked for their gene links using network pharmacological analyses. Results revealed the exciting effects of stigmast-4-en-3-one, isosilybin, kushenol, nobilin, dendrocandin, polydatin, darendoside A, and tribulusamide A as hepatoprotective compounds. The functional groups in FTIR spectra suggest that AOE has a wide range of polyphenolics for therapeutic contribution. Five hub genes namely VEGFA, CYP19A1, MAPK14, ESR1, and PPARG, were found to be strongly involved in protecting and recovering from liver damage. The results demonstrate the prospective immunomodulatory-associated hepatoprotective effects of AOE natural compounds which may be affirmed by advanced molecular analysis.

Keywords: Natural compounds of Aerides odorata, hepatoprotection, Immunomodulation, gene-upregulation, polyphenolics

KEYNOTE SPEAKER

INTEGRATING MULTIDISCIPLINARY METHODS FOR BASIC DRUG DISCOVERY: HARNESSING THE POWER OF NATURAL PRODUCTS RESEARCH

Prof. Dr. Asaad Khalid MA Ahmed

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Abstract

The discovery of bioactive compounds and drug hits in medicinal plants is a challenging process that requires expertise from various disciplines. Bioinformatics has emerged as an essential discipline in molecular biology that encompasses areas such as structural biology, proteomics, and genomics studies. Bioinformatics tools, including molecular modeling and simulation, have become vital for researchers to address the challenges that arise from the enormous amount of data generated by experimental research.

This lecture will provide an overview of our multidisciplinary research on the discovery of natural bioactive hits that target neglected diseases, with a focus on the diverse medicinal plants. We will highlight successful stories from our cell- and target-based research, which have led to the identification of interesting bioactive natural products.

The discussion will also address the challenges of drug discovery research, including predicting toxicity, and optimizing pharmacokinetics. By integrating various disciplines, including bioinformatics and computational techniques, we can enhance our understanding of drug discovery and develop more effective treatments for diseases.

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KEYNOTE SPEAKER

CHALLANGES AND OPPORTUNITIES IN ISOLATION OF CNS ACTIVE SECONDARY METABOLITES FROM PLANTS RELEVANT TO ETHNOPHARMACOLOGY

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Abstract

A great number of secondary metabolites generally found in plants which are daily use in ethnomedicine and as food, may affect the central nervous system (CNS) in mammals because they accumulate in the brain and trigger neuropharmacological effects (desire and/or unwanted). Natural products hold a great promise as potential drug leads to develop neuropharmacological therapies but the lack of critical insights into the translatability related to a knowledge about brain concentration in vivo is a serious limitation. Additionally, the isolation of bioactive compounds from complex plant extracts represents an essential step for their identifcation and further bioactivity assessment. The purifcation of natural products is a complex process, due to the vast array of diverse matrices.

Using state-of the art isolation techniques related to counter current chromatography and a battery of behavioral models in zebrafish and mice we define a workflow that allows to derive conclusions regarding the potential of CNS active natural products and their potential mechanisms of action.

Acknowledgments: This work was supported by National Science Center, Poland, grant number 2017/27/B/NZ4/00917.

KEYNOTE SPEAKER

VALORIZATION OF BIOACTIVE COMPOUNDS IN AGRICULTURAL WASTES AND THEIR FOOD APPLICATIONS

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Abstract

Food waste is defined as the by-products or residues derived from the processing of raw materials into higher-value products. It has been reported that significant amount of waste is generated worldwide each year which leads to environmental, economic and societal problems. The best option is to minimize food waste/by-products, however, whenever it is unavoidable valorization of these wastes could be an important alternative for the solution. More specifically, considering that these by-products contain a variety of bioactive compounds, such as dietary fiber, flavonoids, phenolic compounds, antioxidants, etc. valorization of these compounds could be a significant strategy to produce value-added products. Thus, novel and green approaches may also be used to valorize food wastes and improve their stability and applicability. In this work, valorization of bioactives from wastes as well as methods used to improve their stability and bioavailability are covered together with their applications, limitations and future perspectives.

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KEYNOTE SPEAKER

PHENOLIC COMPOSITION AND BIOLOGICAL ACTIVITY OF *WITHANIA SOMNIFERA* L. COMMERCIAL SAMPLES

Milena Polumackanycz[1], Spyridon A. Petropoulos[2], Tomasz Śledziński[3], Elżbieta Goyke[3], Agnieszka Konopacka[4], Alina Plenis[1], Agnieszka Viapiana[1]

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Withania somnifera Dunal (Ashwagandha) belongs to the Solanaceae family. It originates in north-western and central India and the Mediterranean region of North Africa. Most of its products are used and sold as dietary supplements. Among the various plant parts of W. Somnifera which have been claimed to have a large variety of health-promoting effects, the roots are the most popular. Their powder and preparations are consumed extensively as a functional food for promoting vitality and virility. In the present study, the chemical composition and bioactive activities of the aqueous and hydromethanolic extracts of commercial samples of Ashwagandha were evaluated. For this purpose, the HPLC method was used for the determination of ten phenolic compounds. The antioxidant activity was assessed by DPPH and ABTS radical scavenging activity, and FRAP and CUPRAC assays, while the inhibitory activity against AChE and BChE enzymes was determined using Ellman's method. The antibacterial activity was determined only for aqueous extracts of W. somnifera because of their widespread use as tea infusions. Moreover, the contents of total phenolic, total flavonoid, total phenolic acids, and L(+)-ascorbic acid were determined. Polyphenols and L(+)-ascorbic acid content, as well as the antioxidant activity, were higher in the aqueous extracts than in the hydromethanolic extracts. Moreover, higher amounts of phenolic acids and flavonoids were found in the hydromethanolic extracts compared to the aqueous ones. Finally, the aqueous and hydromethanolic extracts were the most efficient extracts in terms of AChE and BChE inhibitory activities, respectively. In conclusion, although W. somnifera is associated with several beneficial health effects, the products available over the counter do not ensure that they always possess these effects and further control is needed. Moreover, the processing of thestudied products highlighted the importance of the extraction method to the obtained bioactive compounds and by extension to the potential health effects.

Key Words: Ashwagandha, phenolic profile, antioxidant capacity, antimicrobial activity, Solanaceae

KEYNOTE SPEAKER

NATIONAL MEDICINAL PLANTS BOARD BOOSTS CONSERVATION & CULTIVATION OF HERBS IN INDIA

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The National Medicinal Plants Board (NMPB) under the Ministry of Ayush has implemented several initiatives to support the conservation, development, and sustainable management of medicinal plants in India. The National Medicinal Plants Board (NMPB), a wing of the Ministry of Ayush, Government of India, has been actively working towards the conservation, development, and sustainable management of medicinal plants in the country. The NMPB has supported 1498 projects throughout India under the Central Sector Scheme for Conservation, Development, and Sustainable Management of Medicinal Plants. As part of its efforts, the NMPB has supported 103026.32 hectares of land for in-situ and ex-situ conservation and resource augmentation. This includes the establishment of 24,000 herbal gardens, 1175 Joint Forest Management Committees (JFMCs), and 57 nurseries. The NMPB has also developed an e-platform, "e-CHARAK", for the trade of medicinal plants and to provide easy market access. In addition, the NMPB has set up a helpline in collaboration with the Centre for Development of Advanced Computing (CDAC) Hyderabad. Under the National Ayush Mission (NAM), the Ministry of Ayush has supported the cultivation of 140 prioritized medicinal plants in identified clusters/zones across the country. The ministry supported 56,305 hectares of land, 220 nurseries, 354 post-harvest management units, 25 processing units, 42 rural/district collection centers/retail outlets, 10 seed germplasm centers, and 15 demonstration plots among other initiatives. The NMPB provides project-based support for various activities such as survey inventorization, in-situ conservation, ex-situ conservation, support to JFMCs and other communities for value addition activities, research and development, information, education, communication, and promotional activities. The NMPB has also supported 233 projects under IEC activities, 19 projects for the marketing of medicinal plants, and 57 projects for establishing nurseries to raise quality planting material. Through its Regional Cum. Facilitation Centers (RCFCs), the NMPB provides financial and technical assistance to farmers and stakeholders for the development of quality planting material. The NMPB has set up 7 RCFCs across the country to promote medicinal plant activities in different regions. The Ministry of Ayush has also supported 235 workshops, buyer-seller meets, exposure visits, and training programs for 19061 farmers under the IEC activities of the National Ayush Mission scheme.

KEYNOTE SPEAKER

PHYTOPLASMA DISEASES ASSOCIATED WITH MEDICINAL PLANTS AND SPICES IN ASIAN COUNTRIES AND THEIR MANAGEMENT

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Medicinal plants are economically very important crops which are of great value for domestic use and for export all over Asian countries. Medicinal plants and spice crops are used in the traditional medicine and our daily culinary as they are rich in many phytochemicals, that provide health benefits and delicious taste to food dishes. Because of their increasing appliance in pharmaceutical, culinary and cosmetic industry, medicinal and spice crops are currently sharing an important trade in global economy. Phytoplasmas are now becomes globally importance because of producing unspecific symptoms, various losses and diverse epidemiology throughout the world. Phytoplasmas cause diseases in several medicinal plants/spice crops inducing serious economic losses. Therefore, phytoplasma diseases are the major constraints in profitable cultivation of medicinal plants/spice crops and lowers its quantum and quality and gaining international importance. Phytoplasma diseases of medicinal plants occur all over Asian countries, and many of these diseases either were previously of unknown or mistakenly presumed to be induced by virus. So far more than 60 medicinal plant species and 10 different spice crops were reported to be affected with phytoplasma diseases. Phytoplasma diseases of medicinal plants occur all over Asia, however, the majority of reports are from India, China, Iran, Turkey, Korea and Thailand. These diseases differ considerably in geographic distribution and size of the various taxonomic groups and subgroups of the associated phytoplasmas. Newly discovered phytoplasma diseases of medicinal plants/spice crops are increasingly being attributed to phytoplasma infections. This rapid increase of infection may be related to the fact that currently the use of insecticides to control insect vectors of are minimized phytoplasmas is not allowed on medicinal plants/spice crops, whereas insect control and/or management by other, environmentally-friendly means is not fully satisfactory. Changes in the composition of secondary metabolites occurring in diseased plants can be related to the role of phytoplasma infections in triggering plant defense responses in which, however, the levels of valuable phytochemicals are greatly affected. The impact of phytoplasma infections on medicinal plants should be considered in promoting good agricultural practices for cultivation and propagation of these plants. Epidemiological studies are little attempted in case of medicinal plant/spice crop-phytoplasma host combination which is required to be carried out in order to eliminate infected plants to prevent further epidemic spreading. Therefore, the current developed practices in identification and characterization of phytoplasmas infecting medicinal plants/spice crops in Asia should be applied which would be quite helpful in early detection of the disease, understanding phytoplasmas taxonomy, diversity and a sound practical management approaches.

ORAL PRESENTATION

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ORAL PRESENTATION

CAROB (*CERATONIA SILIQUA* L.) SEED EFFECT ON SEMEN QUALITY AND BLOOD PARAMETER IN LORI-BAKHTIARI RAM

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Abstract

Ceratonia silique L., is one of the herbal medicines which contains more than one flavonoid and carotenoid, is widely used for antioxidant purposes. Carob is good for sexual and blood metabolites functions. This study was conducted to investigate the effect of adding hydro-alcoholic extracts of carob seeds to the diet on semen quality and blood parameter in Lori-Bakhtiari Ram. In this study, carob seeds were extracted by percolation method. Thirty Lori-Bakhtiari rams used in current study were divided in control group (no carob extract), treated group (containing 150 mg of carob seed extract in the diet). This experiment was performed in December and March. Sperm was collected from treated and control groups during the experimental month using the artificial vaginal. Sperm motility, were evaluated by the CASA software. Furthermore, Blood samples were collected for evaluation of Fasting Blood Sugar (FBS), Cholesterol (CH) and Triglyceride (TG). After using 150 mg/kg of carob extract on diet during experiment the motility of sperms was significantly increased compared to the control group while, the level of t FBS, CH and TG were significantly decreased ($P<0.05$).In general, it is concluded that the amount of 150 mg/kg of carob extract in the diet of Lori-Bakhtiari ram has a positive effect on sperm quality and blood parameters.

Key word: Carob, Semen Parameter, Blood parameter, Ram

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ORAL PRESENTATION

WILD PLANTS: FOOD AND MEDICINE IN THE MIDDLE EAST

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Abstract

Anthropologists, among other fields, are interested in studying what we eat, how we eat, and why we eat what we eat. The main purpose of this paper is to present examples of different edible plants and their use by Arabs for treatment of diseases according to their way of life. The second purpose is to identify the wild plants and their uses for food, and medicine etc. The third purpose is to document the ethno-botanic data on herbal remedies, which are traditionally used by them.

Methodology: The paper is based on unstructured interviews, and the observation of participants were carried out in the informant homes. Most of the informants were in the age of 25 to 80. This survey involved 40 people, 10 of whom are traditional healers who were interviewed about food and medicinal plants during the last four years, among different communities in the Middle East.

Results: Information about 50 food and medicinal plants used by different Arab communities. This information included names of plants, parts used, which were obtained from 40 people, 10 of whom were healers .This survey is the first work which collected information about food and medicinal plants.

Conclusion: Adult and senior persons have a wide range of herbs used for their diseases as well as for food and diet.
The information obtained in this study can serve as a scientific base for further investigations to determine their desired results and adverse effects. The plants found in this study, while also used for treating diseases, is also worthy to take into consideration that these plants had not been evaluated clinically to approve their safety and efficacy.

Keywords: Plants, food, medicine, healers, culture, Arab.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

TAXONOMY INFLUENCES THE EFFICACY OF PLANT EXTRACTS AGAINST ANTIBIOTIC RESISTANT BACTERIOME

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Abstract

Antimicrobial resistance of the microbiome poses lately a high risk to human and animal patients and also to environment health. The missuse and lack of control in antimicrobial treatments lead to escalation of the antibiotic resistance phenomenon. The research aimed at investigating to which extent the taxonomy of plants intervenes in conditioning the antimicrobial resistance in both ATCC strains (*Escherichia coli* ATCC 25922 and *Pseudomonas* spp. 10145) and clinical isolates. The pathogenic bacteria were obtained from animal patients with diverse pathologies and identified for diagnostic purposes by standard microbiological techniques using rapid biochemical tests: GN 24 (Tody Laboratories, România) for Gram-negative, and GP 24 (Tody Laboratories, România) for Gram-positive strains as *Escherichia coli*, *Pseudomonas putrefaciens*, *Pseudomonas fluorescens,* and *Staphylococcus sciuri, Staphylococcus aureus*. The evaluation of both antibiotic susceptibility and the activity of essential oils against multiresistant bacteria were tested by the Kirby-Bauer disc diffusion method using amoxicillin/clavulanic acid, penicillin, imipenem, gentamycin, streptomycin, florfenicol, cefquinome, erythromycin, tylosin, tulathromycin, oxytetracycline, and doxycycline. The assessment included 10 essential oils derived from plants belonging to families *Lauraceae* (*Cinnamomum zeylanicum*), *Myrtaceae* (*Melaleuca alternifolia)*, *Geraniaceae* (*Pelargonium capitatum)*, *Lamiaceae (Mentha piperita*, *Thymus vulgaris*, *Lavandula angustifolia*, *Ocimum basilicum*, *Salvia glutinosa*). The extracts of *Lauraceae* family showed a significant antimicrobial activity against all strains, at least comparable or even superior to that of the most efficient antibiotics, regardless of their concentration. These results promote the further therapeutic experimentation of cinnamon essential oil as an efficient alternative to antibiotics in clinical trials.

Key words: plant taxonomy, essential oils, clinical isolates, ATCC strains, antibiotic resistance

Acknowledgements
The work was supported by grant ERANET Core Organic Co-fund ROAM Free #249/2021

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ORAL PRESENTATION

AMMOIDES PUSILLA POTENTIAL USE IN CREAM CHEESE PRESERVATION

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Abstract

The main aim of this study was to investigate the potential of *Ammoides pusilla* to improve the quality and the shelf life of cream cheese. The plant aqueous extract phenolic compounds' quantification and antioxidant activities avaluation were conducted. We investigated also the antimicrobial activity of *Ammoides pusilla* essential oil (*A.pusilla* EO) against five strains of food borne pathogen bacteria.
EO has shown to be an effective antimicrobial agent, against all tested bacteria except *Bacillus cereus* for whish only a moderate activity was recorded.

Tested cheese samples were supplemented with two different levels of *A.pusilla* EO, 250 and 500 µl/ kg of pressed curd (S1, S2) and dried plant powder at a rate of 4g / kg of pressed curd (S3).
A kinetic model was built based on yeast and mold population growth, in order to estimate the shelf life of cheese. Sensory and physicochemical properties were monitored during storage.
The use of ammoides pusilla extended the predicted shelf life of cheese samples from four to seven days at refrigeration temperature. The sensory test has shown that cheese samples supplemented with plant powder (S3) were well appreciated by consumers.

Key Words: *Ammoides pusilla,* shelf-life, essential oil, cream cheese.

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ORAL PRESENTATION

OPTIMIZATION OF PROPOLIS EXTRACTION WITH NATURAL DEEP EUTECTIC SOLVENTS USING CENTRAL COMPOSITE DESIGN

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Abstract

Due to its high phenolic content, propolis, an important bee product, is being utilized as a nutritional supplement. This research investigates the feasibility of extracting phenolic compounds from propolis using natural deep eutectic solvents (NADESs), and it uses central composite design (CCD) to enhance the extraction procedure. 109 NADESs were produced and investigated for stability, physicochemical characteristics, and capacity to dissolve phenolic substances, with four of them chosen for optimization utilizing CCD of Response Surface Methodology (RSM). Three independent variables—time, temperature, and ultrasonic amplitude—were tested at five different levels (-α, -1, 0, 1, and +α) to find the best combinations using CCD. Standard ANOVA was used to evaluate the CCD findings and created a quadratic regression equation. Using NADES 76 (proline:citric acid 2:1 (water 15% w/w)), NADES 62 (betaine:malic acid:proline 1:1:1 (water 15% w/w)), and 80% ethanol as a common solvent, the ideal extraction conditions were determined to be 80°C, 135 minutes, and 100% ultrasonic amplitude. Temperature and ultrasonic amplitude were shown to be less successful in extracting phenolic and flavonoid substances from propolis for the other two NADESs examined in this study. The maximum total phenolic and total flavonoid contents calculated for NADESs were, 5344.3 mgGAE/L and 110.3 mgQE/L, respectively. 5284.2 mgGAE/L and 255.1 mgQE/L were the actual values for the maximum total phenolic and total flavonoid content, respectively. An analysis was also conducted using LC-MS/MS to examine the phenolic compound profiles of each Propolis-NADES solution, in order to compare the distribution of each compound type across the different solutions. Compared to traditional solvents, our work has demonstrated that NADESs can be a good option for extracting bioactive content from propolis.

Key Words: Natural Deep Eutectic Solvents, Propolis Extraction, Optimization, Central Composite Desing.

Acknowledgements
The authors wish to thank ‘‘Balparmak R&D Center”, Altıparmak Food Co., for providing all facilities and opening their laboratories to run the experiments and analysis.

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ORAL PRESENTATION

CHYMOTRYPSIN AND TRYPSIN INHIBITORY ACTIVITIES OF SOME PLANTS GROWN IN RIZE (TURKEY)

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Abstract

The serine proteases like chymotrypsin and trypsin located in the gastrointestinal tract have important role in many physiological events such as digestion of proteins, fibrin clots, removal of proteins around cancer cells and protection from inflammatory diseases, cancer, ulcer, etc. On the other hand, due to its role in the digestion of proteins, it can be the target of drugs that can be used in the treatment of obesity. For this reason, research continues on the discovery of serine protease inhibitor compounds, especially from herbal sources.

In the study we designed in this context, it was aimed to investigate the trypsin and chymotrypsin inhibitory activities of six different plants [*Anemone narcissiflora* L., *Arctium minus* (Hill) Bernh., *Caltha palustris* L., *Cruciata laevipes* Opiz, and *Echium italicum* L.] naturally grown in Rize (Turkey). In addition, antioxidant activities of selected plants were determined by three different methods (ABTS, DPPH and CUPRAC). For these purpose 80% ethanol extracts were prepared from flower and leaves of *A. narcissiflora*, flower and aerial parts of *A. minus,* aerial parts and root of *E. italicum*, aerial parts of *C. palustris* and *C. laevipes.* Then, the antioxidant, chymotrypsin and trypsin inhibitor activities of all prepared extracts were determined in vitro.

According to the activity test results, the highest chymotrypsin inhibitory activity was observed with *C. palustris* (81% inhibition), while the highest trypsin inhibitory activity was observed with *C. laevipes* (82% inhibition). The extracts with the highest chymotrypsin and trypsin inhibitory activity were subjected to liquid-liquid separation and the enzyme inhibitory activities of the obtained fractions were examined again with the same methods. The highest chymotrypsin and trypsin inhibitory activities among all fractions were detected in the *n*-hexane fraction of *C. palustris* (77% inhibition) and the dichloromethane fraction of *C. laevipes* (71% inhibition), respectively. Although high antioxidant activity was detected in all extracts in general, *A. narcissiflora* leaf and *E. italicum* root extracts showed prominent activity in all applied antioxidant activity determination methods. Based on these results, it is planned to carry out further studies to determine the compounds responsible for the activity in *C. palustris* and *C. laevipes*.

Key Words: Chymotrypsin, trypsin, Caltha palustris, Cruciata laevipes, antioxidant

Acknowledgements
This research was financially supported by Gazi University Scientific Research Projects Coordination Unit (Project no: TKB-2022-8209)

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ORAL PRESENTATION

BIOLOGICALLY ACTIVE SECONDARY METABOLITES AND MEDICINAL USEAS OF *Stachys schtschegleevii*

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Abstract

Stachys schtschegleevii is a plant with medicinal properties that has been used for treating various diseases for centuries. Recent research has revealed that this plant contains a diverse range of biologically active secondary metabolites, including flavonoids, terpenoids, and phenolic acids. These compounds have been found to possess a wide range of pharmacological activities, such as antioxidant, antimicrobial, anti-inflammatory, antitumor, and antidiabetic effects. One of the diseases that *Stachys schtschegleevii* has shown promising results in treating is rheumatoid arthritis (RA). RA is an autoimmune disorder that primarily affects the joints, causing inflammation, pain, and stiffness. Recent studies have demonstrated that *Stachys schtschegleevii* is effective in controlling RA-related pathological and inflammatory outcomes. Moreover, *Stachys schtschegleevii* has also been found to interact with the active site of the coronavirus protease enzyme, inhibiting the amino acids of the active site during the catalytic process. This indicates that *Stachys schtschegleevii* may have potential applications in the treatment of coronavirus. Although *Stachys schtschegleevii* has been traditionally used in Iran for treating colds and other diseases, it is not well-known in Turkey. This paper aims to highlight the various bioactive compounds present in this plant and their potential applications in the treatment of human diseases. The review will also summarize recent studies on the pharmacological properties of *Stachys schtschegleevii*, including their mechanism of action and potential therapeutic benefits. This information may be useful for researchers and healthcare professionals interested in natural product-based drug discovery and development.

Key Words: *Stachys schtschegleevii*, Rheumatoid arthritis, Covid, Pharmacology.

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ORAL PRESENTATION

IN VITRO DETERMINATION OF COLLAGENASE, ELASTASE, ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES, ANTIOXIDANT ACTIVITIES AND CHLOROGENIC ACID QUANTITATION IN *HELICHRYSUM STOECHAS* (L.) MOENCH AND *HELICHRYSUM STOECHAS* SUBSP. *BARRELIERI* (TEN) NYMAN

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Abstract

The inhibitory effects of ethanol (80%) and aqueous extracts of *Helichrysum stoechas* (L.) Moench and *H. stoechas* subsp. *barrelieri* (Ten) Nyman on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), two enzymes associated with the pathogenesis of Alzheimer's disease (AD), in addition to on elastase and collagenase, which are associated with inflammation and skin aging, were examined. At the same time, the extracts' antioxidant activity was evaluated employing DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and iron ion-chelating activity tests, since oxidative damage plays a role in both the pathogenesis of AD and skin aging. The total phenol and flavonoid contents of the extracts have been determined spectrophotometrically. *H. stoechas* which is collected from Hatay, a city in Türkiye, ethanolic extract and *H. stoechas* which is collected from Izmir, a city in Türkiye, aqueous extract suppressed AChE and BChE in a concentration-dependent manner. Nevertheless, neither of the two plant extracts inhibited elastase or collagenase. Although both ethanolic and aqueous extracts demonstrated excessive antioxidant activity in the DPPH radical scavenging activity and FRAP assays, they were insufficient in the iron-chelating experiment. Chlorogenic acid was identified in the extracts utilizing HPLC; the two extracts with the strongest cholinesterase (ChE) inhibition also had the greatest chlorogenic acid concentration. The ethanolic extract of *H. stoechas* (Hatay sample) and the aqueous extract of *H. stoechas* (Izmir sample) contain fascinating ChE inhibitors that need further research.

Key Words: *Helichrysum stoechas*, enzyme inhibition, Alzheimer's disease, antioxidant activity, chlorogenic acid

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ORAL PRESENTATION

RANCIMAT: A RAPID APPROACH FOR DETECTION OF OXIDATIVE DEGRADATION IN FAT-CONTAINING FOOD PRODUCTS

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Abstract

Amount of food consumption has been increasing rapidly due to the worlf population growth. Therefore, consumption of edible oil, which is one of the basic components of foodstuffs, is increasing fast. Hence, the necessity of delivering the oil to the consumer without deterioration of its current quality has emerged with the increase in the need for oil in the food industry. The deterioration of fat-containing foods is due to oxidation reactions caused by auto-oxidation chain reactions that occur under various conditions (such as heat, light and oxygen). This condition is known as rancidity [1]. Rancidity is one of the major problems affecting the quality of the fat-containing products [2]. So, oxidative stability as one of the most important parameters determining the quality of the foodstuff should be identified [3]. Measuring the oxidative stability of products normally requires months of work. Some instrumental methods such as Rancimat have been developed to measure the oxidation resistance of oil-containing products at increasing temperatures (less than 24 hours and > 100 ºC). On the other hand, determination of rancidity is also performed by conventional methods such as peroxide value measurement. However, it is not possible to identify the shelf-life of the product by these tests only give information about the current status of the product. Rather, Rancimat method accelerates samples to determine if antioxidants are needed to help manufacturers extract the full value from their oils. Rancimat oxidation test is a practical method that does not require extra analysis that consumes a lot of chemical materials and time [4]. In the current study, Rancimat method will be described as an alternative to traditional methods with applications.

Key Words: Rancidity, lipid oxidation, induction time, accelerated oxidation test, fats and oils.

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ORAL PRESENTATION

IS BOVINE MASTITIC BACTERIOME SENSITIVE TO PLANT EXTRACTS?

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Abstract

One of the most impacting diseases of dairy cows worldwide, due to the difficulties to diagnose subclinical development and to treat the clinical disease is represented by mastitis. In this study the several plant extracts and essential oils were assessed for antimicrobial efficacy on bacteria isolated from subclinical cases of mastitis.

Milk samples were obtained from Romanian Spotted extensively raised dairy cows (n=20), cohabiting on the same farm with sheep and pigs, which were diagnosed with subclinical mastitis. The microbiome components were identified by use of classical bacteriological methods and cultivated against *Melissa officinalis* and propolis alcoholic extracts and essential oils of *Rosmarinus officinalis*, *Thymus vulgaris* and *Lavandula angustifolia* in the Kirby-Bauer well diffusion method. *S. sciuri, Staphylococcus spp, E. coli, Enterococcus faecium* were isolated from the milk samples, showing a high MAR index, thus eliminating the possibility of therapy with 12 of the 14 antibiotics tested in some isolates.

The best antibacterial effect was shown by *Thymus vulgaris* essential oil (25±5.6 mm inhibition) versus the weakest effect of *Lavandula angustifolia* essential oil (17.5±8.2mm inhibition). *Staphylococcus* spp. showed the highest sensitivity, while *E. coli* strains were much more resistant. When compared to the essential oil the *Melissa* tincture was less effective (11.3±3.6mm versus 12.3±4.3mm) but comparable to amoxicillin, amoxicillin/clavulanic acid and stronger than cefoperazone. The propolis extracts used to control bacterial growth have shown *in vitro* efficacy, the effect depending on the concentration used.

Both tincture and essential oils proved to be efficient depending more on the strain than on the solvent type. These results are opening the prospect of using plant extracts, essential oils and propolis as an alternative to antibiotics in the treatment of mastitis, leading to safer products, with less antibiotic residues in milk.

Keywords: subclinical mastitis, microbiome, antibiotic resistance, medicinal plants, dairy cows

Acknowledgements
The work was supported by grant ERANET Core Organic Co-fund ROAM Free #249/2021

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

MOLECULAR CHARACTERIZATION OF *Fusarium oxysporum f.*sp *albedinis* FROM SOUTH WEST ALGERIA

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Abstract

The date palm (*Phoenix dactylifera* L.) is the stronghold of oasis ecosystems, by its presence in these dry areas, allowed the maintenance of various forms of animal and plant life. The Bayoud a vascular fusariosis caused by a phytopathogenic fungus *Fusarium oxysporum f.*sp *albedinis* (FOA) threatens the existence of this crop in these arid regions. The present study aims mainly at the analysis of the morphological diversity and the estimation of the FOA pathogen in southwest Algeria by a biomolecular tool (PCR). Surveys carried out in four regions of southwestern Algeria (Saoura, Ksours du nord, Touat and Béchar) allowed the collection of 111 isolates from infected rachis and rhizosphere. The study of the orphological variation within these strains reveals the existence of an important morphological variability, four different morphotypes, cottony type, downy type, mucous type and senescent type were observed. In addition, microscopic observations showed the existence of only microconidia, macroconidia and chlamydospores. Molecular analysis of 27 FO isolates by the specific technique of polymerase chain reaction (PCR) with the specific FOA markers (TL3-FOA28) showed that 20 isolates belong to FOA. Finally, pathogenicity analysis of 18 FOA isolates from PCR results revealed the high pathogenicity of 9 FOA isolates from rachis.

Keywords: Date palm, *Fusarium oxysporum f.*sp albedinis, morphological variability, PCR, Pathogenicity

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ORAL PRESENTATION

SPRAY DRIED NANOEMULSIONS LOADED WITH AVOCADO OIL: INFLUENCE OF HOMOGENISATION PRESSURE

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Abstract

Avocado oil has antioxidant effect due to the high amount of oleic monosaturated fatty acids, promotes the accumulation of HLD cholesterol that gives health benefits to the cardiovascular disease, also it is useful as anti-inflammatory agent in cancer preventation. Chemical instability and poor water-solubility of lipophilic compound such as avocado oil limit their bioavailability and delivery efficency. Nanoemulsions are promising method because of their unique physicochemical and functional properties: high encapsulation efficiency; low turbidity; high bioavailability; high physical stability. In this study, the effect of high-pressure homogenization on the water-in-oil-in water ($W_1/O/W_2$) double emulsions containing avocado oil encapsulated within a complex of whey protein and maltodextrin were investigated in order to produce stable spray dried powders. Maltodextrin (15%) and whey (15%) protein were used for double emulsion and 20% of avocado oil was used in the formation of the emulsion. All of double emulsions including avocado oil were evaluated for particle size and stability tests, and immediately spray dired at 165 °C inlet air temperature. The obtained powders were characterized as encapsulated oil efficiency, particle size, moisture content, water activity, bulk density, tapped density and Carr Index. $D_{(4.3)}$, $D_{(3.2)}$, D_{10}, D_{50} and D_{90} values of the emulsions were 10.53 µm, 4.56 µm, 1.68µm, 7.69 µm and 18.78 µm, respectively; after high pressure application, these values were reduced to 0.28 µm, 0.16 µm, 0.08 µm, 0.22 µm and 0.57 µm. The process yield of double emulsion was 60% in spray drying, this value increased to 87% for nanoemulsion. In additionally, the solubility, kinetic and centrifuge stability of encapsulated avocado oil powder within high-pressure homogenization was %100 while around 83% traditional homogenization method. The results of this study showed that high pressure treatment was found a positive effect on spray-dried emulsions in terms of the encapsulation efficiency which was increased by 37%.

Key Words: Avocado oil, high-pressure homogenization, nanoemülsion, spray drying, encapsulation.

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ORAL PRESENTATION

AN EFFICIENT GREEN METHOD FOR RECOVERY OF POLYPHENOLS FROM OLIVE TREE (OLEA EUROPAEA) LEAVES BY MEANS OF LACTIC ACID-BASED DEEP EUTECTIC SOLVENT

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Abstract

Biowastes generated during the processing of industrial crops and products are often rich in bioactive compounds like polyphenols. Using biowastes to obtain natural antioxidants is a sustainable and environmentally friendly approach that can promote human health while reducing waste. Olive leaf, which is one of the by-products of the olive oil industry, was used as a raw material in this study due to its rich bioactive content.

The current study is aimed at establishing an efficient green media, using an eco-friendly lactic acid-based deep eutectic solvent (DES) system, consisting of a hydrogen bond donor-HBD (glycerol) and hydrogen bond acceptor-HBA (lactic acic). The molar ratio of lactic acid-glycerol mixture was selected 1:1 depending on our previous study [1]. Ultrasound-assisted extraction (UAE) method, a green extraction method, has been used to recover natural antioxidants from olive leaves. The extraction time and amplitude were determined for ultrasound-assisted extraction of bioactive-rich extract from olive leaves prior to routine studies. The Box–Behnken design type of the response surface method (RSM) was employed to investigate the effects of independent parameters (particle size, solid mass and water content in DES) on the total phenolic content (TPC). Bioactive properties of the olive leaf extracts were also assessed depending on total flavanoid content (TFC) and antioxidant activity measured by 2 different assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) methods.

Key Words: Natural antioxidants, biowaste, deep eutectic solvent, green extraction, Box-Behnken-RSM.

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ORAL PRESENTATION

ANTI-QUORUM SENSING AND ANTIMICROBIAL ACTIVITY OF ZINC AND COPPER NANOFLOWERS DERIVED FROM *COTINUS COGGYGRIA* AND *ROSMARINUS OFFICINALIS* PLANT SPECIES

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Abstract
Background: Resistance to microbial pathogens has gained importance day by day. Microbial contamination in cosmetic preparations has become a remarkable issue in recent years due to the expiration date, hand contact, and use of the same product by more than one person. Since very low nanometers are not desired to be applied on the skin in cosmetic preparations, hybrid nanoflowers, mostly micro-sized, which have biotechnological importance, can be used in cosmetic preparations thanks to their high surface area and efficiency. In this study, we aimed to evaluate the hybrid nanoflowers of *Cotinus coggygria* and *Rosmarinus officinalis* plants, which have cosmetic importance, from a microbiological point of view.
Material and Methods: While synthesizing hybrid nanoflowers, metal ion and buffer are used in both copper and zinc nanoflower synthesis. Syntheses are made at different pH and concentrations. After the synthesis process, the copper nanoflower is kept in the refrigerator. Zinc hybrid nanoflower synthesis is done by keeping it in a magnetic stirrer. After the synthesis process, characterization is made from various aspects such as SEM, EDX [1-5]. Then, antimicrobial analysis of these plants and their nanoflowers was performed by agar diffusion method. Then, anti-quorum sensing method was applied against various microorganism. In this method, 15 ml of *Chromobacterium violaceum 12472* culture was inoculated into LB soft agar. Wells with a diameter of 5 mm were opened with a cork drill. 20 µL of the samples were deposited into the wells. It was incubated for 24 hours at 30ºC [6].
Result and Discussion: As a result of the study, antimicrobial and antiquarum sensing effects were observed in hybrid nanoflowers of both plants. With the data we obtained with this study, it has been a pilot study in terms of preventing microbial contamination in biotechnological and cosmetic products.
Key Words: Hybrid nanoflowers, anti-quorum sensing, cosmetic, biotechnology

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ORAL PRESENTATION

NEUROINFLAMMATION IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE AND PHYTOTHERAPEUTICS

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Abstract

Neuroinflammation is an important mechanism that plays an active role in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). In the central nervous system (CNS), the process involved in the inflammatory response is called as neuroinflammation and is characterized by the activation of the immune reaction through increasing the microglia and astrocyte population, as well as increasing different concentrations of cytokines and chemokines. Sustaining of neurogenesis as a mechanism of brain repair, mobilization of neural precursors for repair, remyelination, and even axonal regeneration are involved in neuroprotection, but neuroinflammation may be damaging if it causes neuronal damage. The best-known pathophysiological features of AD are the aggregation of neurotoxic forms of amyloid-β proteins in senile plaques and the accumulation of hyperphosphorylated tau proteins in neurofibrillary tangles. Therefore, drug treatments targeting amyloid-β and tau are used in clinical practice. However, FDA-approved anti-AD drugs have only symptomatic efficiency, and the search for alternative and more effective therapeutic targets for the treatment of the disease continues.

Phytochemicals and their derivatives are natural compounds that provide neuroprotection by helping to prevent the initiation and progression of neurodegeneration by altering pathogenic factors. Epidemiological studies show that phytochemicals affect the main pathogenetic mechanisms of AD by targeting oxidative stress, mitochondrial dysfunction, neurotrophic factor deficiency, apoptosis, and abnormal protein accumulation. Curcumin, ferulic acid, hypericum, and resveratrol, important phytochemicals, have been shown to exert preventive and therapeutic effects on depressive disorders by inducing brain-derived neurotrophic factor (BDNF) expression in the hippocampus. These results suggest that phytochemicals contribute to the activation of signaling pathways for neuroprotection by inducing the biosynthesis of neurotrophic factors (NTFs), the Bcl-2 protein family, and antioxidant molecules. Further elucidation of the molecular mechanisms underlying the anti-amyloidogenic effects of blood-brain barrier (BBB)-permeable phytochemicals besides their neuroprotective activities is crucial for synthesizing novel and multifunctional phototherapeutic compounds with high neuroprotective potential for the treatment of AD. In this context, phytochemicals, also known as anti-AD therapeutics, are suggested as a potential therapeutic strategy for the development of antidepressants and anxiolytic agents for the prevention of neurodegeneration. Accordingly, this review discusses the role of inflammation in the developmental period of AD and phytochemical compound-based treatment strategies for AD. In addition, it is highlighted the importance of research on the development of multi-targeted neuroprotective agents based on the structure of BBB-permeable phytochemicals to improve brain dysfunction and prevent neurodegeneration.

Keywords: Alzheimer's disease, neuroprotection, natural products, herbal medicine, phytochemicals

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ORAL PRESENTATION

IS IT SAFE TO USE ALL THE CINNAMON DERIVATIVES FOR HEALTH PURPOSES?

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Abstract
Cinnamon is obtained by drying the tree bark of the *Cinnamomum* Schaeff. (Lauraceae) genus, which consist of 250 different species have been described around the world. *Cinnamomum verum* J. S. Presl, *C. cassia* (L.) J. Presl, *C. burmannii* (Nees & T. Nees) Blume and *C. loureiroi* Nees are the most well-known species [1]. According to literature and a common knowledge, the cinnamon and its byproducts are used for their effect on lowering the blood sugar [2,3].
In this study, the major chemical content and *in vitro* antidiabetic activity of different commercially available cinnamon samples (cinnamon sticks, tea bags, capsules) were evaluated in order to draw a conclusion wheter they are safe to use for health purposes. Pharmacognosic analyzes (macroscopic-microscopic analysis, total ash assay, loss on drying), chromatographic analyzes (thin layer and high-pressure liquid chromatography) and inhibition assays on diabetes-related enzymes (α-amylase, α-glucosidase, aldose reductase) were performed on those samples.
As a result of the study, it was determined that aqueous and ethanolic extracts of different cinnamon species contained 7.73 - 333,691 mg/g trans-cinnamaldehyde and up to 43,735 mg/g coumarin. The ready-made samples were purchased from the herbalist did not present any trans-cinnamaldehyde or coumarin peaks in the HPLC chromatogram, therefore the compounds could not be detected. Decoction and ethanolic extracts of *C. cassia*, *C. burmannii* and *C. loureiroi* cinnamon sticks were found to contain high levels of coumarin, which could pose a health risk, according to European Food Safety Authority data. The study findings revealed that the origin of the Cinnamon is vital in the use of both food and therapeutic purposes. Besides, all the cinnamon samples were found to have inhibitory effects on α-amylase, α-glucosidase, and aldose reductase enzymes. This research once again revealed the importance of meticulous inspection of the products sold in herbalists.

Key Words: Diabetes, HPLC, α-amylase, α-glucosidase, aldose reductase, *Cinnamomum* sp.

Acknowledgements
We would like to thank Gazi University Scientific Research Projects unit for their financial support (Project code: TEU-2022-7381)

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ORAL PRESENTATION

PHYTOCHEMICAL AND BIOLOGICAL EVALUATION OF SOME ORNAMENTAL *ASTERACEAE* SPECIES

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Abstract

The current study focused on the investigation for the first time in Romania of three particular ornamental *Asteraceae* species from pharmacognostic and biological points of view. The first stage of the study was represented by obtaining the plant material in ecological conditions and analyzing the macro- and microscopic characteristics of the studied species (*Rudbeckia hirta*, *Tagetes erecta* and *Zinnia elegans*). Subsequently, extracts from inflorescences were obtained and analyzed. Fractionation was then carried out, leading to the separation of several extractive fractions, followed by their phytochemical evaluation. The qualitative analysis of the extracts obtained from inflorescences, performed using UHPLC-MS techniques, highlighted the presence of several classes of biocompounds. Moreover, the performed fractionations allowed the isolation of several compounds. The present study reported, for the first time, the existence of new compounds in one of the studied species. The structure of compounds was elucidated by corroborating the results obtained using various spectroscopic analyses.

The evaluation of the antioxidant action showed that the applied separation methods proved to be useful in obtaining selective fractions with increased biological activity compared to the initial extracts, while the antimicrobial testing showed that the extracts present antibacterial activity especially on Gram-positive bacteria, and remarkable antifungal action, comparable to that of the used standards.

The *in vitro* evaluation of the cytotoxic effect exerted on murine fibroblasts allowed the observation of cytotoxic effects only at relatively high concentrations for all investigated extracts. Furthermore, the comparative assessment of the cytotoxicity of the most promising extract and of one of its isolated compounds is another argument that emphasizes the importance of applying various separation techniques in order to obtain compounds with increased biological activity and reduced cytotoxicity.

Key Words: separations, UHPLC-MS, antioxidant assays, antimicrobial activity, cytotoxicity.

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ORAL PRESENTATION

CHARACTERIZATION OF THE NECTAR MICROBIOMES OF *SALVIA CRYPTANTHA* MONTBRET & AUCHER EX BENTHAM AND ITS PARASITE *OROBANCHE ANATOLICA*

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Abstract

This study aimed to investigate the nectar microbiomes of *Salvia cryptantha* and its parasite *Orobanche anatolica* using metagenomic analysis. *Salvia cryptantha* is an endemic species for Turkey. *Orobanche anatolica* is a selective parasite, found only on the genus Salvia and generally on S. cryptantha. Floral nectar is an important resource for pollinators, providing them with carbohydrates and other nutrients.

Nectar samples were collected from the *Salvia cryptantha* and *Orobanche anatolica* plants at Hacettepe University Beytepe Campus in Ankara, Turkey. The study evaluated the microbial diversity and abundance in the floral nectar of three different plant groups during flowering. Nectar collection was carried out using sterile hematocrit tubes and micropipette, and the microbial density in nectar samples was determined using colony-forming unit calculations. Metagenomic analysis was performed using the next-generation sequencing method, and the data produced was converted to raw data (FASTA format) for analysis. Alpha diversity was used to analyze the species richness and diversity of microbial communities in each sample, and Chao1 and Shannon diversity indices were determined using an OTU-based analysis method.

The study found that nectar microbiomes changed during the flowering period and differed significantly between plant groups. The bacterial and fungal densities varied significantly among the different plant groups and between the first and last three days of flowering. The results suggest that microbial diversity and abundance in floral nectar are dependent on the plant species and its stage of flowering, providing valuable insights into the microorganisms present in both host and parasite plants' nectar and their interactions. In conclusion, this study highlights the importance of understanding the microbial communities in floral nectar and their potential ecological impacts. Further investigations on plant-microbe interactions in nectar could provide more insights into the role of microbial diversity in the evolution of floral traits and the functioning of ecosystems.

Key Words: Nectar, Microbiome, Metagenomic analysis, *Salvia cryptantha*, *Orobanche anatolica*

Acknowledgements
The study was carried out within the scope of the project numbered FHD-2021-19484 supported by Hacettepe University.

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ORAL PRESENTATION

HEAVY METAL ACCUMULATION AND CHEMICAL COMPOSITION OF ESSENTIAL OILS OF TANSY (*TANACETUM BULGARE L.*) CULTIVATED ON HEAVY METAL CONTAMINATED SOILS

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Abstract

Comparative research has been conducted to determine the content of heavy metals and the chemical composition of tansy oils, as well as to identify the possibility of tansy (*Tanacetum vulgare L.*) growth on soils contaminated by heavy metals. The field experiment was performed on an agricultural field contaminated by the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. On reaching the flowering stage, the tansy plants were gathered. The content of heavy metals in leaves and inflorescences was determined by microwave mineralisation and analysed by ICP. The oils were obtained from the leaves and inflorescences of the plant by hydrodistillation and analysed by gas chromatography-mass spectrometry (GC-MS) technique. Tansy is a plant tolerant of heavy metals and can be grown on contaminated soils. Heavy metals do not affect the development of tansy and the quality and quantity of oil obtained from it. The Pb, Cd and Hg concentration in oils was below the permissible limits for pharmaceutical purposes. Twenty-two components were identified, representing 98.11-98.56 % of the leaves oil and 98.49-98.88 % of the flowers oil of the total oil components. Oxygen-containing monoterpenes (82-88%) predominated in the tansy oil, followed by oxygen-containing sesquiterpenes (8.7-12.1%), and monoterpene hydrocarbons (1.1-1.4%). Aliphatic hydrocarbon (n-undecane, 2%) was also present in the oil. The oil obtained from inflorescences and leaves of tansy had the same dominant components of trans-chrysanthenyl acetate (46.96–50.89%), trans-thujone (14.45–20.8%), trans-carveol acetate (3.91–6.01%), spathulenol (4.21-5.63%) and alfa-cadinol (3.77–5.83%). The analysed oils belong to the trans-chrysanthenyl acetate chemotype. The essential oil of tansy can be a valuable product for farmers from polluted regions.

Key Words: essential oil composition, heavy metals, polluted soils, tansy.

Acknowledgements
The financial support by the Bulgarian National Science Fund Project DFNI KP-06-H54/7 is greatly appreciated.

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ORAL PRESENTATION

A MODEL OF PURCHASE INTENTION OF COMPLEMENTARY AND ALTERNATIVE MEDICINES: THE ROLE OF SOCIAL MEDIA INFLUENCERS' ENDORSEMENTS

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Abstract

Social Media Influencers (SMIs) are a fashionable way of marketing products by creating electronic word-of-mouth (e-WOM) on social media. The marketing of complementary and alternative medicines (CAMs) by SMIs is becoming increasingly popular and gaining credibility within consumers on social media platforms. Nonetheless, advising about health care products on social media should be examined as it is different from endorsing other kinds of commercial products. The aim of this study is to develop a model that provides the underlying mechanisms of the stimuli of SMIs on social media towards consumers' purchase intention of CAMs.

This study used framework synthesis methods to develop the model. The framework synthesis was conducted by identifying a BeHEMoTh strategy (Behaviour of Interest, Health context, Exclusions and Models or Theories) to systematically approach identifying relevant models and theories and studies relative to the research aim.

This study presents a novel model for understanding the purchase behaviour of CAMs using SMIs as a marketing strategy. The model included two well-known theories (theory of planned behaviour theory and source credibility theory) as well as extensive existing research from a multidisciplinary perspective. The model is exclusively designed to help identify elements affecting perceived source credibility and factors to have an influence over consumers' preferences to purchase CAMs by taking into consideration SMIs' endorsements.

This study provides unique insights introducing new research areas to health literature and offers, new roles for healthcare professionals in this digital era by gaining new skills and competencies, required to provide more credible and accurate information about CAMs. The study also highlights the new marketing era of online health related product endorsements and recommends that policy makers and researchers carefully evaluate the impact of SMI's on the use of CAMs, as well as to regulate the content of these promotional materials.

Key Words: social media influencer, complementary therapies, source credibility, health products, theory of planned behaviour, complementary and alternative medicines

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ORAL PRESENTATION

QUALITY INFLUENCE ON MOROCCAN CROCUS SATIVUS SAMPLES USED FOR FOOD INDUSTRY

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Abstract

In order to be used in therapy, a plant product must meet the conditions of identity and quality imposed by the rules in force. Due to the high price of *Crocus sativus*, frauds with saffron are increasingly being reported, which reach the market in the form of expensive products of questionable quality. The investigated samples came from a local producer in the Taroudant area, Taliouine village, Morocco. Evaluating the quality of the two saffron samples included in the study (sample 1 – whole stigmas and sample 2 – fine saffron powder) we found that both samples represent the genuine vegetable product, according to the rules in force. The UHPLC analysis indicated the existence of some quantitative differences between the identified components, which we believe is justified by the different presentation of the two samples. The sample with the highest amount of safranal and picrocrocin was Sample 1, which can also be justified by the fact that the two components are volatile, which is much easier to lose from the plant material in powdered form (Sample 2). The *in vitro* testing indicated that both samples have good antioxidant activity. Today, however, the dimension of food has been completed by that of medicinal product, vegetable product with therapeutic potential and medicine. Even though credible scientific knowledge about the efficacy and safety of many dietary supplements is inadequate, the appearance on the market of a standardized extract of this plant product elevates saffron to the highest rank, bringing it into the realm of medicine

Key Words: quality control, saffron, chemical composition, antioxidant activity.

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ORAL PRESENTATION

ASSESSING THE SAFETY AND EFFICACY OF DIY-HOMEMADE SUNSCREENS WITH NATURAL, AROMATIC AND HERBAL INGREDIENTS

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Abstract

Sunscreen products are one of the most commonly used products during the summer months, as the exposure to sunlight increases. Every year, new commercial preparations are developed, and we witness improvements in the effectiveness and safety of sunscreen products. However, recently there has been an increasing public acceptance and appetite for "completely natural" and homemade skincare products, including sunscreens. These homemade sunscreens, which are often formulated using aromatic and medicinal herbal preparations, are being touted as safe alternatives to commercial sunscreens on online social media platforms such as Pinterest and alternative health websites.

It is important to note that scientific research has consistently focused on the effectiveness and safety of sunscreen products in the international literature. Moreover, concerns have been raised about the serious risks posed by non-commercial sunscreens, such as those that are "homemade" or "completely natural." Therefore, it is crucial to investigate whether these "DIY" sunscreen products, which are advertised as completely natural and additive-free, pose a threat to public health due to their lack of sun protection properties.

In this study, we will examine the formulations of these proposed products, focusing on their durability, protection against ultraviolet A and B (UVA and UVB) radiation, and average sun protection factor (SPF) values from a toxicological perspective. It is imperative that we emphasize the importance of safety when it comes to sunscreen products, as the use of unsafe sunscreens can lead to serious health risks, such as skin cancer, premature aging, and other skin disorders.

Key Words: Natural skincare products, Homemade sunscreens, aromatic and herbal ingredients, toxicology, UV protection

Acknowledgements: This study is supported by TUBITAK.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

ANTI-INFLAMMATORY, ANTINOCICEPTIVE AND ANTIOXIDANT ACTIVITIES OF SELECTED WEST-AFRICAN ASTERACEAE PLANTS USING *IN VITRO* AND *IN VIVO* METHODS

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Abstract

Launea taraxacifolia (LT), *Solanecio biafrae* (SB) and *Crassocephallum rubens* (CR) are used ethnomedicinally for the treatment of body pains, arthritis, wounds, and fever in West Africa. However, they are yet to be explored for their anti-inflammatory potential. Therefore, the present study was aimed at investigating the *in vitro* antioxidant, *in vitro* and *in vivo* anti-inflammatory potentials of the mentioned plants.

The dried plants leaves were macerated separately in ethanol (96%) and water, resulting in ethanol (EE) and water extract (WE), respectively. The ethanol extracts were fractionated into different solvents of increasing polarity [*n*-hexane, dichloromethane, ethyl acetate (EAF), *n*-butanol (NBF)]. Antioxidant activity of the extracts was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO) scavenging methods. *In vitro* lipoxygenase (LOX) inhibitory activity of the extracts was determined by microtiter assay. Carrageenan-induced hind paw edema model and *p*-benzoquinone-induced abdominal constriction test in mice was used to determine the anti-inflammatory, and antinociceptive activities of the extracts (250 and 500 mg/kg doses), respectively.

Among all extracts, EAF of CR exhibited the highest DPPH antioxidant activity (87%) followed by its NBF (84%) and EAF of SB (84%). The result was similar for NO antioxidant activity with NBF of CR, having the highest activity. The ethanol extract of CR exhibited highest LOX inhibition (91.0%). This result is in tandem with the *in vivo* anti-inflammatory result, where the ethanol extract of CR and SB exhibited a significant anti-inflammatory activity. CREE also exerted noteworthy antinociceptive activity without inducing any apparent gastric lesion at the dose of 500 mg/kg close to that of the reference compound acetylsalicylic acid (200 mg/kg), however, with ulceration.

This study scientifically justifies the traditional use of the aforementioned West African medicinal plants for the treatment of rheumatism for the first time. Further study is ongoing to identify potential anti-inflammatory drug candidates in these plants.

Key words: *Crassocephallum rubens Solanecio biafrae,* Antioxidant, LOX inhibition, Anti-inflammatory, Antinociceptive

Acknowledgement

I.O.O and I.E.O would like to thank the Scientific and Technological Research Council of Türkiye (TÜBİTAK) for the project grant funded through TÜBİTAK-2236 Co-Fund Brain Circulation program. We also thank Gazi University for providing research facilities as hosting institution.

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ORAL PRESENTATION

INVESTIGATION OF ANTIMICROBIAL EFFECT OF *JUNIPERUS COMMUNIS*, *ZINGIBER OFFICINALE*, *PISTACIA LENTISCUS* ESSENTIAL OILS IN COMBINATION WITH CHLORHEXIDINE AND DESIGN OF MOUTWASH FORMULATIONS

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Abstract

In this study, the antimicrobial effects of *Juniperus communis* L., *Zingiber officinale* Roscoe, *Pistacia lentiscus* L. essential oils, which are used ethnobotanically in throat infections, were compared with chlorhexidine, and their antimicrobial effects were investigated by designing binary combinations with chlorhexidine.

Antimicrobial activity experiments were performed against methicillin-resistant *Staphylococcus aureus* and *Streptococcus mutans* microorganisms, which are mouth-throat pathogens, with broth microdilution assay. Then, binary combinations of chlorhexidine and individual essential oils were investigated with the checkerboard method to determine the synergistic effect. Synergistic effective concentrations were loaded into the prepared mouthwash formulations and the effects of the formulations were investigated against the same pathogenic microorganisms by the agar-well diffusion method. As a result of the study, the FICI value of chlorhexidine-*J. communis* essential oil and chlorhexidine-*Z. officinale* essential oil combinations against MRSA were calculated as 0.4326, while the FICI value of the chlorhexidine-*P. lentiscus* essential oil combination was calculated as 3.3413. In this direction, combinations of chlorhexidine with *Z. officinale* and *J. communis* essential oils showed a synergistic effect, while chlorhexidine and *P. lentiscus* essential oils showed antagonistic effects. In formulations, the formulation containing chlorhexidine-*J. communis* synergistic combination was found more effective than the formulation containing chlorhexidine-*Z. officinale* against both *S. mutans* and MRSA.

As a result, in line with the data obtained from this study, the designed combinations of chlorhexine with *J. communis* essential oil can be used successfully against microorganisms causing throat infections. In addition, to the best of our knowledge, in this study, combinations of these oils with chlorhexidine were studied for the first time and a mouthwash formulation was designed.

Key Words: Chlorhexidine, *Juniperus, Zingiber, Pistacia*, synergism

Acknowledgements
This work was supported by TÜBİTAK project number 1139B412101851. Patent application was made with the number 2022/016569 of the results of the study.

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ORAL PRESENTATION

DETERMINATION OF DIFFERENT POPULATIONS IN *HELICHRYSUM* SPP. FROM HATAY FLORA AND EVALUATION OF SOME PROPERTIES

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Abstract

The genus *Helichrysum* is used extensively in the fields of ornamental plant, cosmetic products, food supplements and herbal tea production. *H. stoechas*, *H. plicatum* and *H. sanguineum* species from the *Helichrysum* genus found in the Hatay flora, which are the plant material of the project, are used as diuretic, anti-inflammatory and antispasmodic among the traditional medicine, but they can also be used in medicine and cosmetics due to their volatile and phenolic components. The *H. sanguineum* species found in the flora is local endemic since it is only found in the flora of Hatay in Turkey and the species has been identified as endangered in the IUCN (World Union for Conservation of Nature and Natural Resources) category. As a field study, for *H. sanguineum*, *H. plicatum* and *H. stoechas* villages from Altınözü, Antakya, Samandağ, Defne and Yayladağı districts were surveyed. Soil samples and chemical compositions were determined.

Key Words: Everlasting, *Helichrysum*, neryl-acetate, quercetin

Acknowledgements
This project was supported by HMKU-BAP under the project number 22.GAP.036.

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ORAL PRESENTATION

COMPARISON OF THE CYTOTOXIC AND PROLIFERATIVE EFFECT OF *Laurus nobilis L.* SEED EXTRACTS ON HEALTHY AND CANCEROUS CELLS

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Abstract
Often referred to as bay leaf, *Laurus nobilis L.* is a plant native to the southern Mediterranean region. Located in the family Lauraceae, it is an enduring perennial shrub. Citric acid, carbohydrate, favonoids, eugenol, tannins, steroids, alkaloids, triterpenoids are all present in the chemical composition of bay leaves. Many studies have reported that *Laurus nobilis L.* extracts mainly from the leaves and seeds have many properties such as antibacterial, antifungal, antioxidant, anti-carcinogenic, neuroprotective, and wound healing (1, 2). The aim of this study is to determine the properties of laurel plant seed extracts that inhibit or reduce cancer cell proliferation without showing cytotoxic effects on healthy cells. The laurel plant was collected from the southern Mediterranean region (Hatay-Antakya) of Turkey. Prior to in vitro experiments, bay leaf seeds were pulverized, dissolved in DMSO, and filtered. The chemical composition of the Bay leaf was identified. For in vitro cell viability and cytotoxicity assay adipose-derived mesenchymal stem cells (ADMSC) were used in the study, as well as a human glioblastoma cell line (T98G). MTT (3-(4,5 Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) analysis was performed for cell viability evaluation, and AO (acridine orange) /PI (propidium iodide) staining was performed to detect apoptotic and necrotic cells.Bay leaf solution obtained at different concentrations (2.5 to 200 µg/mL) was applied to ADMSC and T98G cells and their viability and cytotoxicity effects at 24, 48 and 72 hours were examined and compared.It was observed that laurel seed extract did not cause any cytotoxic effect in ADMSC cells and even increased cell proliferation. A decrease in cell proliferation was observed in the T98G cell line. Based on these analyses, it was concluded that laurel seed extract can reduce the proliferation of cancer cells without harming healthy cells.

Key Words: Bay leaf, Cytotoxicity, Laurus nobilis L., Glioblastoma cell line, Adipose-derived mesenchymal stem cells, Cell viability

Acknowledgements
For his assistance in the experimental stages of the study, we are grateful to Cihan Konan. There was no funding for the study.

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ORAL PRESENTATION

IN VIVO SEDATIVE ACTIVITY EVALUATION ON SOME PLANTS USED IN TURKISH FOLK MEDICINE

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Abstract

According to data from the World Health Organization, approximately 10% of the world's population experiences various anxiety problems, and 30% of the adult population suffers from insomnia. There is a tendency to naturally sourced compounds with fewer side effects due to the significant side effects such as abuse, addiction development, amnesia, and cognitive and sexual dysfunction. of conventional drugs used in the treatment of anxiety and insomnia, Accordingly, within the scope of this study, sedative activities of *Anthemis fumariifolia* (Papatya), *Verbascum bombyciferum* (İpek Sığırkuyruğu), *Rubus canescens* (Dirkel), *Avena sativa* (Yulaf), and *Capparis ovata* (Kebere) plants used for sedative purposes among the folk, were evaluated. The dried and powdered plants were extracted with *n*-hexane, etil acetate (EtOAc) and methanol (MeOH), subsequently. In addition, water extracts were prepared from dried plants. Each extract was submitted to bioassay systems; traction test and hole-board test were used for sedative and anxiolytic effects. *C. ovata* MeOH extract was the most active extract on *in vivo* traction test (11,95±5,21 (Re-Establishment Time) (Sec) ± S.E.M) and holeboard Test (22,83±5,49 (Explored Holes During 5 min) ± S.E.M), compared to Lorazepam. Therefore *C. ovata* MeOH extract was subjected to chromatographic methods for the isolation and purification of the compounds. The structures of the compounds were elucidated by means of spectroscopic analysis. At the end of the study, 3 flavonoid glycosides [Rutin (**1**), Quercetin-3-glucoside (**2**) ve Quercetin 3-rhamnoside (**3**)] were isolated. The amount of rutin in the *C. ovata* MeOH extract was determined using HPLC and was found to be 6.5%. This study confirmed the claimed use of the plant against anxiety in Turkish folk medicine. It is thought that the major flavonoid glycosides in *C. ovata* may be responsible for the sedative anxiolytic activity. Further studies continue to elucidate the mechanism.

Key Words: folk medicine, sedative, anxiolytic, isolation, *in vivo* activity, *Capparis ovata*

Acknowledgements
This study was supported by Gazi University Research Foundation [grant number 02/2020-10 (ID: 5966)]

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ORAL PRESENTATION

GC-MS ANALYSIS & ANTI-INFLAMMATORY ACTIVITY OF *HELIOTROPIUM BACCIFERIUM* ESSENTIAL OIL

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Abstract

Phytomedicine is currently an important area of better treatment and has great prospects in many countries. It's cheaper, culturally and socially better known, more compatible with the human physiological system, and has significantly fewer side effects. *Heliotrepium Bacciferum* (*Family Boraginaceae*) It is not only a widespread herb, but also an important medicinal plant. These medicinal herbs are found in the desert of Algeria. The essential oil of this species was extracted by hydrodistillation in and analyzed by GC-MS. It consisted of a mixture of monoterpenes and sesquiterpenes, while the main components of this EOs are Agarospirol (15.15%), 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-.alpha.,.alpha.,4a,8-tetram (9.41%), Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4trimethyl-3-(1-methyletheny (8.96%), tau.-Cadino (6.47%), linalool (5.37%) Cyclohexanol,3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-,[1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)] (5.36%), respectively. The way2drug web source shown that most of the compounds contained in the essential oil have anti-inflammatory properties (Pa) Agarospirol (0.392), 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-.alpha.,.alpha.,4a,8-tetram (0.726), Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methyletheny (0.793), tau.-Cadino (0.566), linalool (0), Cyclohexanol, 3-ethenyl-3-methyl-2-(1-methylethenyl)-6-(1-methylethyl)-, [1R-(1.alpha.,2.alpha.,3.beta.,6.alpha.)] (0.870), respectively and the global anti-inflammatory of the total essentiel oil (0.31). The anti-inflammatory activity of this essential oil was evaluated in vivo on the inflamed left paw by injecting of 1% formalin into the volume of the paw before and after injection at 30 and 60 minute intervals for 180 minutes. Intraperitoneal treatment at a dose of 200 mg/kg body weight. It shows significant inhibition of mouse paw edema from 60 minutes to 180 minutes of experiment with 61.89±13.54%, 85.47± 5.92%, 94.78±2.85% respectively, instead the 150 mg/kg dose shows significant inhibition from 120 minutes to 180 minutes of the test with an inhibition percentage of 50.74 ± 11.83% and 70.81 ± 7.34%, respectively. This study shows that 200 mg/kg is effective in treating formalin-induced acute mouse paw edema, with better efficacy at 150 mg/kg and nonsteroidal anti-inflammatory drugs.

Key words : *Heliotropium Bacciferum*, *Boraginaceae*, Anti-inflammatory, activity, GC-MS, essential oil.

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ORAL PRESENTATION

ENZYME INHIBITORY AND PHYTOCHEMICAL STUDIES ON *Pistacia vera* LEAVES COLLECTED from GAZİANTEP

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Abstract

Pistacia vera L., known as Pistachio, is a species belonging to the Anacardiaceae family. It is reported in the literature that various *Pistacia* species have numerous ethnobotanical uses. In this study, in addition to pancreatic cholesterol esterase enzyme inhibition, the *in vitro* antidiabetic (*α*-glucosidase and *α*-amylase enzyme inhibition) and antiobesity (pancreatic lipase enzyme inhibition) potentials of *P. vera* leaves collected from Gaziantep province were investigated. The phytochemical content of the 80% ethanol extract prepared from *P. vera* leaves was investigated by RP- HPLC technique. While the presence of gallic acid and methyl gallate in the extract was determined by RP-HPLC analysis, the extract was standardized on pentagalloylglucose (1.11 mg/g plant). The inhibitory effects of *α*-amylase, *α*-glucosidase, pancreatic lipase, and pancreatic cholesterol esterase of the 80% ethanol extract were evaluated. The extract had an inhibition value of 100% on the *α*-glucosidase enzyme at a concentration of 2 mg/ml, while at the same concentration this value was 99.70 ± 0.26 for the reference compound acarbose. The *α*-amylase inhibitory activity of the extract at 2 mg/ml concentration was found to be 88.51 ± 3.15%. While the extract reached the inhibition of the highest pancreatic lipase enzyme (57.19 ± 2.86%) at 2 mg/ml; this value was 0.25 mg/mL for inhibition of pancreatic cholesterol esterase enzyme (39.45 ± 1.78%). The findings from the experiments revealed a potent antihyperglycemic and potential antiobesity activity of *P. vera* leaves. In the light of these results, it was thought that *P. vera* leaves could be a potential source for isolation studies directed by antihyperglycemic and antiobesity activity.

Key Words: Antidiabetic; Phytochemistry; *Pistacia vera*; RP-HPLC

Acknowledgements
This study was supported by a grant of Gazi University-BAP (02/2019-32)
Thanks to TUBITAK 2211/A National PhD Scholarship Program

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ORAL PRESENTATION

KEKIK: UNIQUE MULTIFUCTIONAL AROMATIC HERBS FROM TÜRKİYE

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Abstract

Kekik is the common name of some plant species grown in Türkiye flora from different genera of the Lamiaceae plant family of which major components of the essential oils are mostly carvacrol and/or tyhmol. *Thymus* (58 taxa), *Origanum* (26 taxa), *Satureja* (13 taxa), *Thymbra* (4 taxa) and *Coridothymus* (1 taxa) are the main kekik genera represented in Türkiye with a total of 102 different taxa according to the TUBIVES database. All these kekik taxa are locally known by different names and used for various purposes like spice, herbal tea, salad, and traditional medicine because of their strong antimicrobial properties. Apart from low local consumption, some kekik taxa have economic importance, especially for export in large quantities. The most commonly wild-collected, cultivated and commercially important Kekik genus is *Origanum,* and the major species of this genus are as follows: *Origanum onites* L., *Origanum vulgare* L. subsp. *vulgare*, *Origanum vulgare* subsp. *gracile* (K.Koch) Ietsw., *Origanum vulgare* subsp. *hirtum* (Link) Ietsw., *Origanum vulgare* var. *viride* Boiss., *Origanum majorana* L., *Origanum syriacum* L. and *Origanum minutiflorum* O.Schwarz & P.H.Davis. Another economically important Kekik genus is *Thymbra,* and the well-known species of this genus is *Thymbra spicata* L. This kekik genus is mainly wild harvested and traded. Apart from other kekik taxa, this species has been used as a salad and pickled for use in winter before the flowering stage is harvested. *Thymus*, *Satureja* and *Coridothymus* genus kekik plants are mostly locally known and traded in small quantities. Besides other uses, all Kekik taxa have been used for the production of essential oil and distilled water for both homemade use and commercial purposes. Essential oil content and composition of the Kekik plants vary greatly depending on the plant taxa, geographic region, and stage of growth, wild harvested or cultivated. Post-harvest processing and cultivation techniques also affect the quality of final product. Turkish Kekik products are known with their high quality in the world market, and the demand has gradually increased. In addition to being used as a spice in the world of cousins, essential oil of Turkish oregano has a great importance during the COVID-19 pandemic period because of its strong antiviral properties, as well.

Keywords: Kekik, thyme, oregano, *Origanum*, *Thymus*, *Thymbra*, essential oil

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ORAL PRESENTATION

GREEN NANOTECHNOLOGY FOR DIABETES MANAGEMENT

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Abstract
Diabetes mellitus (DM) is a long-standing, challenging, and non-transmissible endocrine disease that has created significant clinical issues. This condition currently affects 387 million people worldwide and by 2040, it is expected to affect more than 640 million people [1]. The aim of this study was to summarize a set of studies regarding the use of nanotechnology based on plant extracts and phytocompounds in the treatment of type 2 DM (T2DM).
Recent developments in nanoparticulate technologies for better drug delivery offer considerable promise for the administration of a variety of active substances and phytocompounds [2]. The use of nanotechnology in DM pharmacological therapy has grown significantly over the last two decades. In order to reduce the adverse effects and dosage frequency while enhancing the effectiveness of oral antidiabetic medications, several drug delivery systems have been researched. For example, metformin nanoparticles were included in a variety of formulations, some of them using polysaccharide nanocarriers (e.g, metformin-loaded alginate nanoemulsion/pectin nanoparticle, alginate/chitosan-coated nanoemulsion for oral insulin delivery) [3, 4].
Another approach for DM treatment is represented by the use of plant-based nanoformulations, given that plants represent an important source of secondary metabolites and the synthesis follows current trends, being eco-friendly and cost effective. Thus, a formulation with reported antidiabetic properties can include silver nanoparticles from *Punica granatum, Ocimum basilicum, Lonicera japonica,* zinc oxide nanoparticles from *Momordica charantia, Urtica dioica, Silybum marianum*, gold nanoparticles from *Cassia auriculata, Gymnea sylvestre* etc. [5]. Moreover, the preparation of nanocarriers for phytochemicals comes with certain advantages, such as: a more targeted action, enhanced availability and stability, dose and frequency of administration reduction. For example, glycyrrhizin, an active component of *Glycyrrhiza glabra's* roots improved the antihyperglycemic and antihyperlipidemic activity in type-II diabetic rats, comparable to metformin. Additionally, a combination of glycyrrhizic acid-loaded nanoparticles and thymoquinone-loaded nanocapsules was used, exhibiting better antidiabetic properties than when administered separately in nicotinamide and streptozotocin-induced T2DM rats [6].
In conclusion, the current paper provides an up-to-date overview of the nanoformulations of plant extracts and phytochemicals used in the treatment of T2DM.
Key Words: diabetes mellitus, plant extracts, phytocompounds, nanoformulations.

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ORAL PRESENTATION

THE EFFICACY AND SAFETY OF BIOAPIGYN® OINTMENT FOR PELVIC MUSCLE TONUS IN PREMARKET/POSTMARKET STUDY IN THE TREATMENT OF FEMALE URINARY INCONTINENCE

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Objective / Purpose: The purpose of this work was the comparison of the clinical efficacy as well as monitoring of safety of Bioapigyn® vaginal ointment for pelvic muscle tonus in alleviating the symptoms of stress, urge and mixed urinary incontinence and vulvo-vaginal disorders in child-bearing and menopausal & postmenopausal women in premarket study compared to three years of post-market clinical follow-up (PMCFU) investigation.

Materials and methods: 66 participants for premarket and 30 per year for PMCFU investigation were recruited and treated once a day with 2.5 mL/day of Bioapigyn® ointment for pelvic muscle tonus for 28 consecutive days. ICIQ-UI SF score, the residual urine volume, the total score of vulvo-vaginal symptoms and vaginal pH were determined before and after the treatment. For statistical evaluation Statistica 11.0 software package was employed.

Results: Compared to baseline values premarket study resulted in 54.9% decrease of ICIQ-UI-SF score, 76.9% decrease of residual urine, 14.2% decrease of vaginal pH and total dissapearence of vulvo-vaginal disorders. During PMCFU investigation ICIQ-UI-SF score was reduced between 65.8% and 72.7% (average 69.2%), residual urine volume from 82.6% to 85.1% (average 83.7%), vaginal pH from 16.4% to 22.4% (average 19.3%) while all the symptoms of vulvo-vaginal disorders disappeared completely in all participants. Although, post-market results are better compared to premarket study the difference was not statistically significant for none of the tested parameters. None of the patients experienced any discomfort or adverse effect including allergic reaction, worsening of the existing or the occurrence of new symptoms during either premarket or post-market treatment.

Conclusion / Discussion: Bioapigyn® vaginal ointment for pelvic muscle tonus alleviate the symptoms of incontinence by tightening and firming of the smooth muscles of the pelvic floor due to the ingredients with smooth muscles contraction/relaxation and astringent activity. Creating acidic, viscous, low water activity vaginal environment resulted in complete disappearance of vulvo-vaginal complaints.

Keywords: urinary incontinence, vulvo-vaginal disorders, honey, herbal macerates

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

KINETIC MECHANISM OF SOUR CHERRY PEEL POLYPHENOL EXTRACTION USING DEEP EUTECTIC SOLVENT

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Abstract

The recovery of rich compounds that occur as waste in the food industry gains importance in terms of increasing the nutritional content of foods and reducing environmental pollution [1]. In the fruit juice and jam industry, sour cherries are processed extensively [2]. Evaluation of sour cherry wastes is considerable in this regard.
In this study, polyphenol extraction of deep eutectic solvents from green solvents was investigated, which has attracted attention recently. For this purpose, a green and environmental method microwave-assisted extraction has been chosen for evaluating the kinetic parameters of the extraction process. The polyphenol content of sour cherry peel wastes has been measured since it is an important resource for the pharmaceutical and food industry. The behavior of the microwave assisted extraction of total phenolic content (TPC) from sour cherry peels has been determined by applying pseudo-first-order and pseudo-second-order kinetic equations, respectively. The most appropiate kinetic model has been found as pseudo-second-order kinetic equation by evaluating the kinetic data. Extraction rate and kinetic parameters show that the application of MAE was reasonable compared to previous processes. Last but not least, the extraction ended very quickly and effectively in terms of energy saving.

Key Words: Deep eutectic solvent; biowaste; sour cherry waste; kinetic modelling; polyphenols.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

CHEMICAL CONSTITUENTS OF *DIANTHUS SUPERBUS*, *MATRICARIA CHAMOMILLA* AND *GLYCYRRHIZA GLABRA*

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Abstract

Currently, one of the main directions of science is the study of plants with medicinal properties, the determination of biological activity and its use for medical purposes. In the present study, the quantitative and qualitative composition of phytochemical components of plants *Dianthus superbus, Matricaria chamomilla* and *Glycyrrhiza glabra* growing in the Republic of Kazakhstan were investigated for the first time. The results of our studies showed the maximum amount of organic acids (0,317 %) and flavonoids (0,296 %) in the plant *Dianthus superbus*. The content of extractive substances *Matricaria chamomilla* (30,01 %) and *Glycyrrhiza glabra L*. (35,3 %), and the content of polysaccharides is higher in plants *Glycyrrhiza glabra L*. (1,475 %) and *Dianthus superbus* (1,434 %). The plant *Matricaria chamomilla* showed good results in the amount of elements needed daily by the human body, including – K (2876,70 µg/ml), Na (646,36 µg/ml), Mg (270,75 µg/ml), Ca (517,55 µg/ml), Fe (38,30 µg/ml) and Zn (2,008 µg/ml). The results of the conducted analyses showed that the studied objects contain a sufficient amount of bioactive substances that can in the future expand the range of effective domestic medicines based on plant raw materials of the Republic of Kazakhstan.

Key Words: *Glycyrrhiza glabra, Dianthus superbus, Matricaria chamomilla*, phytochemical components, macroelements, microelements.

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

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ORAL PRESENTATION

COMPARISON OF THE EFFECTS OF QUERCETIN AND QUERCETIN-LOADED CYCLODEXTRIN FORMULATIONS ON ACUTE PAIN

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Abstract

Quercetin is in the class of flavonols and its chemical formula is 3.3', 4', 5,7-pentahydroxyflavone. It has been shown to have a variety of pharmacological effects, including antioxidant, antiviral, and anticancer properties. Quercetin has also anti-inflammatory and antinociceptive/analgesic properties. However, the low solubility and bioavailability of quercetin adversely affect its effectiveness. Thus, in order to increase the bioavailability and solubility of quercetin, it is aimed to examine the change in the analgesic effect of quercetin by preparing a biologically compatible cyclodextrin formulation that can be targeted to the tissues in this study. Quercetin and quercetin-cyclodextrin complex were administered intraperitoneally at doses of 3, 5, 10, and 20 mg/kg in mice. Time-dependent analgesic effects of pure quercetin and quercetin cyclodextrin complex were compared in hot-plate and tail-immersion tests, acute pain models tested supraspinal and spinal organization of pain respectively. Quercetin at the doses of 5, 10, and 20 mg/kg was found to increase pain thresholds against thermal stimuli in tail immersion and hot plate tests in 30-180 min. time interval. Only the effect of 20 mg/kg quercetin weakened at 180 min. The effect at 3 mg/kg was observed only in hot-plate test at 120 min. Cyclodextrin complex prepared with 3 mg/kg quercetin showed significant analgesic effect in 30-180 min time interval in both two tests. Additionally, the analgesic effect of the cyclodextrin complex prepared with 20 mg/kg quercetin was not weakened in both tests like pure 20 mg/kg quercetin at 180 minutes. Thereby, it can be concluded that application of quercetin in cyclodextrin is provided to improve pharmacokinetic properties such as bioavailability of quercetin. Thus, the efficacy and duration of action of quercetin were enhanced. This study displays the analgesic potential of quercetin by spinal and supraspinal regulations and the advantages of application in cyclodextrin formulation for pain relief.

Keywords: Cyclodextrin, quercetin, pain, tail-immersion, hot-plate.

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ORAL PRESENTATION

ANTIMICROBIAL AND ANTIBIOFILM-FORMING ACTIVITY OF EXTRACTS OF MEDICINAL PLANTS FOUND IN THE UKRAINIAN CARPATHIANS

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Abstract

The problem of formation of microorganisms' resistence to antibiotics determines the topicality of searches for alternative antimicrobial agents. Herbal substances that from long ago have been used in folk and conventional medicine look epsecially promising in this respect due to a number of advantages, including their antimicrobial and antioxidant activity and availability of a broad spectrum of biologically active substances, micro- and macroelements.

We have analysed the antimicrobial activity of 20 ethyl and methyl extracts from 10 medicinal plants (*Hypericum perforatum* L., *Craraegus laevigata* L., *Chamaenerion angustifolium* L. *Scop., Equisetum arvense* L., *Potentilla erecta* L., *Arnica montana* L., *Achillea millefolium* L., *Symphytum officinale* L., *Centaurium erythraea Rafn.,* and *Artemisia absinthium* L.) by inhibition zones indicators determined by agar diffusion method, and minimum inhibitory concentrations with regard to reference and clinical strains of opportunistic pathogenic microorganisms. The analysis of antibacterial activity of extracts of medicinal plants showed the anti-staphylococcal activity of extracts of *Arnica montana* L. inflorescences, *Equisetum arvense L.* shoots, and *Potentilla erecta L.* rhizome. Among the methyl extracts, the antimicrobial activity was shown by extract of *Potentilla erecta* L. rhizome. The study of antibiofilm-forming ability of extracts of *Potentilla erecta L.* rhizome proved the highest effect of its ethyl extract among all extracts under study. Ethyl extract (0.1 %) was proved to reduce the biofilm-forming properties of *S. aureus* by 91.7 % compared with the control. In a number of cases, the activity of antibiofilm-forming properties of methyl extracts was lower than that shown by ethyl extracts.

The results of our studies indicate to the prospects of using a number of medicinal plants as a source of substances with antibiofilm-forming activity that may also be used in combination with substances with antimicrobial activity; whilst other plants that have both antimicrobial and antibiofilm-forming activity may be used independently as preparations with a synergic – antimicrobial and antibiofilm-forming effect.

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ORAL PRESENTATION

ALKALOIDS OF PLANTS *HAPLOPHYLLUM GRIFFITHIANUM* AND *HAPLOPHYLLUM RAMAZISSIMUM*

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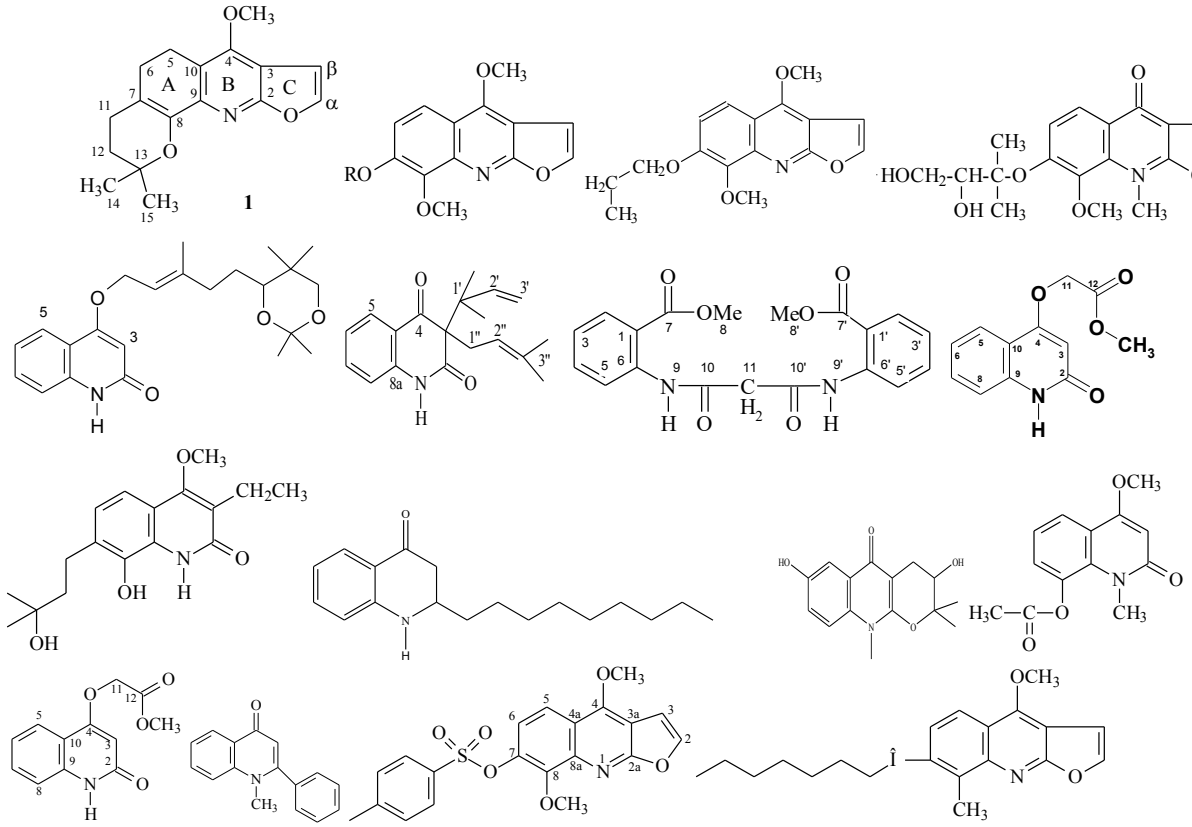
The pharmacological studies of quinoline alkaloids and their derivatives isolated from plants of *Haplophyllum* genus *(*fam. Rutaceae), which are unique source of various alkaloids, showed that they are low toxic and have wide spectrum of pharmacological action. Most of them possess by inhibitory action on CNS, sedative, sleeping, anticonvulsant, estrogen and other effects.

In the last period, alkaloids of 6 plant species of the Rutaceae family have been studied. Alkaloids of the plants *Haplophyllum perfaratum, H.griffithianum, H.ramossimum, H.pedicellatum, H.acutifolium, Ruta graveolens, Dictamnus angustifolus* were studied. The study of 2 new plant species has begun.

The structures of the isolated new alkaloids were established on the basis of the study of spectral datas (UV-, IR-, ^{1}H and ^{13}C-NMR, DEPT-spectra) and X-ray diffraction data from the plants.



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ORAL PRESENTATION

PHYTOCHEMICAL PROFILE AND BIOLOGICAL ACTIVITIES OF *LIGULARIA NARYNENSIS*

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Abstract

Ligularia is a genus of perennial grasses of the Compositae family, which has about 180 species, mainly distributed in Central and East Asia. There are 17 species registered in Kazakhstan. More than 27 species of *Ligularia* have been used as traditional medicinal herbs to treat fever, pain, inflammation and intoxication, as well as to improve blood circulation. In the course of phytochemical studies, it was found that this genus is an important source of sesquiterpenes, many of which exhibit antibacterial and antitumor bioactivity. *Ligularia narynensis* is a perennial herb growing in Almaty region of Kazakhstan and in Xinjiang province of China.
Studies of the chemical composition of the medicinal plant *L. narynensis*, collected in Almaty region, Kazakhstan, were continued. Ethanol, petroleum ether, dichloromethane, ethyl acetate extracts and an aqueous part from the whole *L. narynensis* plant were obtained. The separation of the dichloromethane extract of the medicinal plant *L. narynensis* was carried out by chromatographic methods using various adsorbents (silica gel, AB-8, ODS, Sefadex). The results of TLC and LC-MS studies showed the presence of sesquiterpene lactones, triterpenoids, lignans. One new compound and ten known compounds were isolated from *L. narynensis*. The structure of natural compounds was determined using modern physico-chemical methods (chromatographic methods, MS, NMR spectroscopy) by comparing their spectral data with the literature data. All compounds were isolated from this plant for the first time. The cytotoxicity of all isolated compounds was evaluated.

Key Words: *Ligularia narynensis,* sesquiterpene lactone; triterpenoids; lignan; cytotoxicity.

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

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ORAL PRESENTATION

MICROSCOPIC CHARACTERIZATION AND PROXIMATE ANALYSIS OF *BELOSYNAPSIS VIVIPARA* LEAVES WITH IDENTIFIED ALKALOIDAL COMPOUNDS THROUGH HPTLC

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Abstract

Objectives: *Belosynapsis vivipara* (Dalzell) C.E.C. Fisch. (F: Commelinaceae) is commonly known as spiderwort, distributed throughout Western Ghats regions in India. The plant is rare in scientific platform due to endangered species in India. The aim of the present study was carried out with the microscopic method and SEM analysis for determination of the anatomical arrangement and histological nature of the leaves and various proximate analyses followed by HPTLC analysis for determination of alkalidal constituents present in the said plant leaves.

Methods: Scanning Electron microscopy (SEM) was carried out for confirmation ofmicroscopic characters. Proximate alaysis such as moisture content, ash content, crude fibre content, crude fat content, protein content and carbohydrate content were also determined as per the statdard methods. HPTLC analysis was carried out using ethyl acetate and methanol (9:1) mobile phase at 470 nm.

Results: Microscopic analysis showed the pesence of stomata, non glandular covering trichomes, collapsed parenchymatous cells, fibers, sclereids, perisperm cells and all these parts were confirmed with SEM analysis. Further, HPLC analysis showed the presence of betacyanin (betanin) in the ethanol leaves. TLC identification confirmed the Rf of betanin was 0.53.

Conclusions: Powder microscopy and SEM analysis result showed the similar anatomical characters present in the BV plant leaves. Further, HPTLC analysis showed the presence of betanin in the leaves when scanned with 470 nm. The TLC plate was aslo confirmed the presence of betanin with Rf value of 0.53. This study revealed the authentication of the plant and will avoid adulteration with other genus of *Belosynapsis*.

Key Words: *Belosynapsis vivipara*, HPTLC, microscopy, SEM, TLC

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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF THE ENDEMIC *MENTHA LONGIFOLIA* SUBSP. *CYPRICA GROWING IN CYPRUS*

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Abstract

Mentha longifolia subsp. *cyprica* (Heinr. Braun) Harley endemic to Cyprus, traditionally used to treat tonsilities and as an inhalant for headaches. In this study the antioxidant and antimicrobial activity of the essential oil and the extracts of the aerial parts *M. longifolia* subsp. *cyprica* is reported for the first time. The essential oil profile was determined by GC and GC/MS, 26 compounds were identified. The main components were pulegone 71.5%, 1,8-cineole 9.5%, menthone 5.0% and limonene 3.4%. The antioxidant activity of the the extracts was determined by DPPH radical scavenging method and total phenolic content using FCR. According to the DPPH method the methanol extract revealed the highest antoxidant activty with 0.109 mg/ml than ethyl acetate extract with 0.368 mg/ml and *n*-hexane extract with 1.737 mg/ml. The total phenol amounts of the extracts determined by spectrophotometric method using FCR equivalent to gallic acid showed that the highest total phenol content (60.9±0.034 mg gallic acid/g extract) was determined in the methanolic extract and the *n*-hexane extract (10.1±0.023 mg gallic acid/g extract) showed the weakest. The antimicrobial activity of the essential oil and extracts were screened for their antibacterial and anticandidal effects against selected microorganisms. All the samples demonstrated weak to moderate inhibition effects (>2000 to 62.5 µg/mL) against all tested microorganisms compared with standard agents. *E. coli, S. marcescens* and *K. pneumonia* were the most resistant strains against tested plant extract. They were not inhibited by extract at the maximum concentration used in the assay (2000 µg/mL). *Pseudomonas aeruginosa* was the most sensitive strain to tested samples (MIC, 500 to 1000 µg/mL). According to the results, the essential oil and extracts of the *M. longifolia* subsp. *cypriaca* showed better inhibition effects against *Candida* species (MIC, 62,5 to 500 µg/mL). Especially *C. utilis* was inhibited by the essential oil at the concentration of 62,5 µg/mL (MIC).

Key words: *Mentha longifolia*, essential oil, GC-GC/MS, Plant extracts, antimicrobial activity, antioxidant activity

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ORAL PRESENTATION

ANTI-ENZYMATIC ACTIVITIES AND NEUROPROTECTIVE EFFECTS OF CARVACROL AND P-CYMENE IN SH-SY5Y CELLS

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Abstract

Medicinal plants can be useful in treatments of manyl disorders, among them also Alzheimer's disease (AD) and diabetes mellitus [1]. Recent studies highlighted a possible relationship between AD and type II diabetes mellitus (T2DM). In fact, T2DM was recently identified as a possible independent risk factor for AD [2]. Several essential oils rich in carvacrol and/or *p*-cymene have been used for different aims due to the activities shown by these two compounds [3,4]. Nowadays, there are no previous studies on the possible activities of natural substances on both Central Nervous System (CNS) and enzymes involved in diabetes. This research investigated carvacrol and *p*-cymene possible anti-cholinesterase, anti-α-amylase, and neuroprotective effects. The antiacetylcholinesterase and anti-α-amylase activities were evaluated at different concentrations using Ellman and Dineshkumar's assay, respectively. Carvacrol and p-cymene possible effects on CNS was determined in SH-SY5Y neuroblastoma cells. First of all, maximum non-toxic dose was determined using an MTT assay, then the neuroprotective effects were evaluated on H_2O_2-induced stress in SH-SY5Y cells, studying the expression of caspase-3 using Western blotting assays. Carvacrol showed inhibitory activities against both cholinesterases with IC_{50} values of 3.8 µg/mL for acetylcholinesterase and of 32.7 µg/mL for butyrylcholinesterase. The anti-α-amylase activity of carvacrol resulted in an IC_{50} value of 171.2 µg/mL. Moreover, after a pre-treatment with 50µg/mL of carvacrol and *p*-cymene, the expression of caspase-3 was reduced respect to cells treated with H_2O_2 alone. Carvacrol and *p*-cymene showed *in vitro* anti-enzymatic properties and may act as neuroprotective agents against oxidative stress.

Key Words: carvacrol; *p*-cymene; Central Nervous System; amylase; cholinesterases

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ORAL PRESENTATION

THE POST-HARVEST TECHNOLOGY CHALLENGES IN MAPS SECTOR IN ALBANIA

Alban Ibraliu, M. Rupa

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ORAL PRESENTATION

PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY STUDIES ON THE UNDERGROUND PARTS OF *TRACHYSTEMON ORIENTALIS*

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Abstract

Trachystemon orientalis (L.) G. Don, known as "kaldirik, hodan, ıspıt" in Türkiye, is an edible plant that grows in Eastern Bulgaria, Western Caucasus, and Black Sea Region (1). It is used as a food and folk medicine among the people (2,3). This study aimed to perform phytochemical and antioxidant activity studies on the extracts prepared from the underground parts of the plant. The isolation studies were carried out on the chloroform, ethyl acetate, and remaining aqueous subextracts prepared from the methanol extract from the underground parts of the plant. The structures of the compounds isolated were identified by ^{1}H NMR and ^{13}C NMR techniques. FRAP and CUPRAC assays were performed to determine the antioxidant activity on the methanol extract and the chloroform, ethyl acetate, and remaining aqueous fractions prepared from the methanol extract. One phenolic acid (rosmarinic acid) was isolated from the ethyl acetate fraction and one phenolic compound (3-(3,4-dihydroxyphenyl)-2-hydroxypropanoic acid (danshensu)) and two arylnaphthalene lignans (globoidnan B and rabdosiin) from the remaining aqueous fraction. These compounds were isolated from the underground parts of *T. orientalis* for the first time. The ethyl acetate fraction showed the highest activity in the FRAP and CUPRAC tests (794.818±8.999, 583.06±5.882 µM trolox equivalents (TE)/g), respectively. The rosmarinic acid may be responsible for this activity.

Key Words: *Trachystemon orientalis*, Boraginaceae, phytochemistry, isolation, antioxidant activity

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EVALUATION POTENTIAL ANTIHYPERGLYCEMIC EFFECTS OF FERULA MERVYNII AND FERULA ORIENTALIS ROOT EXTRACTS

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Abstract

Numerous *Ferula* species have been traditionally used for therapeutic purposes in alleviating diabetic problems (1,2). In this study, in vivo antihyperglycemic effects of root extracts prepared from *Ferula orientalis* (FOE) and *Ferula mervynii* (FME), an endemic species for Türkiye, and ferutinin (FT), the main compound of Ferula species, were investigated. In vivo antihyperglycemic effect was evaluated in male Balb/c mice by using oral glucose tolerance test (OGTT) and streptozotocin (STZ)-induced type 1 diabetes model. In the OGTT method; FOE (200 mg/kg), FME (200 mg/kg), FT (100 mg/kg), glibenclamide (GLB, 5 mg/kg) or vehicle (10% DMSO) was administered orally 30 min prior to glucose solution (2 g/kg) and blood glucose levels were measured at different time intervals. To induce diabetes, STZ (50 mg/kg; i.p) was administered to mice for five consecutive days and FOE (200 mg/kg), FME (200 mg/kg), FT (100 mg/kg), GLB (5 mg/kg) or vehicle (10% DMSO) was orally given for 14 days. Fasting blood glucose levels of the mice were measured on the 8th and 15th day of the experiment. Serum insulin level and caspase-3 expression in pancreatic tissue were detected by ELISA and western blot, respectively. FOE, FME, FT and vehicle treatments did not exhibit antihyperglycemic effects in OGTT and diabetic mice. However, GLB treatment caused a significant decrease in blood glucose levels in both models ($p<0.05$). A decrease in serum insulin levels and an increase in pancreatic caspase-3 expression were detected in diabetic mice ($p<0.05$). Extracts and FT treatments did not alter these parameters. In conclusion, we found that FOE, FME and FT at tested doses did not possses in vivo antihyperglycemic effects.

Key Words: Diabetes, *Ferula mervynii*, *Ferula orientalis*, ferutinin, in vivo, streptozotocin

Acknowledgements
This study supported by the grants from the Scientifc Research Project Coordination Unit of Karadeniz Technical University (Project No. THD-2022-9970)

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ORAL PRESENTATION

ANALYSIS OF INTERACTION KINETICS OF ASCORBIC ACID: BSA COMPLEX AND ANTIGLYCATION POTENTIAL

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Abstract

Antioxidants are compounds that can neutralize harmful molecules called free radicals, which can contribute to the glycation process. Vitamins can help protect cells and other structures in the body from damage caused by free radicals. Vitamin C is a powerful antioxidant that can help protect the body from glycation. In the present study, interaction kinetics of ascorbic acid (AA)-BSA complez and non-enzymatic glycation system of serum albumin and glucose for day 28 at 37 °C has been studied. The glycation process was checked with the measurement of fructosamine content (NBT method), carbonyl content (DNPH method), and total advanced glycated end products (AGEs) spectroscopically. The aggregation of amyloid β-structure was measured with Thioflavin-T assay in glycation-treated BSA. The results indicate that the AA had a very stable nteraction with BSA and also showed suppression of generated early-stage, Amadori products (35.01%), carbonyl content (20.07%), and late-stage, advanced glycation end-products (24.55) in the glycation system. The glycation-induced aggregation in presence of AA was prevented by 23.91% in the glucose-mediated glycation system. Therefore, these findings suggest that AA can be used as a therapeutic drug for antiglycation as well as anti-aggregation.

Key Words: Advanced glycation end-products, Aggregation. Ascorbic acid, Glycation

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ORAL PRESENTATION

MEDICINAL, ECOLOGICAL AND ECONOMIC POTENTIAL OF MAJOR HALOPHYTES

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Abstract

The increase in soil salinity and drought are the most important problems worldwide, especially for agricultural production. As the problems of salinization are increasing day by day, both the evaluation of these areas and their economic importance and potential for use increase the importance of salt tolerant plants. Halophytes, which are salt-tolerant species, provide a good alternative as a crop when compared to the effort and budget for remediation of such areas. Halophytes which survive even in salinity conditions higher than 0.5%, have adapted to areas where other plants can not survive and where high salinity conditions prevail. They also have traditional uses as food, medicine, industrial raw material, as a feed and fuel plant. In Turkey, they are consumed as vegetables, especially in coastal regions. In our country, salty areas formed by natural and human influence cover a very large area and are generally accepted as useless barren areas. However, these areas have the potential to be used in halophyte farming without great effort. The production of halophyte plants as food, animal feed, various medical and any industrial product will also benefit these areas. In this study, taxa that are used worldwide as food, naturally grown in our country, and taxa that have relatives in our country and their potential uses are given.

Key Words: Halophyte, salinization, medicinal, salt tolerant, economical, ecological

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ORAL PRESENTATION

SYNTHESIS, STRUCTURE, AND CATALYTIC ACTIVITY OF A NEW ZINC-CATECHIN COMPLEX

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Abstract

The mechanism of action of flavonoids is based on the structural peculiarities of these natural compounds and on the active sites of the site of action. Thus, based on studies in the literature that indicate the actions and indications of flavonoids, the mechanism of action of their complexes with different metals, although still incompletely elucidated and outlined to justify the action of these products, they enjoy popularity and a wide spectrum of uses. The method for obtaining the catechin-zinc complex used catechin and zinc salt in a 1:1 molar ratio. The catechin was dissolved in methanol under continuous stirring, then the zinc salt was added gradually over 30 minutes, then the reaction was refluxed for a period of 3 hours. Various systems were tried to adjust the pH, but the best results were obtained with NaOH 1N (pH=8.5). After removing the solvent by filtration, a yellowish white precipitate was obtained which was washed several times with acetone and dried in an oven. Then it was used for structural confirmation, UV-VIS absorption spectroscopy, morphological analysis and EDAX. The IR spectrum of the zinc-catechin complex was recorded in the range 400-4000 cm^{-1} by the ATR technique. The infrared spectrum of free catechin showed the ν(C=O) band shifted from 1657 cm^{-1} to 1598 cm^{-1} upon binding with Zn(II) ion. A new ring was formed in the complex which accentuated the conjugative effect, thus the characteristic IR absorption band ν(C=C) moved from 1619 cm^{-1} to 1509 cm−1. The vibrations at 634 cm^{-1} observed in the complex implied the presence of the O-Zn bond, which was not present in free catechin. FTIR method confirmed the structure of the synthesized complex. The antioxidant potential of the obtained complex was better than the activity of Zn, but lower than catechin.

Key Words: catechin-zinc complex, structural confirmation, FTIR, antioxidant effect

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ORAL PRESENTATION

BIO-INSPIRED ELECTRODE FABRICATION FOR HIGH PERFORMANCE ENERGY STORAGE SYSTEMS

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Abstract

Increasing energy consumption and worldwide environmental problems lead to high interest in renewable energy resources. Recently, energy storage and conversion systems such as batteries, supercapacitors, photovoltaic devices, fuel cells, etc., are at the forefront of technological progress. For this perspective, researchers have focused on the fabrication of novel electrodes and electrolyte systems. Lately, bio-inspired energy storage devices gain immense attention due to their biocompatibility, abundant resources, low cost, availability and low toxicity [1-2].

In the present work, the electrode materials composed of biological components were prepared for energy storage devices. Amino acids such as histidine and L-proline were used to fabricate electrode materials. Hydrothermal synthesis was performed to obtain two different active electrode materials composed of histidine and L-proline, respectively. The other components of active electrode materials was chosen in carbon-derived samples and conducting polymers. The prepared electrode was investigated electrochemically by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and galvanostatic charge-discharge (GCD) techniques [2]. The characterization of electrode materials was performed diffuse reflectance infrared Fourier transform Spectroscopy (DRIFT) and atomic force microscopy (AFM) techniques.

Key Words: Bio-insoired supercapacitor, wearable, energy storage, L-proline, histidine, carbonaceous material.

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ORAL PRESENTATION

AMINO ACID DERIVED ELECTROSPUNNED ELECTRODES FOR SUPERCAPACITOR APPLICATIONS

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Abstract

Portable, wearable energy storage devices with high safety and unlimited cycle life have gained increasing attention for the development of next-generation personal electronics. The application areas of these systems range from implantable medical devices to electrochemical sensors, from solar energy conversion to stimulators and actuators. One of the most important types of energy storage systems is supercapacitors. Their main advantages of them are high power–energy densities, robust cycle life, and rapid charging capabilities. Their scalable dimensions make them attractive for a variety of applications in biological systems such as electronic skins, capsule endoscopies, deep brain stimulators, cardiac pacemakers, and transcorneal electrical stimulators, etc. [1-3]

In this study, to prepare a biocompatible supercapacitor, histidine amino acid-functionalized graphene oxide and polyaniline ternary nanocomposites will be used as the active material of the electrode. And the electrospinning technique was carried out to coating of the current collector surface. The ink was prepared in DMF as the solvent. The fiber morphology and chemical characterization of electrospunned film was investigated by atomic force microscopy (AFM) and Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) techniques, respectively.

Electrochemical behavior of the prepared films was studied by the electrochemical workstation. Cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS) techniques were applied for the evaluation of electrochemical performance. The electrode performance was evaluated by a two-electrode system, while the supercapacitor prototype was investigated by three-electrode system.

Key Words: Biosupercapacitor, biocompatibility, energy storage, wearable electronics

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ORAL PRESENTATION

DEVELOPMENTAL TOXICITY AND *IN VIVO* GENOTOXICITY OF *SALVIA SUFFRUTICOSA* METHANOL EXTRACTS

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Abstract

Salvia suffruticosa Montbret & Aucher ex Benth. is a member of Lamiaceae family and widely used for nutrition/ traditional medicine all around the world. It has been used to treat asthma, bronchitis, cough, depression, inflammations and skin diseases since ancient times. It is known that secondary metabolites obtained from *Salvia* species have many activities such as anti-inflammatory, hepatotoxic, cytotoxic/antitumor, anticancer, antioxidant. According to the previous findings, main active molecules of *S. suffruticosa* were found as camphor, camphene and 1,8-cineol. The toxicity analyses of every medicinal plant should be evaluated to assure their safety profiles. Although *in vitro* antioxidant, antibacterial, cytotoxic and neurobiological effects of *S. suffruticosa* were studied before there isn't any study showing the *in vivo* toxicologic effects in literature. In this study the methanol extracts of *S. suffruticosa* (50 mg/L) were prepared and *in vivo* developmental toxicity and genotoxicity analyses were performed by somatic mutation and recombination test (SMART), *in vivo* DPPH test and *in vivo* developmental toxicology test using *Drosophila melanogaster* as a model organism. The results showed that the plant extract caused similar spot frequencies like negative control ($p>0.05$) and the coadministration of the extract with H_2O_2 (6,5 µg/mL) was able to protect organism from the genotoxic effects of H_2O_2. *In vivo* DPPH and developmental toxicology tests also showed that *S. suffruticosa* methanol extract was able to protect the healthy organism's antioxidant capacity and didn't affect the puparation, survival and eclosion rates of *D. melanogaster*. To sum up, *S. suffruticosa* methanol extracts were found as antigenotoxic/ non-toxic in this study and should be further evaluated for its potential for the development of new drugs in future.

Key Words: *Salvia suffriticosa*, *In vivo*, Toxicity, Genotoxicity, *Drosophila melanogaster*

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ORAL PRESENTATION

DISTRIBUTION OF SEABUCKTHORN (*Hippophae spp*. Linn) IN NEPAL

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Abstract

The review of herbaria voucher specimens recorded three species of Seabuckthorn in Nepal: *Hippophae salicifolia* D.Don, *H. tibetana* Schlecht and *H. rhamnoides* L. However, the former two species have frequently been collected and reported from the 24 northern mountainous districts. We carried out MaxENT modeling to assess the present and future distribution of these two species. The MaxENT result verified the distribution of species only from 18 districts, resulting in skeptic on its distribution in 24 districts. To appraise the distribution record of the species in country, ground truthing, intensive consultations and field observations are immediate. National level survey of the species' distribution, use and conservation complements the conservation initiatives adopted by the agencies. Precise prediction of the distribution of species using MaxENT, ecological survey and community consultations is useful for decision makers, especially for those whose conservation and management activities range for national level.

Keywords: Seabuckthorn, modelling, mountains, conservation and distribution

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ORAL PRESENTATION

PROPIONIC ACID: PHARMACOLOGICAL PROPERTIES, MOLECULAR MECHANISMS AND HEALTH BENEFITS

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Abstract

Propionic acid, also known as propanoic acid, is a naturally occurring short-chain fatty acid. It can be produced through different methods, including chemical synthesis and fermentation. In fermentation processes, certain bacteria produce propionic acid as a metabolic byproduct. Chemical properties of propionic acid contribute to its versatility in various applications, including food preservation, pharmaceuticals, agricultural products, and industrial processes. On the other hand, propionic acid possesses various pharmacological properties and exerts its effects through multiple molecular mechanisms, which contribute to its potential health benefits. In this review, there is an overview of pharmacological properties, molecular mechanisms, and associated health benefits of propionic acid. The exact molecular mechanisms underlying propionic acid's pharmacological properties are diverse and context-dependent. They involve interactions with various signaling pathways, receptors, enzymes, and transcription factors, including but not limited to those mentioned earlier such as inhibition of cyclooxygenase (COX), activation of peroxisome proliferator-activated receptor gamma (PPARγ), and modulation of G-protein coupled receptors (GPCRs). While propionic acid shows promising potential and has been investigated in various contexts, it is essential to note that further research is needed to fully understand its molecular mechanisms, optimize its therapeutic applications, and evaluate its safety and efficacy in clinical settings. Taken together, consulting scientific literature and healthcare professionals can provide the most up-to-date and accurate information regarding potential health benefits of propionic acid.

Keywords: Propionic acid, health benefits, biological effects, metabolism, dietary intake

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ORAL PRESENTATION

A STUDY ON THE GUM ISOLATION OPTIMIZATION IN DIFFERENT FENUGREEK GENOTYPES

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Abstract

Gum content is an adhesive, emulsifier, thickener, geller, bulker and stabilizer agent used in food material. This agent has been identified as a food hydrocolloid in the food industry. Fenugreek has more quality gum content compared to other plants as guar, tara and locust bean gums. This plant is an annual plant belonging to the Fabaceae family, and it can be used in different industries such as food, cosmetics, medical or etc. So, this study was conducted to determine gum content variability of the different fenugreek genotypes (18 genotypes, 3 cultivars) depending on the different applications under different growing (irrigated and dryland) conditions. These applications are ordered as temperatures (30, 60, 90 °C), time (1, 3, 5 h), pH (pH=3, pH=10) and flour:water ratio (1:30 and 1:60 g/mL). The gum content of fenugreek genotypes showed high huge variation among the applications and growing conditions. The gum content of fenugreek ranged from 1.44% to 91.43% under different growing conditions. The highest gum content was found from PI 426971 genotype (91.43%) in 90 °C, 5 h, pH 10 and 1:60 flour: water ratio and followed by PI 251640 genotype (87.67%) in 90 °C, 5 h, pH 10 and 1:30 flour: water ratio under irrigated conditions. The highest gum contents were found from PI 660995 (in 90 °C, 5 h, pH 10 and 1:30 flour: water ratio) and PI 426971 (in 90 °C, 5 h, pH 10 and 1:60 flour:water ratio genotypes with 84.14% and 83.02% under dryland conditions, respectively.According to result of the study, the high fenugreek gum content was obtained from high temperature (90 °C), time (5 h) and pH=10 (basic media) under both irrigated and dryland conditions.
In a conclusion, the different applications affected the gum content of different fenugreek genotypes under different growing conditions.

Key Words: Fenugreek, gum content, optimization.

Acknowledgements
This study was part of the Ph.D. thesis of Mahmut Camlica. The authors thank the Scientific and Technological Research Council of Tuürkiye (Project code: 219O465) (TUBITAK) for providing financial support and the United States Department of Agriculture (USDA) for supplying seeds of fenugreek genotypes.

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ORAL PRESENTATION

IMPACT OF ORGANIC AND INORGANIC FERTILIZERS APPLICATION ON THE ANTIBACTERIAL ACTIVITIES OF DILL (*ANETHUM GRAVEOLENS* L.)

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Abstract

Dill (*Anethum graveolens* L.) is an annual aromatic herb that belongs to Apiaceae family. Dill is traditionally used for promoting digestion, and treating stomach ilments and colic. In this study, the effects of different levels of farmyard manure (FM) and ammonium nitrate (AN) applications were investigated on the antibacterial activities of dill. Diferent doses FYM (750, 1000, 1250 and 1500 kg/da) and AN (3, 6, 9 and 12 kg/da) were applied by sowing. The ethanol, n-hexane, and dichloromethane extracts obtained were evaluated for their potential as antibacterial agents against six bacterial strains, including three gram-positive strains (*S. epidermidis, S. aureus, E. faecalis*), three gram-negative strains (*E. coli, P. aeruginosa, K. pneumoniae*), and one fungus (*C. albicans*). The tested minimum inhibitory concentration (MIC) values for the gram-positive and gram-negative strains ranged from 39 µg/ml to 1250 µg/ml. Ethanol and hexane extracts showed significant antibacterial activity against all tested bacterial strains while no inhibitory effect on *S. epidermidis* was observed. The highest antibacterial activity against all tested bacterial species was observed with the 750 kg/da FYM and 6 kg/da AN, 12 kg/da AN applications. In conclusion, dill extracts can be used as antibacterial agent increases food safety.

Key words: Farmyard manure, Ammonium nitrate, *Anethum graveolens*, Antibacterial activity

Acknowledgements
This study is part of a MSc Thesis and it was supported by Scientifc Research Project Fund (2017.10.07.1205), Faculty of Agriculture, Bolu Abant Izzet Baysal University, Türkiye.

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ORAL PRESENTATION

AMBURANA ACREANA DUCKE: SECONDARY METABOLITES AND INHIBITORY POTENTIAL OF BRAZILIAN LEISHMANIASIS

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Abstract
Amburana acreana, popularly known as 'Cumaru-de-cheiro', is native to Brazil and used in folk medicine for the treatment of diseases of the respiratory system. However, information on the chemical composition of the species and its antiparasitic potential are still incipient. The aim of this work was to isolate and identify secondary metabolites obtained from the leaf extract of *A. acreana* and to evaluate their antileishmaniasis activity. Approximately 300 g of leaves were collected in the city of Ji-Paraná – RO, Brazil. The extract (88.3 g) was obtained through cold remaceration with 94% ethanol in 4 cycles of 24h. Then, the extract was resuspended with methanol/water (9:1) and partitioned with hexane, chloroform (CAA) and ethyl acetate solvents. The CAA partition (14.7 g) was maintained on the chromatographic column, using silica gel 60H (Acros, 0.063-0.200 mm) as the stationary phase and, mobile phase, hexane/acetone, in a polarity gradient. Four substances were isolated, which had their structures identified through the analysis of ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (one and two-dimensional), obtained in Bruker spectrometers, models DRX-500, using Tetramethylsilane as an internal reference standard. Through the analysis of the spectrometric data obtained, combined with the comparison with data described in the literature, it was possible to identify the isolated substances such as Coumarin [(**1**), 1,2-benzopyrone)], *p*-hydroxybenzoic acid (**2**), vanillic acid [(**3**), 4-hydroxy-3-methoxybenzoic acid], and Amburoside B [(**4**), 4-*O*-β-D-glucopyranosyl-benzyl vanylate]. Amburoside B was tested as a potential inhibitor of allosteric Superoxide dismutase from Leishmania braziliensis (LbSOD) by thermal change assay (ThermoFluor). It was found that this compound, compared to the negative control, achieved an average offset of -1.0 °C, this means that its interaction with LbSOD prioritizes the unfolded conformations of the protein. Further studies with Amburoside B on LbSOD should be conducted to fully explore its capacity of allosteric modulation and protein disturbance as a new alternative of treatment for Leishmania.

Key Words: Cerejeira, Amburosídeo, Phenolics compounds, Leishmania, Secundary metabolites, Ethnopharmacology.
Acknowledgements: UEFS, CAPES, CNPq e UFG.

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ORAL PRESENTATION

NATURAL COMPOUNDS IN SPECTROPHOTOMETRIC DETERMINATION OF ALUMINIUM

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Despite the use of aluminum in everyday life, medicine and some other industries, its increased amount harms the body, most of all affects the central nervous system. As a result, severe consequences can occur (Alzheimer's disease, Parkinson's syndrome, autism, etc.). Therefore, aluminum metal belongs to the number of "metal poisons", since its toxic dose can lead to both chronic and acute poisoning, which can even result in the death of a living organism.

And therefore, aluminum and its compounds can be considered the object of chemical-toxicological research. To identify the cause of fatal poisoning, research is carried out, consisting of several stages: extraction of a poisonous substance or its metabolite from biological materials; purification of the extracted substance from foreign compounds; proof and determination of the target substance based on various research methods.

In view of this, one of the tasks assigned to us was the determination of aluminum quantitatively in certain objects of poisoning. For chemical-toxicological analysis, the most acceptable is the photometric method. Therefore, we directed further research to the application of the spectrophotometric method for determining Al^{+3} in the biological objects.

In this study, saponin which is a naturally occurring compound that are widely distributed in all cells of legume plants was used to detect the metal.

The essence of the invention lies in the fact that in the method of spectrophotometric determination of Al in its salts' solutions, including its conversion into a complex compound with aluminon and 3rd component - saponin, in an acidic medium, an Al solution with a pH of 1-2 is added 10 times the amount of aluminone, 0.5 ml of a 1% solution of saponin and water up to 10 ml of volume. Spectrophotometric measurement is carried out on a spectrophotometer, λ=490 nm, l=1 cm.

The method for the spectrophotometric determination of Al in the presence of saponin has some advantages. Firstly, the accuracy, sensitivity, and selectivity of the determination of Al^{3+} sharply increase in comparison with known methods due to the use of steroid or triterpene saponins as surfactants. Secondly, it is used as a surfactant - saponin, obtained from plant materials in sufficient quantities in a simple way.

The essence of this technique was presented to the relevant organizations and we obtained the Euroasian patent (036947 (19.01.2021)).

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ORAL PRESENTATION

COMBINED EFFECT OF PROBIOTICS AND TAMARIX GALLICA EXTRACT AGAINST SOME PATHOGENIC STRAINS

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Abstract

Our present work was undertaken to evaluate the antibacterial activity of phenolic extracts of *Tamarix gallica* growing in the region of Tiaret (Algeria) in combination with a probiotic bacterium "*Bifidobacterium breve*" against three pathogenic strains: *Staphylococcus aureus; Pseudomonas aeruginosa and Escherichia coli.* The extracts of *Tamarix gallica* leaves were obtained by three organic solvents; methanol (80%), ethanol (80%) and distilled water. The total polyphenols contents were measured using a Folin-Ciocalteu reagent assay. The methanolic extract gave the highest value compared to the other solvents (105 ± 0.72 mg GAE/g) and showed inhibitory effects against all the tested clinical isolates with DZI values of 20, 16.5 and 16 mm for *S. aureus*, *E. coli* and *P. aeruginosa* respectively. However, the obtained results revealed that *B. breve* produced inhibitory substances capable of inhibiting the growth *of* these bacterial strains. The combined effect between *T. gallica* extract and *B. breve* supernatant inhibited all the pathogenic germs tested with a DZI ranging from 23 to 40 mm for the most sensitive strain *S. aureus* followed by 14 to 25 mm for *P. aeruginosa* and 10 to 18 mm for *E.coli*. As a conclusion, the combination between the extract of the leaves of *T.gallica* and *B. breve* strain gave highest synergistic effect in comparison with the activity of the extract (*T. gallica*) alone and the probiotic (*B. Breve*) alone.

Key words: Extracts, *Tamarix gallica*, Antibacterial activity, *Bifidobacterium breve*, Combined effect

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

DIVERSITY OF SOME ORCHIDS SPECIES IN ALGERIA EVIDENCED BY MORPHOMETRIC AND ECOLOGIAL DATA.

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Abstract

The Orchidaceae are the largest family of monocots flowering plants, distributed worldwide mostly in tropical regions. The Mediterranean basin is an important center of diversification of the two subfamilies Orchidoideae and Epidendroideae which representing key groups within Asparagales order. Tubers, rhizoma and leaves were used in herbal medicine and, recently, its peculiar bioactive compounds are involved within huge anticancer potential. These fascinating plants are remarkable with regard to their range of evolutionary and adaptive strategies. In North Africa, the taxonomy of orchids still controversial due to high hybridism and polymorphism of populations expressed at morphological and ecological levels. Conversely, very few studies are devoted to the systematic inventory of the Algerian and North African taxa. Plant material sampled from various bioclimatic sites was subjected to macro-and micro-morphometrically measurement of 20 vegetative and floral characters, and data matrix was subjected to multivariate analyses (Principal component analysis). Anatomical cuts were performed on fixed Leaves, stems and tubercula then micrographs were produced. Results explain significant variability of flower segments correlated with diverse tubercula structures suggesting occurrence of different breeding strategies. Morpho-anatomical analysis revealed distinctive groups structured in a different bioclimatic gradient, suggesting adaptation. Result emphasizes the importance of comparative vegetative and floral morphology and anatomy for the inventory and caracterization of critical populations that conservation most be based on systematic evolutionary approach. Taxonomic and phytogeographical details are provided on several critical endangered species from Algeria.

Key Words: Orchidaceae, systematic, Algeria, morphology, ecology, anatomy.

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

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

THE EFFECT OF SALICYLIC ACID ON SOME GROWTH PARAMETERS OF STRAWBERRIES GROWN UNDER DIFFERENT SHADE CONDITIONS

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In the present study, the effects on the biochemical activities of strawberries were determined by increasing the plant resistance against environmental stresses and by applying salicylic acid to strawberry plants grown by using shading nets with different permeability. The experiment was performed as a pot experiment in the growing period between April and September 2022 with 3 replications and 10 plants in each replication. Different shading conditions (0%, 35%, 55%, and 75%) and doses of salicylic acid (0.0, 0.1, 0.25, 0.5, and 1.0 mM) were applied to Albion neutral-day strawberry (*Fragaria X ananassa* Duch.) cultivar by foliar spraying in the experimental greenhouse. The plants were harvested in two different developmental stages (i.e., the flowering period and the fruiting period). The total phenolic, total flavonoid, chlorophyll, carotenoid contents, and MDA contents were determined in the harvested leaf samples. The lowest phenolic content was obtained from the control application (166.75 mg GAE/g) during the flowering period, and the highest was determined as 252.57 mg GAE/g from the 55% shade application during the fruiting period. The total amount of flavonoids was the lowest (79.95 mg QE/g) in the 35% shade application during the fruiting period, and the highest was detected (186.37 mg QE/g) in the samples taken from the plots where no shade was applied during the flowering period. The lowest total chlorophyll content of 0.35 mg/g TA was obtained from the control application during the flowering period, and the highest was obtained from the 3.59 mg/g TA and 35% shade application during the fruiting period. The lipid peroxidation amount (MDA) was obtained at the lowest level (0.66 nmol/g TA) in the 55% shade application and the highest (2.88 nmol/g TA) was obtained from the plants harvested during the fruiting period from the plot where no shade was applied.

Keywords: shade, strawberry, salicylic acid, leaves, growth parameters

Acknowledgment: We are thankful to the Scientific Research Projects Unit (BAP) of Amasya University for providing support to this research, with the number of FMB-BAP 22-0575 BAP Project. This study is a part of Cavit BURSA's master thesis.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF *GUAVA (PSIDIUM GUAJAVA)* LEAVES AND BARK AGAINST *FUSARIUM OXYSPORUM*: A TOMATO WILT PATHOGEN

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Abstract

The emergence of antifungal resistance is a major attribute among plant pathogens. Therefore, bio fungicides are mostly used to control fungal plant diseases because of their eco-friendly nature and cost-effectiveness. This study aimed to assess the antifungal activities of crude extracts of leaves and bark of *P. guajava* against *Fusarium oxysporum*. The leaves and bark of the experimental plant were collected from Kucha Woreda, Gofa Zone of Ethiopia. All the collected plant samples were washed properly, dried under shade, and ground to powder. The bioactive components were extracted from the powder using ethanol and acetone. The antifungal activities of the extracts were evaluated using the agar well diffusion method, and the inhibitory zones were recorded in millimeters. The agar diffusion method assessed the plant extracts' minimum inhibitory concentration (MIC) against F. oxysporum. The standard antifungal drug, Mancozeb, was used as a positive control, and the distilled water as a negative control. The bioassay studies of the crude extracts were undertaken at four different concentrations (60, 80, 100, and 120 mg/ml). The results revealed that the crude extracts of ethanol and acetone had antifungal activities against *F. oxysporum* in a concentration- dependent manner. The ethanol extracts of leaves had a high growth inhibitory effect at a concentration of 120 mg/ml with zones of inhibition of 19.16 mm. At the same time, acetone extracts of the bark recorded the lowest growth inhibitory effect at a concentration of 120 mg/ml with zones of inhibition of 16.5 mm. The positive control Mancozeb at a 100µg/ml concentration inhibits fungal mycelium by a mean zone of inhibition of 23.98 mm. The crude extracts of ethanol leaves and bark of P. guajava inhibit *F. oxysporum* growth with the minimum inhibitory concentration of 70mg/ml and 90 mg/ml while, crude extracts of acetone leaves and bark had 80 mg/ml and 100 mg/ml of MIC. Generally, this study proves antifungal activity for P. guajava and provides a scientific basis for their traditional use. Pure chemical compounds and antifungal activity against many fungi should be studied to use as sources and templates for the synthesis of drugs to control fungal pathogens of plants.

Keywords: Antifungal activities, Minimum Inhibitory Concentration, *F. oxysporum*, *Psidium guajava*, Tomato wilt.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

CULTIVATION STUDIES FOR TAURUS SNOWDROP (*GALANTHUS ELWESII* HOOK.)

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Abstract

Galanthus elwesii Hook is one of the most important species of Amaryllidaceae family, from which bulb plants are exported. The present study design was based on *G.elwesii,* which is an important export product and was conducted in Suluova district of the city of Amasya in the growing season of 2022-2023 to cultivate its species. The study was established according to the Random Blocks Trial Design with four replications. The number of emerging plants (number), flowering rate (%), leaf length (cm), number of leaves (number), number of fruits (number), fruit setting rate (%), bulb diameter (cm), bulb weight (g), number of bulbs (number/plant), ratio of young bulbs (pieces/plant), bulb yield in the plot (g), bulb yield per decare (kg/da), phenolic compounds, and total phenolic and flavonoid substances were investigated. The total phenolic substance content, total flavonoid substance amount, and the phenolic components of *G.elwesii* were determined with the Folin-Ciocalteu, aluminum chloride, and HPLC methods, respectively. The number of emerging plants was found to be 299.75 ± 61.62, flowering rate 53.12% ± 17.80, leaf length 9.45 ± 0.23 cm, number of leaves 2.01 ± 0.10, number of fruits 55.5 ± 24.28, fruit setting rate 35.08% ± 10.56, bulb diameter 1.63 ± 0.07 cm, bulb weight 3.30 ± 0.39 g, number of bulbs 159.87 ± 87.27, new bulbs ratio 1 unit/plant, and bulb yield per plot was found to be 285.98 ± 22.68 g and bulb yield per decare 1.900 kg/da. The major component of the snowdrop extract was determined as Gallic acid in the study (4.9 ppm/1mg extract). Total phenolic substance and total flavonoid substance contents were lowest at the beginning of the flowering period and at the root (0.0099 mg GAE/g, 0.0002 mg QE/g, respectively). The highest amount of total flavonoid substance was in the flower at the beginning of the flowering period (0.005 mg QE/g), and the total amount of phenolic substance was found in the bulbs (0.06214 mg GAE/g) in the post-flowering period. Because of the fact that the export of snowdrops through nature collection reached high levels in our country, it was made compulsory to cultivate this valuable species to protect it.

Keywords: Plant development period, Kaempferol, Snowdrop, Quercetin

Acknowledgement: We are thankful to Scientific Research Projects Unit (BAP) of Amasya University for providing support to this research, with the number of FMB-BAP 22-0574 BAP Project.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

THE SYNTHESIS AND SPECTRAL CHARACTERIZATION OF NOVEL BIOACTIVE QUINONE COMPOUNDS

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Abstract

Quinone molecules are chemical compounds which they are commonly present in nature and also named as endogenous compounds of biological importance in humans. Quinones and their analogues, including 1,4-naphtho-quinone, azanaphthoquinone, anthraquinone, and their like, use many oxidative agents, regioselective reactions, and novel catalytic synthesis pathways [1]. Quinones are used in many drugs for the purpose of clinical cancer treatment, such as anthracyclines [2]. In this context, at the moment antitumor quinones have big significance on many researches with a wide range of synthetic reactions and antitumor activity evaluations.

Quinones administer their cytotoxic/antitumor potential in the mechanisms which can be explained by two chemical properties: first one is the redox cycle of quinine molecule by consecutive reduction and reoxidation which results in occurence of reactive oxygen species (ROS) and free radicals, and the second one is electrophilic arylation of critical cellular nucleophiles.

The aim of this study, heteroatom substituted naphthoquinones were synthesized according to Michael addition mechanism. The structures of these quinone products were purified through column chromatography method. Their structures were characterized by using various spectroscopic techniques such as microanalysis, FT-IR, ^{1}H NMR, ^{13}C NMR and MS.

Key Words: Quinone; Bioactive compounds; Organic Synthesis; Spectroscopy

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

OPTIMIZATION OF QUALIFIED NURSERY TREE PRODUCTION AND FERTILIZATION RELATIONSHIPS IN MASTIC TREE

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Abstract

Mastic tree (*Pistacia lentiscus*) is found in flora of Turkey, besides the other countries having coast on the Mediterranean. Alhough, the variety *P. lentiscus* var. *chia* from which the gum mastic is being economically obtained, has been grown only in Chios Island of Greece. It is a known fact that, mastic tree had also been grown in Çeşme Peninsula in the past, where the ecologoical conditions are identical. In recent years some studies conducted by related organizations aiming on to restart the mastic tree growing in proper ecologies of our country have became prominent. In mastic tree, which is a biologically dioecious plant, gum mastic production has been conducted only from male plants. For this reason, only vegetative methods should be used for propagation of this plant. The traditional propagation method of mastic tree depends on planting of cuttings, that prepared from the relatively old shoots, directly to the places where the plantation will be established. In different studies, besides the clonal differences, depending on the application of root promoting auxins and the rooting media used, percent rooting up to 80% was obtained in leafy semi-hardwood cuttings was exposed. Despite some questionable aspects of other propagation methods such as budding/grafting, air layering and micro-propagation, they also have different problems need to be discussed. In mastic tree and relative species, it's a common practice that using some organic and inorganic substrate mixtures together with soil in growing young nursery trees. It is a known fact that the growing media particularly enriched with mineral fertilizers, positively affect the nursery tree quality parameters. From this point of view, to expose the effects of slow releasing fertilizers which became prominant in recent years on nursery tree growth. Slow releasing fertilizers applied to growing medium have been significantly increased the nursery tree quality in many woody plant species. In optimization of qualified nursery tree production, the importance of fertilization at nursery tree level together with proper propagation methods have discussed in this paper.

Key Words: *Pistacia lentiscus*, fertilization, propagation, nursery tree

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ORAL PRESENTATION

TRAGOPOGON DSHIMILENSIS K. KOCH EXTRACT INDUCES WOUND CLOSURE IN DIABETIC RATS

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Abstract

Wound healing agents aid the body's natural healing process and promote wound closure. Members of the *Tragopogon* L. family are Turkish medicinal plants used to treat wounds. In this study, we explore the time-dependent effect of *T. dshimilensis* K. KOCH extract on wound closure in diabetic wound repair. Using a Soxhlet apparatus, plant specimens were extracted with methanol. Using a rotary evaporator, the methanol solvent was evaporated after extraction. There were 36 Wistar rats used in the experiments. Animals were separated into three major groups: non-diabetic (NDM), diabetic (DM), and *T. dshimilensis* (ETD). A single intraperitoneal injection of streptozotocin induced diabetes. On all animals, full-thickness dorsal skin excisions were made. In the ETD group, wounds were treated with 50 mg/kg *T. dshimilensis* methanolic extract. There was no treatment administered to the NDM and DM groups. Throughout the process of wound healing, wound areas were photographed and computed. On day seven, the wound areas in the ETD-treated group lowered significantly ($P<0.001$) when compared with the DM group. *T. dshimilensis* extract accelerates wound closure in diabetes-induced rats, and can be used to develop novel medications for diabetic wounds.

Key Words: diabetes, wound closure, wound healing, Tragopogon dshimilensis

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

A COMPARATIVE EVALUATION OF PLANT EXTRACTS' INHIBITING EFFECTES ON PORTED BACTERIA IN PIGS AND SMALL RUMINANTS

Aurel Vasiu[1], Marina Spînu[1,2], Sergiu Zablau[1], Diana Ioana Olah[1], Emoke Pall[1,2], Carmen Dana Şandru[1,2], Anca Secara[1], Gheorghiță Duca[2]

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Abstract

Continuously increasing antimicrobial resistance urges for research in identifying new active compounds agains bacterial pathogens. This research aimed to monitor the potential of traditional medicinal plants in efficient control of antibiotic resistant bacteriome in swine and small ruminants raised on low-input outdoor farms from North Western Romania.
Aerobic bacterial strains (n=14) originating from the nasal cavities of extensively raised swine and sheep 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 aromatogram technique was applied to alcoholic extracts *Calendula officinalis, Saturaja hortensis*, *Coriandrum sativum*, *Artemisia absinthium*, *Cucurbita pepo*, *Allium sativum* and essential oils of *Anethum graveolens, Zingiber officinale, Geranium spp.* and *Lavandula angustifolia*.
The antibiogram indicated a multiple antibiotic resistance (MAR) index > 0.2 in 86% of the pigs and 33% in sheep (overall MAR=0.34 and 0.13, respectively). In pigs the highest average of inhibition diameters was 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); in sheep the amikacin was the most efficient. Out of the plant extracts, *C. pepo* was the most effective in pigs (12 out of 14 strains) while the in sheep, the highest efficacy was recorded for the essential oil of *Geranium spp.* (26.87±8.2 mm) and the lowest activity was noted for the *Anethum graveolens* oil (15.04±2.2 mm).
These plants could enhance the welfare of the animals by reducing the potentially pathogenic, antibiotic resistante bacterial load, as an alternative to classical antibiotic therapy. These results open the perspective of using essential oils in the prevention of staphylococcal infections.

Key words: low input farms, sheep, pigs, plant extracts, antimicrobial resistance

Acknowledgements
The work was supported by grant ERANET Core Organic Co-fund ROAM Free #249 / 2021 and by PPILOW project of the European Union's Horizon 2020 programme, grant agreement 816172.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

PLANT SPECIES' DEPENDANT *IN VITRO* IMMUNE RESPONSE AND CHANGES IN THE PORTED BACTERIOME IN SMALL VERSUS LARGE RUMINANTS

Emoke Pall[1,2], Carmen Dana Şandru[1,2], Marina Spînu[1,2], Gheorghiță Duca[2], Diana Ioana Olah[1], Florina Marian[1], Mariana Rusu[2], Aurel Vasiu[1]

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Abstract

Low-input farms, where cohabitation of various species is frequent, ensure welfare by providing outdoor free-roaming and also offering the opportunity for expression of physiological behaviour of the animals. The study aimed at investigating the bacteriome and the immune potential of cohabitants (bovine and sheep) from a low input farm. The research was carried out on nasal swabs and blood samples from bovine (n=7), and sheep (n=17) cohabiting on the same low-input farm. The swabs were subjected to clasical bacteriology techniques and bichemical identification by API tests (Bio Merieux France). To monitor the *in vitro* blast transformation capacity of lymphocytes, aliquots of blood were mixed 1:4 with RPMI1640 (Sigma Aldrich, USA), further 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 37ºC for 72 h. The glucose residue was quantified by spectrophotometry (SUMAL PE2, Karl Zeiss, Jena) and blastogenic indices (SI%) were calculated. The groups were compared by Student's t test for statistical significance of the results. *P. aeruginosa, A. hydophila/caviae, E. cloacae, Pasteurella pneumotropica/M. haemolytica, Sphingomonas paucimobilis* were isolated from bovine with an average MAR index of 0.288, while *P. oryzihabitans, P. aeruginosa, P. fluorescens/P. putida, E. cloacae, Shigella spp.* and *Ewingella americana* with a MAR index of 0.37 were isolated from sheep.

The spontaneous SI was higher in cows (55.7± 10.3%) while PHA induced SI was higher in bovine (53.2±9.21%) and sheep (37.81±5.08%). The *Symphytum officinale* extract did not influence the SI in either bovine or sheep.

Considering the similar influential factors acting on cohabiting animals on a low-input farm, there was a species-specific immune system-controlled MAR resistance of the bacteriome, which the *Symphytum officinale* extract could not influence.

Key words: bovine, sheep, bacteriome, *in vitro* cellular response, MAR index, *Symphytum officinale*

Acknowledgements
The work was supported by grant ERANET Core Organic Co-fund ROAM Free #249/2021

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION

IN VITRO ANTIDIABETIC AND ANTICHOLINESTERASE ACTIVITIES OF VARIOUS PARTS OF SOME *SALVIA* SPECIES GROWN IN TURKEY

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Abstract

Salvia species make up approximately one-quarter of the Lamiaceae family, with over 900 species worldwide, and are highly diverse. Some *Salvia* species are popular as aromatic herbs, essential oils, medicinal plants, ornamental plants, or as a source of food due to their good flavors [1-3]. It was herein examined *in vitro* antidiabetic and anticholinesterase activities of 21 extracts prepared with methanol obtained from roots, flowers and aerial parts of 7 *Salvia* species (*Salvia verticillata* L., *Salvia huberi* Hedge, *Salvia hydrangea* DC. ex Benth., *Salvia staminea* Montbret & Aucher ex Benth., *Salvia syriaca* L., *Salvia aethiopis* L., and *Salvia sclarea* L.) growing in Turkey. The antidiabetic activities of extracts were evaluated against both α-glucosidase and α-amylase enzymes. Anticholinesterase activity of the extracts was tested against both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using a microplate-reader assay based on the Ellman method. The roots extracts had noticeable inhibition against α-glucosidase. The *S. hydrangea* root extract showed the best inhibitory activity against α-glucosidase (79.92 %) when compared with acarbose (74.72 %) at 5 mg/mL concentration. Afterward, root extracts of *S. aethiopis* (63.82 %), *S. huberi* (59.51 %), and *S. staminea* (58.07 %) displayed potential inhibitory activity, respectively. In α-amylase inhibitory assay, the root extract of *S. sclarea* (42.83 %) just showed potential inhibitor activity when compared with acarbose (67.87 %) at 5 mg/mL concentration. Most of the extracts had no activity against AChE at 5 µg/mL, and BChE at 500 µg/mL, when compared with donepezil (97.36 %, and 97.96 %, respectively). However, further studies are needed to determine the active compounds responsible for these activities and to evaluate their potential for use in the treatment of diabetes, Alzheimer's disease, and other related diseases.

Key Words: *Salvia* sp., Lamiaceae, enzyme inhibition, antidiabetic, anticholinesterase

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ORAL PRESENTATION

PHENOLIC PROFILE, ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF ETHANOLIC EXTRACTS OF *FERULAGO HUMILIS*

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Abstract

Ferulago genus (Apiaceae) is represented by about 50 species in the world, 35 of which are naturally distributed in Türkiye. Various studies have been carried out on the biological activities of many species belonging to this genus until today. *Ferulago* species have been used since ancient times as a digestive, carminative and analgesic in the treatment of various gastrointestinal disorders and haemorrhoids. According to the literature survey, Ferulago species are used in our country for bronchitis, various skin diseases and depression, to increase body resistance and as an immune enhancement. *F. humilis* is an endemic species naturally distributed in Türkiye. In our study, the phenolic contents, antioxidant capacities and cytotoxic effects of the extracts obtained from the *F. humilis* species by two different techniques were evaluated. In line with the data obtained at the end of the study, 11 phenolic components in the extracts were determined, and it was determined that the extracts had the antioxidant capacity and showed dose- and time-dependent cytotoxic effects in the HL60 cell line. After this preliminary screening, it is aimed to carry out further studies to determine the compounds responsible for the activity and to experimentally confirm the observed activity.

Key Words: Endemic, Kılkuyruk, Medicinal plants.

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ORAL PRESENTATION

ITS SEQUENCE ANALYSIS IN *CARTHAMUS TINCTORIUS* GENOTYPES

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Abstract

Carthamus L. (Asteraceae) is represented by about 20 species in the Eastern Mediterranean and Irano-Turanian regions. The origin center of the genus is considered to be the eastern part of the Mediterranean region. *Carthamus tinctorius* L. ($2n = 2x = 24$), commonly known as safflower, is one of humanity's oldest crops. The species is economically important as it is used by the public as a natural food colourant as well as for its medicinal properties. In this study, the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA (nrDNA) were analyzed in twenty-nine safflower genotypes. The parsimony and bayesian analyzes were performed using PAUP and MrBayes programs, respectively. As a result, it was determined that there are no differences were found in safflower genotypes in terms of ITS sequences. This is consistent with the occurrence of a population genetic bottleneck during domestication, as reported in the literature. The findings of this study will be beneficial for *Carthamus tinctorius* in breeding programs in Türkiye as well as in other countries of the world.

Key Words: Asteraceae, Safflower, Türkiye.

Acknowledgements
We would like to thank TUBITAK (Project No: 117Z222) and S.U. BAP (Project No: 19401172) for their financial support.

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ORAL PRESENTATION

ISOLATION OF ENDOPHYTIC BACTERIA FROM PINTO PEANUT (*Arachis pintoi*) IN CAN THO PROVINCE WITH ANTIBACTERIAL ABILITY ON *Erwinia* spp.

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Abstract

Introduction: The Pinto peanut (*Arachis pintoi*) belongs to the Bean family (*Fabaceae*) native to South America. With good tolerance to drought and waterlogging, pea grass can be grown all year round in Vietnam, but it is best in spring and autumn for the Northern provinces, and rainy season for the Central, Central Highlands, and Southern provinces. Some research the world shows that planting *Arachis pintoi* interspersed with coffee helps to provide nitrogen for the plant and reduces the amount of N_2O released from the soil compared to conventional grass cover (T. J. Rose *et al.,* 2019). Intercropping *Arachis pintoi* in grasslands helps to release 50% of N and P residues in the soil for 130 days in the dry season and 20 days in the rainy season. Therefore, it has created favorable conditions for microorganisms to dissolve P in the soil to develop and restore degraded grasslands (C. A. Oliveira *et al.,* 2002). *Arachis pintoi* is considered to be a plant with high botanical resistance. The antagonistic potential of *Arachis pintoi* has been studied for the germination and development of tomato (*Solanum lycopersicum*) and pepper (*Piper nigrum*) seeds (F. Monteles *et al.,* 2011). The results also showed that the extract from *Arachis pintoi* inhibited the germination and growth of *Ageratum conyzoides* L., *Comnyza canadensis*, *Echinochloa crushgalli*, and *Echinoloa colonum* (Z. Chaohua *et al.,* 2006). All plants are firmly believed to contain endogenous bacteria (A. Christina *et al.,* 2013). Currently, there are many studies on endophytic bacteria in medicinal plants and plants, endogenous bacteria have the ability to help plants grow well but also have the ability to produce natural antibacterial compounds (G. A. Strobel, 2003), stimulate favors the host plant to produce metabolites (P. R. Hardoim *et al.,* 2008) and is also capable of producing compounds with antiviral properties even including human immunodeficiency virus (A. Christina *et al.,* 2013). Endophytic microorganisms inhabiting the medicinal plants synergistically produce pharmaceutically important metabolites in their host plants (R. Vijayalakshmi *et al.,* 2016). However, no study has been found on endophytic bacteria in Pinto peanut (*Arachis pintoi*) with antibacterial activities. Therefore, the topic of isolating endogenous bacteria in Pinto peanut (*Arachis pintoi*) with antibacterial activity is urgent.

Objective: This study aimed to isolate the endophytic bacteria in Pinto peanut (*Arachis pintoi*), preliminary characterize the isolated endophytes, and test the antibacterial activity of these groups of bacteria.

Materials: Using Root, stem, leaf, and fruit samples of the Pinto peanut (*Arachis pintoi*) in Can Tho province, necessary tools and equipment, and *Erwinia chrysanthemi* 3937 bacteria causing diseases in plants.

Methods: Disinfect the specimen surface according to the method of Xiaomei Yan *et al.*, (2018) with slight modification. Isolation of endophytic bacteria by the method of Celiwe Innocentia Nxumalo *et al.*, (2020). Antibacterial activity was tested by the disc diffusion method according to the method of Bauer (1966).

The principal results: The results showed a total of 33 endophytic bacteria were isolated from Pinto peanut (*Arachis pintoi*). Eight strains showed antibacterial activity against *Erwinia chrysanthemi* 3937. Results after 24 hours of the antibacterial test showed that the average value of the highest sterile ring diameter was 22.7mm (see **Fig. 1**). Based on the recording of 16S rDNA gene sequences, bacterial strain RhC2 belongs to *Burkholderia*, and has the closest relationship to *Burkholderia* sp. AFS000453.

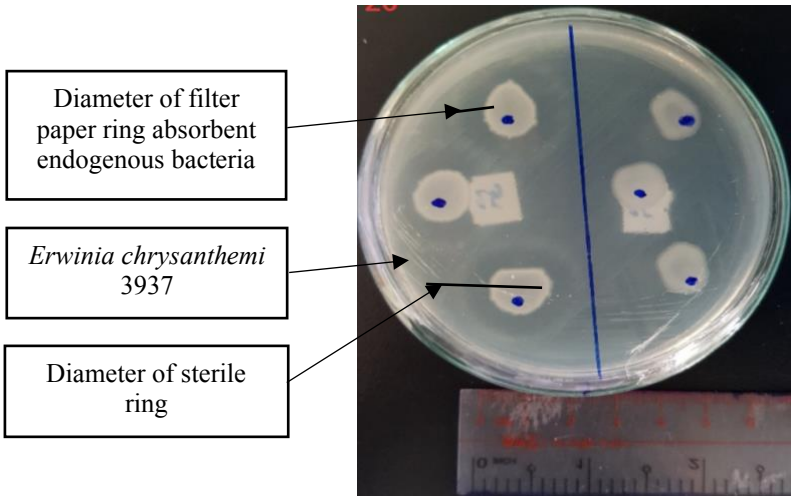

Fig. 1 Sterile ring to investigate the antibacterial activity of endophytic strains of *Arachis pintoi* on *Erwinia chrysanthemi* 3937

Major conclusions: Endogenous bacterial strains isolated from Pinto peanut (*Arachis pintoi*) have antibacterial activity. In the preliminary screening of diverse colonies, different shapes, colors, margins, and textures were observed. Some endogenous bacterial strains showed strong antibacterial activity when tested for antibacterial activity by the agar plate diffusion method. In the future, it is possible to use secondary metabolites from these endogenous bacteria to produce new potential natural herbicides.

Keywords: *Arachis pintoi*, antibacterial activity, endophytic bacteria, isolation, strains.

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ORAL PRESENTATION

ANATOMICAL AND PHYTOCHEMICAL COMPARISON BETWEEN LEAVES OF THE RARE SAPONARIA SICULA RAF. GROWING IN TWO DIFFERENT SICILIAN SITES

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Abstract
The genus *Saponaria* L. (Caryophyllaceae) comprises 42 accepted species [1], mainly distributed in temperate Eurasia [2], and known as valuable medicinal plants. *S. sicula* Raf. is a rare species, that in Sicily grows exclusively on the carbonate stony slopes of Madonie Mountains (SsM), and on volcanic sands of Mt Etna (SsE) (Pignatti 2017).
The aim of the present study was to compare anatomical and phytochemical features of the leaves from the two different populations, for highlighting the influence of the environmental conditions on plant secondary metabolites expression. Anatomical investigation revealed that leaves from SsM had a higher amount of oxalate calcium druses in the mesophyll, and showed a more intense blue-green staining with Toluidine blue O, indicating a higher content of phenolic substances. Phytochemical analyses of the two hydroalcholic extracts confirmed the higher content of total phenols in SsM (8.56 ± 0.57 g of gallic acid equivalents/100 g of dry extract *vs* 6.54 ± 0.16 g GAE/100 g DE). Among them, flavonoids represented the most abundant compounds (6.09 ± 0.17 g of rutin equivalents/100 g DE *vs* 5.31 ± 0.32 g RE/100 g DE). Furthermore, the evalutaion of the content of flavan-3-ols (0.60 ± 0.02 g of catechin equivalents/100 g DE *vs* 0.28 ± 0.08 g CE/100 g DE) and proanthocyanidins (0.93 ± 0.06 g of cyanidin equivalents/100 g DE *vs* 1.13 ± 0.08 g CyE/100 g DE) allowed to calculate the polymerization index of the extracts (0.65 and 0.25 for SsM and SsE, respectively), which highlighted a low amount of polymeric tannins. These findings are in agreement with the antioxidant activity of the two extracts, evaluated by DPPH, TEAC, FRAP and ORAC assays. Indeed, in all the assays, SsM showed the best antioxidant activity (IC_{50} from 2.75 to 477.30 µg/mL) with respect to SsE (IC_{50} 4.04-477.21 µg/mL).

Keywords: leaf anatomy and histochemistry; phytochemical analyses; antioxidant activity.

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ORAL PRESENTATION

TOTAL PHENOLIC CONTENT, ANTIBACTERIAL AND ANTIOXIDANT INVESTIGATIONS OF *CYSTOSEIRA FOENICULACEA* BROWN MACROALGAE COLLECTED FROM THE MEDITERRANEAN (ANTALYA)

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Abstract

Cystoseira foeniculacea macroalgae contains several medicinal traditionally used and pharmacologically explored. However, *C. foeniculacea* has not been well valorized.
So, the aim of the present study was to evaluate the antibacterial and antioxidant activities, total phenolic (TPC) and flavonoid (TFC) contents of *C. foeniculacea* different extracts. To achieve the objectives of this study, ethanol, methanol and aqueous extracts of *C. foeniculacea* were prepared. Then, antibacterial activity was evaluated against gram pozitive bacteria *Staphylococcus aureus* ATTC 43300, *Bacillus cereus* ATTC 11778, *Sarcina lutea* ATTC 9341 and gram negative bacteria *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Klebsiella pneumoniae* ATTC 70603, *Salmonella enteritidis* ATTC 13076 by broth microdilution methods. The antioxidant activity was evaluated by DPPH scavenging assay, ABTS, CUPRAC and metal chelating activity.
TPC and TFC of the extracts were calculated as 14.38±1.22- 37.75±1.05µg GAEs/mg extract and 68.43±0,60 – 30.13±1. 87µg QEs/mg extract, respectively. BHT (Butyryl hydroxy toluene) and BHA (Butyryl hydroxy anisole) and EDTA were used as standard in the total antioxidant activity determination methods we applied to *C. foeniculacea* extracts and the highest ABTS radical scavenging activity in ethanol extract of *C. foeniculacea* (IC_{50}: 37,91±0,73µg/ml), metal chelating activity values of *C. foeniculacea* in ethanol extract (IC_{50}: 138,86±0,71µg/mL) ml). CUPRAC activity in ethanol extract ($A_{0.50}$:150,0±0,12 µg/mL). DPPH scavenging assay did not show any significant activity in any extract of *C.foeniculacea*.
The highest antimicrobial effect against the tested pathogens was seen in the methanol extract of the *C. foeniculacea*, and the most effective strain was determined as gram (-) bacteria Pseudomonas aeruginosa (ATTC 27853) (MIC: 1.562 mg/ml). The water extract did not show antimicrobial activity against any test pathogen.
Therefore, *C. foeniculacea* could be a good source for the identification of antioxidant and antibacterial drugs. In addition, the observed findings could open new horizons on the ethnobotanical usages of *C. foeniculacea*. But, further investigations are required to identify and isolate bioactive compounds from this macroalgae as well the investigations of their biological effects.

Key Words: Antibacterial, antioxidant, macroalgae, Mediterranean.

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ORAL PRESENTATION

BIOACTIVITY-GUIDED FRACTIONATION FOR ENZYME INHIBITORY AND DNA INTERACTION EFFECTS OF *PRIMULA AURICULATA* LAM.

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Abstract

Objective / Purpose: A total of 12 taxa belonging to 8 species of the *Primula* genus are grown in Türkiye [1]. *Primula* species are known to be a source of phenolic compounds, and triterpenoid saponins, which have been shown to have many pharmacological and biological activities, including antioxidant, anti-inflammatory and anti-tumor activities [2]. *Primula auriculata* Lam. is a perennial herb that grows in wet alpine meadows [3]. It is registered that the white powder obtained from the flowers of this species is used in the prevention and treatment of eye infection, Chlamydia infection, and glaucoma in Iran, and it is used in sinusitis, cancer, and as a wound healer in Türkiye [3,4]. Within the scope of the study, it was aimed to determine various biological activities of the aerial parts of *P. auriculata*. **Material and Methods:** The aerial parts of *P. auriculata* were extracted with 80% methanol. The crude methanol extract was dispersed in water and partitioned with *n*-hexane, dichloromethane, and *n*-butanol, respectively to obtain sub-extracts. Active sub-extracts were fractionated by column chromatography. 2,2-diphenyl-1-picrylhydrazyl radical scavenging, acetylcholinesterase, butyrylcholinesterase, tyrosinase, and α-glucosidase inhibitory activities of crude aqueous methanol extract, sub-extracts, and active fractions were investigated using spectrophotometric methods. Furthermore, the DNA-damage protective properties of extracts were investigated using electrophoretic methods. **Results:** The *n*-butanol extract, which was found to have the highest tyrosinase inhibitory effects (62.94 ± 1.40% at 200 µg/mL) among the extracts, demonstrated radical scavenging activity of 82.54 ± 0.77% and 87.23 ± 0.19% at 400 and 500 µg/mL, respectively. In this case, it was determined that the extracts did not damage plasmid pBR322 DNA at the studied concentrations. **Conclusion / Discussion:** This is the first study that reports the enzyme-inhibitory effects of *P. auriculata*. Our data indicated that compounds found in *n*-butanol extract seem to be responsible for the tyrosinase inhibitory effect.

Key Words: *Primula auriculata*, tyrosinase, α-glucosidase, DNA interaction, cholinesterase inhibitory, bioactivity-guided fractionation

Acknowledgements: This study was supported by The Scientific and Technological Research Council of Türkiye, TÜBİTAK (TÜBİTAK 2209-A program) (Project number: 1919B012208826).

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ORAL PRESENTATION

PECULIARITIES OF INTRODUCTION INTO CULTURE IN VITRO SALVIA ROSMARINUS SPENN

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Abstract

Since the earliest time, the humankind has been using essential oils in medicine, perfumery, food industry, etc. At the same time, quantitative and qualitative oil contents in a plant are dependent upon a number of different biological and environmental factors, which is why the ratios of biologically active substances may differ strongly from harvest to harvest.

This is why, plant standardization and introduction of phenotypes with high essential oil contents and determined biochemistry is of big importance. *In vitro* propagation of plants – hyper-producers of biologically active substances – is one of the techniques commonly used to obtain qualitatively and quantitatively stable plant materials.

The purpose of our study was to establish the biochemical composition of rosemary essential oil from the collection of the Botanical Gardens of Uzhhorod National University, study the peculiarities of its introduction into tissue culture, and *in vitro* cultivation with the aim of receiving bipolar explants and callus culture.

The biochemical analysis of the said essential oil showed high contents of the following substances: α-pinene, camphor, cineol and p-cymene, giving grounds to refer this oil to chemotype 4.

For the introduction of rosemary into culture, apical meristem activation method was used. As primary explants, young shoots from five-year-old donor plants were taken. The most preferable were up to 1 cm long fragments with lateral and apical buds. March – May were found to be the best period for introduction of rosemary into tissue culture. It was established that the best sterilizing reagents are 3% solution of sodium hypochloride and 1% solution of silver nitrate. The sterilization time was 10 and 20 minutes, respectively. The microcuttings were preliminarily cleaned of dust and dirt with detergent; a three-time washing with sterile distilled water was mandatory after the sterilization. The aseptic explants were planted on hormone-free Murashige and Skoog agar medium with a 0.7% content of agar-agar. Sucrose (0.3%) and meso-inositol (100 mg/l^{-1}) were added additionally; pH of the medium equalled to 5.7–5.8.

To obtain bipolar regenerant plants and callus, modified Murashige and Skoog mediums were used, supplemented with phyto-hormones of auxin and cytokinin activity type. The test-tubes with the microcuttings were cultivated at light intensity of 3,000–4,000 lx, air temperature 24±2 ºC, air humidity ~70%, and a 16-hour photo-period.

Keywords: rosemary, microclonal propagation, biochemistry, biochemical composition, essential oil.

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ORAL PRESENTATION

INVESTIGATING DIFFERENT COFFEE TYPES ON THE ENZYMES OF BREAD STALING RELEASED BY BAKER'S YEAST

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Abstract

One of the former biochemical activities in the world is the bread making by fermentation of yeast. *Saccharomyces cerevisiae* (or baker's yeast) is the main dough raising agent by providing carbon dioxide in bread and other similar items. Fermentative activity is considered to be important in the bread making since it improves the surface, taste, flavor, structure, and molding of the bakery product carried out by the activity of baker's yeast, providing uniform dough raising with relevance to CO_2 generation. The effect of yeast modification by using different strategies during the bread-making process has gained importance in recent years. Bread staling is a complex period that is composed of aroma loss, changes in mouthfeel, crumb hardening, and crumbliness formation, decreasing consumer acceptance of bakery products caused by changes in crumb, characterized by loss of aroma, changes in mouthfeel, toughening of the crust, firming of the crumb etc. In this study the role of different coffee types(Kenya, Guatemala, Terebinth berry, Cumin and Carob) on baker's yeast activity was studied by adding one gram of them in the standard yeast extract broth growth medium with respect to control. After confluent yeast growth was observed, 10 mL aliquot was taken, centrifuged, microfiltered and different amounts were added to bread dough before baking. and some physicochemical assays and enzyme activity tests were done, confirmed by bread counts. It was found that the protease activity was improved due to Guatemala coffee addition to yeast growth medium.

Key Words: Baker's yeast, Bread, Protease, Enzymes.

Acknowledgements
This study was supported by BAIBU-Scientific Research Projects BAP # 2016.03.01.1066

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ORAL PRESENTATION

TARGTING STAT3 SIGNALING WITH ESSENTIAL OILS: A POTENTIAL STRATEGY FOR ADJUVANT CANCER THERAPY

Muhammed Ashiq Thalappil [1], Elena Butturini [1], Alessandra Carcereri de Prati [1], Ilaria Bettin [1], Lorenzo Antonini [2], Filippo Umberto Sapienza [2], Stefania Garzoli [3], Rino Ragno [2,3], Sofia Mariotto[1]

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Abstract

Natural compounds, such as essential oils (EOs) from aromatic plants, are gaining importance as a potential alternative chemotherapeutic drugs or adjuvant therapy due to their multimodal activities and minimal toxicity to healthy cells. Signal Transducer and Activator of Transcription 3 (STAT3) plays a crucial role in the development and progression of cancer, thereby making it an attractive target for cancer therapy. The objective of this study was to evaluate the anti-STAT3 activity and cytotoxic effects of various EOs using the DU145 human prostate cancer cell line as an *in vitro* model with constitutive STAT3 activation. EOs from *Pinus mugo, Lavandula angustifolia, Pinus sylvestris,* and *Cupressus sempervirens* were found to be highly effective in inhibiting STAT3 activation and inducing cytotoxicity. Importantly, Pinus mugo EO (PMEO) demonstrated a low level of cytotoxicity in non-transformed human fibroblasts, indicating its potential as a specific and effective treatment for cancer cells. The molecular mechanism of PMEO's anti-STAT3 activity was evaluated using spectrophotometric and fluorometric analyses and the consequent biological effects were assessed through western blotting, qRT-PCR, flow cytometry, and wound healing assay. The results indicate that PMEO can rapidly decrease the intracellular levels of glutathione and increase the levels of reactive oxygen species (ROS). Pre-treatment with N-acetyl-cysteine (NAC), a cell-permeable ROS scavenger, blocked the inhibitory effects of PMEO on STAT3. Inhibition of STAT3 activation reduced the expression of proliferative and anti-apoptotic genes at mRNA and protein levels, leading to the inhibition of cell migration and apoptotic cell death. Additionally, a combination treatment revealed that PMEO acts synergistically with cisplatin in inducing cytotoxicity in cancer cells. Taken together, our findings suggest that targeting STAT3 signaling using EOs, specifically PMEO, holds promise as a potential therapeutic strategy and adjuvant therapy in cancer treatment [1].

Key Words: STAT3; apoptosis; essential oil; oxidative stress;Synergism;Adjuvant therapy.

Acknowledgements

We acknowledge the funding from the Italian Ministry for Research and Education (FUR2020MS) and Sapienza University of Rome (RM11916B8876093E, RM120172B8EB30C5, RM12117A89F5B8BB) and the "Centro Piattaforme Tecnologiche" (CPT) of the University of Verona for the Real Time PCR facility.

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POSTER PRESENTATION

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POSTER PRESENTATION

COMPARISON OF ANTIOXIDANT ACTIVITIES OF *ROSMARINUS OFFICINALIS* L. EXTRACTS AND COMMERCIAL PRODUCTS

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Abstract

Objective / Purpose: Rosemary (*Rosmarinus officinalis* L.) from the Lamiaceae family is an important medicinal plant with antimicrobial, antioxidant, antiviral, and immunostimulant effects [1]. Products prepared from rosemary extract are used in European Union member countries and in the United States due to their antioxidant activities [2] As a result of the biological activity studies on phytochemical and antioxidant activity on the rosemary plant in the literature, it was stated that the effect was not caused by a single substance in the plant, but by its extract containing phenolic substances [1,3]. No study was found in which the antioxidant activities of the preparations containing *R. officinalis* extract sold in Türkiye and the extracts with different properties obtained from the plant were compared. In our study, it was aimed to develop the extract with the highest antioxidant capacity from rosemary. **Material and Methods:** Two commercial products containing rosemary extract offered for sale in Türkiye were obtained. Aerial parts of *R. officinalis* were collected from Trabzon and Aydın. Rosemary samples were macerated with 100% methanol, 70% methanol, acetone, ethyl acetate, and 30% ethanol for 4 hours at room temperature, separately for each sample according to the collection area. In addition, it is aimed to prepare extracts with high phenolic content by using acidic hydrolysis and basic hydrolysis methods. At the end of the period, the obtained extracts were filtered and concentrated to dryness in a rotary evaporator at 40˚C. Antioxidant activities of all samples were investigated by in vitro phosphomolybdenum reducing antioxidant capacity (PRAP), ferric-reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. In addition, the total phenol values of the extracts were calculated. **Results:** Antioxidant activities of the prepared extracts were found to be higher than commercial products at DPPH assay. Extracts prepared by acid hydrolysis method from samples collected from both localities showed higher PRAP activity than other extracts and commercial products. **Conclusion / Discussion:** This study is the first study that compares the antioxidant effects of commercial products and rosemary extracts with different properties. Our study has important findings about obtaining products with high antioxidant effects from rosemary.

Key Words: *Rosmarinus officinalis*, rosemary, antioxidant, commercial product

Acknowledgements: This study was supported by The Scientific and Technological Research Council of Türkiye, TÜBİTAK (TÜBİTAK 2209-A program) (Project number: 1919B012204393).

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03-05 May 2023, Ankara-Türkiye
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POSTER PRESENTATION

EFFECT OF EXTRACTION CONDITIONS ON BIOACTIVE COMPOUNDS RECOVERY FROM *CARDOPATIUM CORYMBOSUM* AERIAL PARTS AND FRUITS

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Abstract

The purpose of this study was to assess the effect of extraction parameters including temperature, particle size, and solvent on the yield of polyphenols from *Cardopatium corymbosum* aerial parts. The different parameters except for particle size have shown a prominent effect on phenolic compounds recovery. The highest recovery yield was achieved using hydro-ethanolic maceration.

This paper also sought the establishment of the optimal conditions for hydrodistillation extraction of fruits' essential oil using response surface methodology (RSM) based on central composite design (CCD). The plant: solvent ratio (X_1: 1:7–1:18), condensate flow rate (X_2: 1-2 ml/min), granulometry (X_3: 1.5-2.5 mm) and extraction time (X_4: 30-180 min) were applied as design factors.

For CCD design, the coefficient of determination R^2, and adjusted R^2 of 0.909, 0.819, respectively obtained demonstrate that the model developed can effectively explain the observed data. Only linear terms of X_1, X_3, and X_4 were statistically significant ($P < 0.05$). The predicted optimal essential oil yield was 0.75 % using plant: solvent ratio of 1:9 (m/v), granulometry of 2.5 mm, and an extraction time of 180 min.

Key Words: *Cardopatium corymbosum*, phenolic extract, essential oil, central composite design.

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PRODUCTION OF SOME PHARMACEUTICAL PRODUCTS BASED ON MEDICINAL PLANTS (*MENTHA PULEGIUM* AND *ARTEMISIA*)

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Abstract

Many plants or products derived from them are used in the personal care industry. The use of cosmetics is universal and certainly dates back to very ancient times. To this end, our work is focused on the preparation of some cosmetic products (cream, mouthwash, massage oil, etc.) based on the essential oils of some medicinal plants (*Mentha pulegium* and artemisia). The extraction of essential oils is carried out by hydrodistillation. Separation, quantitative (by volumetric dosing) and qualitative (by TLC) identification of essential oils. Finally, the realization of two galenic preparations based on the essences obtained.

Key Words: Pharmaceuticals, essential oil, medicinal plants, *Mentha pulegium*, *artemisia*.

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POSTER PRESENTATION

EVALUATION OF THE USE AND QUALITY OF OLIVE LEAVES SAMPLES IN DIABETES

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Abstract

Olea europaea L. (Oleaceae) is a fruiting tree with nutritional, therapeutic, and commercial value due to its bioactive components. Leaves of the *O. europea*, is popularly used to lower blood sugar [1,2]. The aim of the study was to investigate the antidiabetic activity, quality, and content of olive leaf samples in variety of forms (plant leaf, ready-made extract, and capsule). Aqueous and ethanolic extracts of the leaf samples were prepared. Pharmacognosic (macroscopic and microscopic analysis, total ash assay, loss on drying), and chromatographic analyzes (thin layer chromatography and high-pressure liquid chromatography), moreover inhibition assays on diabetes-related enzymes (α-amylase, α-glucosidase, and aldose reductase) were performed on those samples.
As a result of the analysis, it was found that the aqueous and ethanol extracts prepared from olive leaf samples contained 190.31-374,295 mg/g oleuropein. The amount of ready-made extract from herbalists contained much lower oleuropein (50.952 mg/g) compared to the olive leaf extracts, which were prepared in the laboratory. All four olive leaf samples had inhibitory effect on α-amylase, α-glucosidase, and aldose reductase enzymes. Although oleuropein content was very low compared to the others, the ready-made extract from herbal also demonstrated inhibition on the diabetes related enzymes. The fact that the supplements that we obtained from the pharmacies exhibited *in vitro* antidiabetic activity with satisfied chemical content suggested that the pharmacists might be more selective in product selection that they sold. As a conclusion, according to the *in vitro* results obtained from the limited samples, olive leaf derivatives can be effective to lower the blood sugar levels, depends on the dose. Further studies are needed with variety of olive leaves and their byproducts necessary to establish reliable content, dose, and effect on the usage of olive leaves in diabetes.

Key Words: Diabetes, HPLC, α-amylase, α-glucosidase, aldose reductase, *Olea europea*

Acknowledgements

We would like to thank Gazi University Scientific Research Projects unit for their financial support (Project code: TEU-2022-7381).

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POSTER PRESENTATION

DESIGN OF SOME BIOACTIVE QUINONE ANALOGUES AS MOLECULAR CANCER AGENTS

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Abstract

Many of the quinones derivatives in nature or sythetically analogues in laboratory are of valuable biological importance. For example, it is named that Daunomycin, a metabolite of Streptomyces peucetius bacteria, is an antibiotic that strongly inhibits the development of experimental tumors and is used for treatment against various animal tumors [1]. 1,4-Naphthoquinone and its derivatives have antibacterial and antitumor properties thanks to their aromatic stability. They form the central chemical structure of many known natural organic substances, especially vitamin K [2]. In the quinone nucleus, the presence of 5-chloro- or 5-hydroxy-substituents or unsaturated it is known from the literature that having a side chain and branching gives antitumor activity [2].

Quinones are also stable because they are highly conjugated and have energy compared to hydroquinones are more balanced. In most cases, they occur in living organisms. Quinones are converted to di-hydroxyphenols or hydroquinones by reduction. The conversion provides a suitable oxidation–reduction system. Quinones have these properties because of the reversible biochemical reduction–oxidation reactions (electron transfer) they play an important role in the living cells [2].

We report that heteroatom substituted-1,4-naphtoquinone derivatives were yielded by the 2,3-dihalo-1,4-naphthoquinone (DHNQ) as starting material with some (R_2-NH-,R-NH_2-) nucleophiles acording to Michael addition mechanism. These reactions mixed were seperated and purificated by column chromatography method. Their chemical structures were determined by various techniques such as FTIR spectroscopy, nuclear magnetic resonance (^{1}H-/^{13}C-NMR), MS, UV-Vis and microanalysis. Morever, their some potential antimicrobial and anticancer activities were investigated.

Key Words: Quinone; Bioactive compounds; Organic Synthesis; Spectroscopy

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POSTER PRESENTATION

CARDENOLIDE GLYCOSIDES FROM ROOTS OF *CYNANCHUM ACUTUM L.* GROWING IN GEORGIA

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Abstract

Cynanchum acutum (L.) is a plant species belonging to Cyanchum genus. This genus comprises of about 200 species in *Asclepiadaceae* family and is distributed worldwide. *C. acutum* is quite poisonous with few medical applications. The alcohol extract of aerial parts of *C. acutum* was reported to have many biological and pharmacological effects.

We have received new data during the study of *C. acutum* of the Georgian flora. Column chromatography was carried out on Diaion HP-20, Sephadex LH-20 and silica gel yielding 3 individual cardenolide glycosides. The full chemical structures were determined by using one- and two-dimensional nuclear magnetic resonance spectroscopy (^{1}H, HSQC, HMBC, COSY) and mass spectrometry. These compounds are the known substances: lanatoside A, lanatoside C and corchorusoside C.

Cardenolide glycosides, the new class observed for the genus cynanchum are definitely interesting.

Key Words: *Cynanchum acutum*; Cardenolide glycosides; Lanatoside A and C, Corchorusoside C

Acknowledgements: We thank to the Shota Rustaveli National Science Foundation of Georgia for financial support (YS-19-168).

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ETHNOBOTANICAL STUDY, PHENOLIC CONTENT, AND *IN VITRO* ASSESSMENT OF UROLITHIC ACTIVITY OF *Petroselinum crispum* (Mill.) EXTRACTS – ALGERIA.

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Abstract

Several studies have reported the traditional medicinal uses of *Petroselinum crispum*, against kidney stone formation. The present work aims to (1) determine the traditional use, and (2) evaluate the phenolic composition of aerial parts extracts of *Petroselinum crispum* and their effects on the dissolution of kidney stones. An ethnobotanical survey was conducted in a remote area of Tlemcen-Algeria using a semi-structured questionnaire between January and April 2023. The polyphenols and flavonoids were extracted by maceration in an ethanol-water mixture (70:30) and evaluated by spectrophotometry. In the dissolution test, an aqueous extract was prepared and then a volume of each extract was added to the kidney stones, after each week the stones were washed, weighed, and then reintroduced again into new extracts.

The ethnobotanical investigation has shown a traditional use of *Petroselinum crispum* as a remedy for renal calculi. Total polyphenols and flavonoid content were 37.34 EAG / g of dry extract, and 35.93 mg EQ / g of dry extract respectively.
On the other hand, the extracts were also effective in dissolving kidney stones after five weeks.

Key Words: Ethnobotanical, Total polyphenols, Flavonoids, Urolithiasis, Kidney stones, Petroselinum crispum.

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MANAGING MEDICINAL AND AROMATIC PLANT RESULTS: A COMPREHENSIVE DATABASE FOR PHARMACEUTICAL AND TOXICOLOGICAL ANALYSIS

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Abstract

Research in medicinal and aromatic science laboratories often involves isolation, identification, and characterization of bioactive compounds from natural sources, particularly those with potential applications in medicine and healthcare. A wide range of analytical techniques, including chromatography, spectroscopy, *in vitro – in vivo* bioassays, and also toxicity tests to investigate the chemical and biological properties with the safeties of these compounds, and to understand how they interact with biological systems at the molecular level.

However, many different species, and different test results creates a great challenge to keep track of results even within one research laboratory.

In this study, we introduce a practical offline database and software program for recording medicinal and aromatic science experimental results in laboratories. The program uses a familiar input file, (Microsoft Excel), so users do not need to learn a new software. By implementing a standardized data recording system and evidence file for each plant, even if the same plant has different synonyms, different parts, and originates from different regions, all data can be collected and accessed through a single platform. This system allows for the integration of experiment results, including chromatograms, spectra, images, etc., and facilitates data management and analysis.

The presented database provides a significant advantage by allowing immediate access to pertinent information regarding laboratory experimentation results during collaborative efforts with diverse research groups and departments. It's always-on data archiving features will prevent any experiment replications and also eliminates the potential loss of time during the data evaluation process.

Key Words: medicinal and aromatic plant science, laboratory results, database, application

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ANATOMICAL AND ULTRASTRUCTURAL INVESTIGATIONS ON *HYPERICUM HETEROPHYLLUM* VENT.

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Abstract

The genus *Hypericum* L. (Hypericaceae, Guttiferae, Clusiaceae) consists of nearly 500 species including trees, shrubs and herbs which grow naturally in different geographic origins of the world from equator zone to Scandinavian countries. Species of the genus have been used as folk remedy in worldwide in treatments of wounds, neuralgia, menstrual disorders, sciatica, arthritis and gastro-intestinal diseases etc. for centuries.

Turkey is an important center for *Hypericum* species which are known as "sarı kantaron, binbirdelik otu, mayasıl otu". The genus is represented by 107 taxa in 20 sections, and 46% of which are endemic, in Turkey. On the contrary of world literature, however, there are only few scientific studies on those species, conducted in our country. *H. heterophyllum* Vent., which is used as herbal medicine and endemic to Turkey, was reported to be the only species under the Sect. *Heterophyllum* Robson.

Hypericum species have glands and secretory canals, which are significant in the characterization of the genus. However, literature survey showed that anatomical properties of *H. heterophyllum* is still lack. As a part of our ongoing studies on *Hypericum* species growing in Turkey, in this study, we aimed to explain anatomical features of root, stem and leaves and ultrastructure of secretory structures of *H. heterophyllum* using light microscopy and scanning electron microscopy.

Key Words: *Hypericum heterophyllum*, endemic, anatomy, ultrastructure.

Acknowledgements
Merve Has would like to thank the Higher Education Institution (YÖK) for providing the opportunity for a PhD scholarship in the field of YÖK 100/2000-Pharmaceutical Studies.

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POSTER PRESENTATION

THE CHEMICAL COMPOSITION OF *JUNIPERUS OXYCEDRUS* AND TRADITIONAL USES OF JUNIPER SPECIES GROWN IN TURKEY

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Objectives: The genus *Juniperus* which belongs to Cuprassaceae family has many native trees and shrubs commonly known as 'juniper'. There are 75 species grown worldwide, 7 of which are grown in Turkey. Mostly, berries and leaves of several Juniper species such as *J. drupacea, J. excelsa, J. foetidissima, J. communis* and *J. oxycedrus* have been used in folk medicine in Turkey. They have been consumed as an antiseptic, diuretic, afrodisiac, analgesic. *J. oxycedrus* was used traditionally in Turkey to treat prostate, menstrual problems, bronchitis, asthma, cold and also used for different skin problems like scabies, eczema, ringworm. The aim of this research is to examine the traditional uses of *Juniperus* species of Turkey and to determine the main chemical constituents of the essential oil which was obtained from *J. oxycedrus* berries.

Methods: Literature survey was conducted by using PubMed, WoS, ScienceDirect and Google Scholar databases to investigate ethnobotanical uses of Turkey's Juniper species. The essential oil of *Juniperus oxycedrus* berries was obtained by hydrodistillation method using Clevenger apparatus. Then, GC-MS analysis was done to identify its main chemical constituents.

Results: The main chemical components which were identified in the essential oil of *J. oxycedrus* berries are α-pinene, myrcene, limonene, D-germacrene.

Conclusion: Junipers are naturally grown plants in Turkey. They have been used in traditional medicine to cure respiratory diseases, prostate problems, menstrual problems, skin diseases. *J. oxycedrus* is one of the *Juniperus* species grown in almost every region of Turkey. The hydrodistillation method was used to produce the plant's essential oil. The chemical constituents were analyzed by using GC-MS. It is found that *J. oxycedrus* is rich in terpenes like α-pinene, myrcene, limonene, D-germacrene.

Key Words: *Juniperus*, *Juniperus oxycedrus*, Juniper essential oil, traditional use, hydrodistillation

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TRADITIONAL USE OF *SIDERITIS TROJANA* AND ITS ANTI-INFLAMMATORY EFFECT

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Objectives: *Sideritis* L., is a member of the Lamiaceae family, represented by approximately 185 species worldwide, represented by 45 species, 34 of which are endemic, in Türkiye. *Sideritis* species are used in folk medicine as herbal teas as nervous system stimulants, carminatives, anti-inflammatory agents, antispasmodics, analgesics, sedatives, and antitussives. *Sideritis trojana* Bornm. is an endemic plant to Kazdağı mountains in Türkiye. According to ethnobotanical data, the plant is used in Türkiye to treat kidney ailments, stomach complaints, abdominal pain, and sore throats. *S. trojana* has been researched for its antioxidant, antimicrobial, antidiabetic, antifungal acetylcholinesterase inhibitory, insecticidal, antigenotoxic activities. Phytochemical profile of the plant revealed the presense of phenolic compounds such as flavonoids and phenylethanoid glycosides as well as essential oil and diterpenes in the previous studies. Consequently, assumed as an excellent source of phenolic compounds with significant antioxidant activity and enzyme inhibitory potential is *S. trojana* and antiinflammatory activity has been investigated in this study.

Method: 25 g herba part of *S. trojana* was measured and added 250 ml ethanol. Yield was measured as 3.74%. COX-1 and COX-2 inhibitory activity of ethanol fraction of *S. trojana* extract was measured by using a Colorimetric COX Inhibitor Screening Assay Kit. By observing the colorimetric appearance of oxidized N,N,N,N-tetramethyl-p-phenediamine at 590 nm, the peroxidase activity is measured. The average COX-1 & 2 inhibition was calculated by taking the mean of COX inhibition activity. Aspirin was used as a positive control. It was calculated the percentage of inhibition after each test was conducted three times.

Results: Overall, it was found that the mean COX-1 and COX-2 inhibition activity of *S. trojana* were 34.33± 0.09% and 40.21 ± 0.39%, respectively.

Conclusion: The study's findings support the traditional uses *S. trojana*. Further studies are advised to prove other traditional uses.

Key Words: *Sideritis, Sideritis trojana,* antiinflammatory activity

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PHYTOCHEMICAL STUDY, NUTRITIVE VALUE AND ANTIOXIDANT ACTIVITY OF PHENOLIC EXTRACTS FROM DESERT PLANT *HALOXYLON ARTICULATUM* BIOSS.

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Abstract

The aim of this study is to the estimation of the nutritive values, phytochemical study and the antioxidant activity of flavonoid extract, tannins and crude extract from the aerial part of *Haloxylon articulatum* Bioss. Which grow in South East of Algeria.

According to the results obtained from the estimation of nutritive value, the plant is rich in carbohydrates and lipids and very poor in proteins. The results also showed a quantitative difference for the phenolic compounds. The total polyphenols were highest content in the crude extract 16.105±0.768 mg GAE/g Extract, it is followed the content of flavonoids by 10.362±0.036 mg QuE/g Extract. Chromatographic analysis by HPLC of crudes extract has identified five phenolic compounds out of 71 peaks as: Chlorogenic acid, Vanilic acid, Vanillin, Rutin and Naringin.

The antioxidant activity was evaluated by three tests (The DPPH radical scavenger assay, the reducing power assay, and the phosphomolybdnum method), the results showed that flavonoid extract had the best scavenging than the other extracts in DPPH radical scavenger assay and the reducing power assay (IC_{50}: 140±0.04 µg/ml, $EC_{0.5}$: 190.5±0.006 µg/ml, respectively). The tannic extract demonstrated the greatest effectiveness in the total antioxidant activity (116.093±0.018 µg EAsA/g Extract).

Key Words: *Haloxylon articulatum* Bioss., Nutritive values, HPLC, Antioxidant activity.

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A TRITERPENE FROM *LAVANDULA STOECHAS* L. LEADS TO SIGNIFICANT ALPHA-GLUCOSIDASE ENZYME INHIBITORY ACTIVITY

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Abstract

The *Lavandula* L. genus is in the Lamiaceae family and includes more than 39 species (1). *Lavandula* essential oils, which have been shown to have sedative, anti-inflammatory, antioxidant, antimicrobial, and insecticidal activity belong to its oxygenated monoterpenes and irregular monoterpenes. It has been reported to be flavonoids, hydroxycinnamic acid derivatives, and triterpenes, the major non-volatile components of the plant. Hydroxybenzoic acids, coumarins, and benzofurans are its other secondary metabolites (1, 2). The use of *Lavandula* species in traditional medicine, treatment for pain, inflammation, rheumatism, gastrointestinal diseases, diabetes, central nervous system disorders, insomnia, epilepsy, cancer, and infectious diseases has been registered (3). Due to ethnobotanically usage of the flowering aerial parts of *L. stoechas* L. subsp. *stoechas* for the treatment of diabetes disease and its blood sugar reducing effect, this study is planned for determine the potential antidiabetic effect of polar extract, fractions and pure substances obtained from the *L. stoechas* L. subsp. *stoechas*.

The aqueous EtOH (70%) extract of the powdered aerial parts of *L. stoechas* L. subsp. *stoechas* was subjected to liquid-liquid extraction with *n*-hexane to remove chlorophyll and other pigments. The remaining aqueous EtOH extract was chromatographed on a polyamide column to yield five main fractions (Fr. A-E). Fr. E with high *α*-glucosidase enzyme inhibitory effect was subjected to silica gel column chromatography to obtain LS-E1. The structure of the isolated compound was identified as ursolic acid comparing with the literature findings and 1D-, 2D- (COSY, HMQC, HMBC) NMR spectra.

According to the results, it was determined that the ursolic acid obtained from Fraction E showed higher *α*-glucosidase inhibitory activity than standard acarbose.

Key Words: *Lavandula stoechas*, Lamiaceae, ursolic acid, *α*-glucosidase

Acknowledgements

This study was supported by Hacettepe University Scientific Research Projects Coordination Unit (TDK-2018-17578).

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POSTER PRESENTATION

CHEMICAL CHARACTERIZATION OF ESSENTIAL OIL COMPONENTS OF *HYPERICUM* L. SPECIES GROWING IN TURKEY

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Abstract

Turkey has the richest flora in the temperate zone with a level of endemism and a unique biodiversity. On the other hand, because of the long history hosted many cultures, Turkey has a rich ethnobotanical cultural heritage. Species of the genus *Hypericum* L., belongs to Hypericaceae (Clusiaceae, Guttiferae), are known as kantaron, sarı kantaron, binbir delik out, yara out, kılıç otu etc. in our country and used to treat a variety of conditions and diseases such as burns, wounds, haemorrhoids, diarrhorea, ulcers and as antidepressant etc. both in the world and in our country.

The genus *Hypericum*, inclusive about 500 species, has spread in various parts of the world. In Turkey, which is an important gene center for the genus, it is represented by 107 taxa in 20 sections, and endemism ratio is 46%. Phytochemical studies indicated that *Hypericum* species are rich in acylphloroglucinol derivatives, naphthodiantrones, flavonoids, xanthones, tannins and essential oils. This study aimed to determine chemical compositions of the essential oils obtained by hydrodistillation from the aerial parts of nine *Hypericum* species, *H. empetrifolium* Willd., *H. heterophyllum* Vent., *H. microcalycinum* Boiss. & Heldr., *H. montbretii* Spach, *H. olympicum* L. f. *olympicum*, *H. origanifolium* var. *origanifolium* Willd., *H. origanifolium* var. *depilatum* (Freyn & Bornm.) N. Robson, *H. perforatum* L. and *H. scabroides* N. Robson & Poulter., which were identified simultaneously by gas chromatography and gas chromatography-mass spectrometry.

Key Words: *Hypericum*, essential oil, GC-GC/MS.

Acknowledgements
This study was supported by Anadolu University Scientific Research Projects Commission under the grant numbers 2204S031 and 2205S057.

Merve Has would like to thank the Higher Education Institution (YÖK) for providing the opportunity for a PhD scholarship in the field of YÖK 100/2000-Pharmaceutical Studies.

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POSTER PRESENTATION

DIPLOTAXIS TENUIFOLIA (L.) DC: AN IMPORTANT HONEYBEE PLANT WITH VARIOUS ECOLOGICAL AND BIOLOGICAL ROLES

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Abstract

Wild Rocket (*Diplotaxis tenuifolia* (L.) DC) (Brassicaceae) is a perennial ruderal plant represented by 5 species in the Flora of Turkey, flowering during spring and autumn. It is widely grown in roadsides and fallow areas, especially in Marmara, Black Sea regions and Central Anatolia. It is used in many parts of the world as human food, animal feed, vegetable oil plant, pasture plant, herbal medicine, landscaping, and beekeeping, which is one of the three commercially cultivated rocket species, which demand and production has recently increased in Mediterranean countries. It is a mustard style plant with pungent, spicy tasting leaves that are grown for salads and herbal garnishes. It is full of antioxidants such as phenolic compounds and glucosinolates which give wild rocket its bitter taste and strong scent that protect cells against free radical damage such as certain cancers, including breast, prostate, lung, and colon cancers. Depending on its long vegetation period, it is an important potential plant in terms of beekeeping due to its nectar and fragrant. Besides its use as a crop, as with a lot of herbs, it attracts bees like no other plant; and can be used as good border plants and recommended as a beneficial insectary plant in habitat management of agro-ecosystems due to its long flowering duration and its attractiveness to pollinators, bees and hoverflies. It can be used in landscaping works at a very low cost as it is drought resistant and can grow in all kinds of habitats and does not require much maintenance. *Diplotaxis tenuifolia*, which has these features, is of great importance to ensure the continuation of the population instead of being considered as a wild plant and being uprooted in order to make room for landscaping around establishments or in empty areas.

Key Words: *Diplotaxis tenuifolia,* honeybee, functional food, antioxidant

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POSTER PRESENTATION

L-PROLINE-SUPPORTED NANOCOMPOSITES IN ELECTRODE FABRICATION FOR HIGH ENERGY STORAGE SYSTEMS

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Abstract

Batteries and supercapacitors are the cutting edges of energy storage technologies. When compared to batteries, supercapacitors have higher power density, long cycle life, and rapid charge-discharge capabilities. Very recently, a new type of supercapacitor attracted intense attention for use in biological systems. For this aim, especially, biological samples such as enzymes, amino acids, bacterial medium, etc. are extensively used as electrolyte material or electrode active material during the fabrication process of supercapacitor devices [1-2].

In the present study, an active electrode material for energy storage devices was synthesized using L-proline-derived nanocomposites on wearable conductive substrates. Hydrothermal synthesis was adopted to prepare the active electrode material composed of L-proline, a multi-walled carbon nanotube, and a conductive polymer. The fabricated electrode was evaluated electrochemically using cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS) techniques [2]. The characterization of electrode materials was performed diffuse reflectance infrared Fourier transform Spectroscopy (DRIFT) and atomic force microscopy (AFM) techniques.

Key Words: Biosupercapacitor, wearable, energy storage, L-proline, multi walled carbon nanotube

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03-05 May 2023, Ankara-Türkiye
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POSTER PRESENTATION

EFFECT OF ELECTROSPINNING PARAMETERS ON ELECTRODE PERFORMANCE FOR ENERGY STORAGE APPLICATIONS

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Abstract

Supercapacitors are one of the most important energy storage systems that allow long cycle life, high energy-power density, and rapid charge-discharge capability. In the last decade, they have received much attention due to their unique application area such as portable, wearable, flexible energy storage units for the next generation of personal electronics. Especially, their eco-friendly, biocompatible, and low-cost alternatives are favored to use in living organisms [1].

In the present work, a supercapacitor prototype was fabricated with amino acid-functionalized graphene oxide (GO) and polyaniline (PANI) nanocomposites to obtain active electrode material for the supercapacitor. The fabrication process was performed by electrospinning technique. The effect of electrospinning parameters such as applied voltage, the distance between the needle and collector, and flow rate on supercapacitor performance were evaluated [2].

Atomic force microscopy (AFM) and Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) techniques were applied for the characterization of active electrode materials. Electrochemical behavior of the electrospunned films was studied using cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS) techniques.

Key Words: Amino acid, supercapacitor, biocompatibility, energy storage

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POSTER PRESENTATION

IN VITRO ASSESSMENT OF NEUROBIOLOGICAL EFFECTS OF COMMERCIAL COCOA POWDER SAMPLES FROM TÜRKİYE*

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Abstract

Cocoa powder is obtained from *Theobroma cacao* L. (Malvaceae), which is often used for flavoring in food industry. It is also well-known to be rich in polyphenols, which are beneficial for human health including neuroprotection. As cholinergic hypothesis proposed for pathology of Alzheimer's disease (AD), cholinergic deficit has been reported due to hydrolysis of acetylcholine by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In this study, inhibitory effect of ethanol extracts of the cocoa powders with different brands purchased from several supermarkets in Ankara (Türkiye) against AChE and BChE closely related to AD was evaluated. Moreover, the impact of the same extracts on the elimination of free radicals, which affect the formation and progression of AD, was studied. The antioxidant effects of the samples were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and metal-chelating activity assays. Total phenolic and total flavonoid contents in the extracts were also determined by Folin-Ciocalteu and aluminium chloride colorimetric methods, respectively. Among our samples, the sample coded as RY12 had a high AChE inhibition activity by 56.46 ± 2.12% (inhibition% ± standard deviation, S.D.) at a final concentration of 200 µg/mL. On the other hand, the sample coded as RY7 had a strong BChE inhibition activity by 41.29 ± 0.09% with a final concentration of 200 µg/mL. DPPH radical scavenging activity, FRAP, and metal-chelating activity experiments revealed that the ethanol extracts had modest antioxidant activity. The total flavonoid content of the samples could not be determined since the concentration of quercetin in all samples except of RY5 was less than 0.016 mg. According to the results of total phenol quantification calculated on the basis of gallic acid in the extracts, where each sample was determined to contain moderate phenol content. The results of the study showed that the ethanol extracts of cocoa powders purchased from the markets and used by the public in daily life have a moderate level of cholinestarase inhibition and have antioxidant activity at some extent that can be evaluated. Further studies are in progress in our laboratory to determine which substances are responsible for antioxidant and antialzheimer activities.

Key Words: Cocoa powder, antioxidant, cholinesterase inhibition, Alzheimer's disease.

*This study was performed as graduation thesis of Rahmancan Yurduseven, who was then 5th year student at Faculty of Pharmacy, Gazi University under supervision of Prof. Dr. Ilkay Erdogan Orhan.

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POSTER PRESENTATION

ASSESSMENT OF THE PREVENTIVE ACTIVITY OF *Pinus brutia* TEN. AGAINST *IN VIVO* ACUTE LUNG INJURY MODEL

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Abstract
Acute lung injury is an inflammation-induced lung complication and progresses into acute respiratory distress syndrome, which has a more severe course and is distinguished by diffuse infiltration. Worldwide, acute lung injury and acute respiratory distress syndrome are common occurrences that are linked to high death and morbidity rates. In Türkiye, *Pinus brutia* Ten. (Pinaceae) is extensively distributed and has a long history of use in treating lung and respiratory illnesses like cough, bronchitis, asthma, and pneumonia. *Pinus* extracts also possesses anti-inflammatory and antioxidant properties in addition to its ethnobotanical uses. In the present work, lung injury was induced by lipopolysaccharide (LPS) in Sprague Dawley rats to examine the prophylactic effect of aqueous-methanol extracts of *P. brutia* leaves and cones. Oral dosages of 100, 200, 400 mg/kg of the extracts and dexamethasone (1.5 mg/kg) were given one hour before LPS injection. The bronchoalveolar lavage fluid was examined for the differential white blood cell counts. The levels of the cytokines interleukin (IL)-6, IL-8, and tumor necrosis factor-alpha were also assessed. In addition, lung tissue histopathological analysis, total antioxidant status, total oxidant status, and peroxiredoxin-1 gene expression analyses were carried out. The findings showed that due to their anti-inflammatory and antioxidant properties, both cone and leaf extracts demonstrated protective effects in acute lung damage. At 200 mg/kg dose of cone extract, the preventive effect is noticeable. The results of High Performance Liquid Chromatography (HPLC) revealed that the concentrations of vanillic acid, catechin hydrate, and taxifolin in both extracts were different. The cone extract had higher concentrations of catechin hydrate (0.12 g/100 g) and vanillic acid (0.02 g/100 g) than the leaf extract (0.04 g/100 g and 0.001 g/100 g, respectively). The quantity of taxifolin was determined to be 0.28 g/100 g in leaf extract and 0.11 g/100 g in cone extract.
Key Words: Acute lung injury, HPLC, inflammation, phenolic compounds, *Pinus brutia*, Pinaceae

Acknowledgements
The present study was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) with the project number of 221S105. We would like to thank TÜBİTAK for the financial support.

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POSTER PRESENTATION

PHENOLIC COMPOUNDS, ANTIOXIDANT, ENZYME INHIBITORY AND ANTIMICROBIAL ACTIVITIES OF *LIMONIUM CASPIUM*

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Abstract

The genus *Limonium* Miller, belonging Plumbaginaceae, consists of 25 halophytic species and 2 varieties in Turkey. The resistance of halophytic plants to salt stress is explained by their physiological properties as well as having a strong antioxidant system. It is also known that the synthesis and accumulation of phenolic substances, which are considered the most important antioxidant compounds in plants, increase due to salt stress.

Considering that the use of natural antioxidants has gained importance in the treatment of skin diseases and obesity in recent years, it was aimed to investigate antioxidant, tyrosinase and lipase enzyme inhibitory activities and antimicrobial properties on skin pathogens as well as phenolic composition of halophytic *L. caspium* (Willd.) Gams. For this purpose, methanol extract and its n-hexane, dichloromethane, ethlyacetate and water fractions were prepared.

LC–MS/MS analysis showed that, tannic acid was the most abundant phenolic acid while hyperoxide was the most abundant flavonoid. Ethyl acetate fraction exhibited the highest total phenolic content, DPPH radical scavenging activity and total antioxidant capacity. The extract and fractions showed moderate growth inhibitions against the Gram-positive and Gram-negative bacteria and fungi tested. Weak pancreatic lipase inhibition was observed with hexane fraction. Hexane and ethyl acetate fractions exhibited highest tyrosinase inhibitory activities.

Key Words: *Limonium caspium*, LC-MS/MS, phenolics, DPPH, TAC, tyrosinase, pancreatic lipase, antimicrobial.

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POSTER PRESENTATION

EVALUATION OF ANTIOXIDANT CAPACITY AND TOTAL PHENOLIC CONTENT OF DIFFERENT *DORYCNIUM SANGUINEUM* EXTRACTS

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Abstract

The genus *Dorycnium* is belonging to the Fabaceae family and represented by 13 species in the world and 7 species in Türkiye. Although there are some studies investigating the biological activities of different Fabaceae species, limited research was on *Dorycnium* species. In this study, it was tried to determine the antioxidant potential and total phenolic contents of the extracts obtained by different methods from *Dorycnium sanguineum*, an endemic species belonging to the genus *Dorycnium*. For this purpose, the antioxidant potential of different extracts prepared from the plant was determined by the DPPH test, and the total phenolic content was determined by the Folin-Ciocalteu assay. In line with the obtained data, it was concluded that the extract obtained with methanol both has antioxidant potential and is richer in terms of total phenolic content.

Key Words: Endemik, Kızıl Kaplanotu, Türkiye.

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POSTER PRESENTATION

SCREENING OF SOME PLANT-DERIVED COMPOUNDS AGAINST ENZYMES ASSOCIATED WITH ALZHEIMER'S DISEASE BY *IN VITRO* AND *IN SILICO* METHODS

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Abstract

Alzheimer's disease (AD) is a progressive disorder that causes the degeneration of cells in the brain, characterized by decreased thinking and independence in personal daily activities. AD, the most common cause of dementia, has become a significant health problem with the increasing elderly population, especially in developed countries. The current number of patients is predicted to double every 5 years and reach up to 152 million by 2050. The disease, a problem with its social and economic dimensions, has risen to 6th place among the causes of death since there is no definitive treatment. In addition to the fact that AD is a multifactorial disease, there are two critical hypotheses about its cause: cholinergic and amyloid hypotheses. Although cholinesterase inhibitors and *N*-methyl-D-aspartate antagonists (NMDA) are used to relieve the symptoms of the disease, they do not treat or prevent it. Therefore, current research focuses on understanding AD's pathology by targeting various mechanisms such as abnormal tau protein metabolism, β-amyloid, inflammatory response, cholinergic and free radical damage, and it is aimed to develop treatments that can stop or change the course of the disease. In addition to synthetic compounds, there is also acetylcholinesterase (AChE) inhibitors of plant origin, such as galantamine, the alkaloid discovered in the bulbs of *Galanthus nivalis* L., which is known as "kardelen" in Turkish. For this reason, herbal sources and their secondary metabolites have an important place in AD research. Recently, it has been revealed that the enzyme protein tyrosine phosphatase 1B (PTP1B) has a regulatory role in different processes in the central nervous system, many of which are related to AD. Increased PTP1B activity is associated with faulty neuronal insulin and leptin signaling pathways impaired in AD. Therefore, research on PTP1B inhibitors to correct disrupted signaling pathways is one of the new treatment approaches for AD. Given this information, we screened the inhibitory effects of some natural compounds against PTP1B, AChE, and butyrylcholinesterase (BChE) enzymes, which are used as targets in the cholinergic hypothesis, using *in vitro* methods. The active ones (quercetin, betulinic acid, maslinic acid, oleanolic acid, and ursolic acid) were examined to detect their interactions with related enzymes at the molecular level by *in silico* experiments.

Key Words: Alzheimer's disease, acetylcholinesterase, butyrylcholinesterase, enzyme inhibition, protein tyrosine phosphatase 1B, *in silico*

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POSTER PRESENTATION

AN ANNOTATED CHECKLIST OF THE ENDEMIC ALLIUM SPECIES IN THE MEDITERRANEAN REGION: TAXONOMIC REMARKS AND EVOLUTIONARY TRENDS.

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Abstract

The genus *Allium* L. is a key group within the petaloid monocots, that taxonomic diversity covers more than 800 spontaneous species predominantly Mediterranean. This genus is remarkable for its rarity and endemism; however, its taxonomy remains controversial, suggesting a complex evolutionary history. Materials from published sources and our own expeditions have been used; informations were provided for all inventoried taxa such as nomenclature, synonyms, distribution, chromosome number and infrageneric position. The results show that endemic species are mostly members of the sections *Codonoprasum* Rchb., *Scorodon* Koch and *Brevispatha* Valsecchi. These species are cryptic, often restricted to coastal cliffs and rupicolous habitats. This fragmented distribution reflects the paleohistory of the Mediterranean flora. Despite the abundance of taxonomical works on these endemics, often diploid and relict species, their evolutionary relationships and origin remain unexplored. Within the framework of conservation of this endemic precarious species, results emphasize the importance of a global analytic approach of accumulated data, while a massive damage of naturel habitat occurs insidiously.

Key Words: Endemism, *Allium*, systematic, Algeria, diversity, evolution.

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

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POSTER PRESENTATION

ANTIMICROBIAL ACTIVITY AND ANTI-BIOFILM EFFECTS OF SOME MEDICINAL PLANT EXTRACTS

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Abstract

Antimicrobial resistance continues to be a significant problem worldwide. Therefore, researchers are trying to solve the problem of resistance by developing antimicrobial agents, antiseptics, and disinfectants with different formulations. In our study, the methanol extracts of the aerial parts and roots of *Echium vulgare* L., *E. plantagineum* L., and *E. orientale* L. species form the family Boraginaceae and the methanol extracts of the leaves and the fruits of *Phytolacca americana* L. (Phytolaccaceae) will be evaluated for their antimicrobial activities (MICs) and anti-biofilm effects using microdilution method and anti-biofilm assay, taking into account the cytotoxic (IC_{50} : 3-8 µg/mL; HCS Cell Line, HSNB cell, NIH-3T3 cell; MTT) doses determined. The effects will be investigated against yeast-like fungi *Candida albicans, C. parapsilosis*, and *C. krusei*, as well as bacterial strains such as *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii* and the results will be evaluated by comparison with controls.

Key Words: pharmaceutical microbiology, antimicrobial, antibiofilm, *Echium*, *Phytolacca,* disinfectant

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INVESTIGATION ON CYTOTOXIC ACTIVITY OF *CENTAUREA DRABIFOLIA* SSP. *FLOCCOSA*

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Abstract

The genus *Centaure* L. is one of the larges genera of Asteraceae family, which represented about 206 taxon in the flora of Turkey, with the endemism rate to 64 % [1, 2]. Many species of the genus have long been used traditionally for their tonic, expectorant, antipyretic, antidiareic, emmenanogue and appetizing effects in Turkey [3]. Various studies have shown that *Centaurea* species have important biological activities such as antimicrobial, anti-inflammatory, antiulcerogenic, cytotoxic and antiprotozoal activities [4]. The main secondary metabolites of *Centaurea* species are represented by triterpenes, flavonoids and lignans, and especially they are also known to have sesquiterpene lactones which has important cytotoxic properties [5].

In the current study, the methanol extract and its petroleum ether, $CHCl_3$, EtOAc and n-BuOH fractions of endemic *Centaurea drabifolia* subsp. *floccosa* (Boiss.) Wagenitz & Greuter were investigated for their cytotoxic activity against LNCap (human androgen dependent prostate cancer cell line) and NIH-3T3 (Swiss mouse embryonic fibroblast; non-cancerous cell line) cell lines by MTT method after 24 h treatment. For the positive control, the cells were treated with the chemotherapeutic drug cisplatin which has been widely used in the treatment of a range of cancers. According to the results; the $CHCl_3$ fraction is most active fraction and it has a significant cytotoxic activity with 15.34±0,76 µg/mL IC_{50} value on LNCap cell lines. All the tested extract and fractions showed lower cytotoxicity on NIH-3T3 cell line than LNCap cell line. It is the first cytotoxic activity study of *Centaurea drabifolia* subsp. *floccosa* against prostate cancer cell line. Our future studies will be hold on isolation and structure elucidation studies to determine, test the compounds responsible for the activity.

Key Words: Centaurea, cytotoxicity, MTT, LNCap

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MESMAP – 9
ABSTRACTS & PROCEEDINGS BOOK
03-05 May 2023, Ankara-Türkiye
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ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF *GUAVA (PSIDIUM GUAJAVA)* LEAVES AND BARK AGAINST *FUSARIUM OXYSPORUM*: A TOMATO WILT PATHOGEN

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Abstract

The emergence of antifungal resistance is a major attribute among plant pathogens. Therefore, bio fungicides are mostly used to control fungal plant diseases because of their eco-friendly nature and cost-effectiveness. This study aimed to assess the antifungal activities of crude extracts of leaves and bark of *P. guajava* against *Fusarium oxysporum*. The leaves and bark of the experimental plant were collected from Kucha Woreda, Gofa Zone of Ethiopia. All the collected plant samples were washed properly, dried under shade, and ground to powder. The bioactive components were extracted from the powder using ethanol and acetone. The antifungal activities of the extracts were evaluated using the agar well diffusion method, and the inhibitory zones were recorded in millimeters.

The agar diffusion method assessed the plant extracts' minimum inhibitory concentration (MIC) against *F. oxysporum*. The standard antifungal drug, Mancozeb, was used as the positive control, and the distilled water as a negative control. The bioassay studies of the crude extracts were undertaken at four different concentrations (60, 80, 100, and 120 mg/ml). The results revealed that the crude extracts of ethanol and acetone had antifungal activities against *F. oxysporum* in a concentration- dependent manner. The ethanol extracts of leaves had a high growth inhibitory effect at a concentration of 120 mg/ml with zones of inhibition of 19.2 mm. At the same time, acetone extracts of the bark recorded the lowest growth inhibitory effect at a concentration of 120 mg/ml with zones of inhibition of 16.5 mm. The positive control Mancozeb at a 100µg/ml concentration inhibits fungal mycelium by a mean zone of inhibition of 23.9 mm. The crude extracts of ethanol leaves and bark of *P. guajava* inhibit *F. oxysporum* growth with the minimum inhibitory concentration of 7mg/ml and 9mg/ml while, crude extracts of acetone leaves and bark had 8mg/ml and 10mg/ml of MIC. Generally, this study proves antifungal activity for P. guajava and provides a scientific basis for their traditional use. Pure chemical compounds and antifungal activity against many fungi should be studied to use as sources and templates for the synthesis of drugs to control fungal pathogens of plants.

Keywords: Antifungal activities, Minimum Inhibitory Concentration, *F. oxysporum*, *Psidium guajava*, Tomato wilt.

1.INTRODUCTION

1.1. Background of the study

Tomato (*Lycopersicon esculentum* Mill.) belongs to the family *Solanaceae*. It is one of the most popular vegetables and a major horticultural crop is grown worldwide and occupies a prominent position in the world's vegetable economy (Gebisa *et al*., 2017). The plant originated in Central America and was later distributed worldwide by explorers. It is one of the most edible vegetables widely grown for high nutritional value. It is the next most consumed vegetable after potato, ranking first and the most popular garden vegetable in the world. About 153 billion tons of tomatoes were produced in 2009 (FAO, 2009). China is the largest producer worldwide, with a total production of 33.9 billion tonnes, followed by the United States with 13.7 billion tonnes, India with 11 billion tonnes, Turkey with 10.3 billion tonnes, and Egypt with 9.2 billion tonnes. Nigeria is the 13th in the world and the second largest producer in Africa, next to Egypt (Bawa, 2016). Tomato is an economically important crop among vegetable crops in the country. Central Statistics Authority of Ethiopia (CSA) reported that Ethiopia is the world's 84th largest producer of tomatoes (Tadele, 2016).

Tomato, for fresh consumption, is produced under greenhouse conditions and in open fields. The crop provides high economic importance in West Shewa of Ethiopia. Ethiopia's total area coverage under tomato production is predicted to be more than 7,225 hectares, with a total production of 555,143 tons (CSA, 2013). In Ethiopia, in the 2015 summer cropping season, only tomato production was estimated to be 30,699.95 tons from 5,026.68 hectares (CSA, 2015). It is consumed in every household in different styles, but in certain areas, such as Wollo, Hararge, Shewa, Jimma, and Wellega, it is also an important co-staple food (Ambecha *et al.,* 2012). It can be eaten either as fresh or processed into different products. It is medicinally potentially healing wounds because of the antibiotic properties found in ripe tomatoes. The tomato crop is a good source of Vitamins such as A, B, and C (Baloch, 1994).

However, many constraints affect the productivity and quality of tomatoes, such as bacterial wilt, tomato spotted wilt, *Verticillium* wilt, and *Fusarium* wilt. The most common diseases of tomatoes include early blight, anthracnose, bacterial wilt, tomato spotted wilt, *Verticillium* wilt, and *Fusarium* wilt (Bawa, 2016). Bacteria cause wilt diseases (*Pseudomonas* sp.) and fungi (*Verticillium* sp. and *Fusarium* sp.) (Bawa, 2016). The main symptom of *F. oxysporum* on tomatoes was wilting the tomato in seedlings and adult plants. The tomato plant infected with *F. oxysporum* produces wilt in older leaves and afterward turns yellow. Leaf yellowing can occur on one side of the plant, and gradually most leaves turn yellow and wilt. *Fusarium* wilt is one of the cultivated tomatoes' most important and widespread diseases. The fusarium is a soil-borne pathogen in the class Hyphomycetes that causes tomato wilt (Ajay and Shashi, 2012).

The most common method for effectively controlling tomato wilt disease is using chemical fungicides such as benomyl, carbendazim, copper sulfate, copper oxychloride, Mancozeb, and prochloraz (Song and Goodman, 2002). Using chemical fungicides has been extensively exploited, leading to environmental and toxicological complications (Gurjar *et al*., 2012). However, they are high-cost and capable of creating problems for the environment and human and animal health in all areas of the world. They may lead to developing resistance in pathogenic fungi to common fungicides (Barhate *et al*., 2015). Microbial and botanical fungicides have gained attention as proxies for chemical fungicides. Plant extracts and essential oils have been reported to be effective antimicrobial agents against food and stored grain fungi, foliar pathogens, and soil-borne fungal phytopathogens (Singha *et al*., 2011).

Medicinal plant extracts are promising as alternative control means because of their antimicrobial activity, non-phytotoxicity, and biodegradability. Plants produce a lot of secondary metabolites, many with antifungal activity (Singha *et al*., 2010). Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, Sulfur compounds, saponins, cyanogenic glycosides, glucosinolate, tannin, and saponins. Although hundreds of medicinal plants are used medicinally in different countries as a source of many potent drugs, the vast majority have not been adequately explored against plant pathogenic fungi. Plants are the sources of natural fungicides that make excellent leads for new bio fungicides development (Gomez *et al*., 1990; Talibi *et al*., 2012; Padmaja and Yogesh, 2016).

Several plant extracts possess antimicrobial properties and are, therefore, being exploited to achieve control over various plant ailments (Singha *et al*., 2010). Some antibiotic constituents or some unknown substances contribute to the inhibitory activities of the extracts. Recently, plant extracts drawn from the parts of certain plant species have been successfully tested to demonstrate their antifungal activities. Among these, *P. guajava*, also known as common guava, is an important food crop and medicinal plant widely used in folk medicine around the world (Yariko and Kouji, 2010). Many studies have demonstrated the ability of *P. guajava* to exhibit hepatoprotective, antimicrobial, and antifungal and support its traditional uses (Shirur *et al*., 2013).

1.2 Statement of the problem

The lower yield for farmers in the production of tomato crops mainly are diseases, pests, and sub-optimal fertilization. The most important factors responsible for the low productivity of potatoes and tomatoes are fungal diseases and insect pests. Tomato is infected by many fungi, including *F. oxysporum*, *Alternaria solani*, *Rhizoctonia solani,* and *Colletotrichum musea,* which cause disease in tomatoes from the time of germination up to harvest (Bawa, 2016).

The diseases caused by fungi are fusarium wilt, late blight, early blight, anthracnose, tomato spotted wilt, and *Verticillium* wilt, which led to the collective losses of tomatoes up to 80% (Agrios, 2000). Fusarium wilt was estimated to cause 30 to 40% yield loss in tomatoes (Anita and Rabeeth, 2009). Synthetic fungicides are used as primary means for the control of plant disease all over the world. Farmers worldwide cultivate tomatoes using chemical fungicides, the most convenient way of managing the fungal disease of tomatoes (Song and Goodman, 2001). Although chemical fungicides increase the productivity and quality of tomato production, their inappropriate and non-discriminatory use imposes risks on humans, animals, and the environment (Kumar *et al*., 2007). Increased usage of different chemical-based products to control these pathogens has resulted in problems like the residual effect of chemicals on increased resistance of pathogens to the chemicals and environmental pollution. Thus, alternative control methods are needed as the use of bio-control agents and the use of organic extracts from plants. Using plant-derived products in agriculture has revealed a low toxicity effect on mammalians, less effect on the environment, and has won wide public acceptance (Prince and Prabakaran, 2011). Using plant extract to manage tomato wilt disease is the most effective, eco-friendly, easily biodegradable, and cheap (Alam *et al.,* 2002). Therefore, the present study was designed to evaluate the efficacy of crude extracts from *P. guajava* leaves and bark against the fusarium wilt of tomato.

2. MATERIALS AND METHODS

2.1. Description of the study area

The study area is located at a distance of 444 km south of Addis Ababa,275 km from Hawassa (regional city of SNNPR), 185 km from Arbaminch (Zonal city), and 62 km from Wolaita Sodo town. The geographical coordinates of the site are 06º28’38.76” N latitude, 37º28’3.37” E longitude, and an altitude of 1356 meters above sea level and the average annual rainfall is 1350 mm and the mean annual minimum and maximum temperatures 21 °C and 25 °C respectively (Kucha Woreda Agricultural Office 2017 unpublished report).

2.2. Study design

The cross-sectional study determined the antifungal crude extract of *P. guajava* against *F. oxysporum* that causes wilt in tomatoes from February 2021 to May 2021 at Wolaita Sodo Post-Graduate Microbiology Laboratory.

2.3. Sample size determination

The samples of *P. guajava* leaves and bark in this study were taken by the non-random method. As a result, the sample size determination regarded in the study needs no representation of selected groups. Therefore, the experiment was carried out in triplicate for result precision (Biswas *et al*., 2013).

2.4. Chemicals and instruments

The solvents of analytical grade reagents ethanol 96% and acetone 99.5% were purchased from the atomic chemistry laboratory in Addis Ababa, Ethiopia. Sodium hypochlorite (NaOCl), Mancozeb (C8H12MnN4S8Zn), and the nutrient for media preparation PDA (potato dextrose agar) and potato dextrose broth (PDB), cotton swab, aluminum foil, Whatman No.1 filter paper (90cm), Petri plates were purchased. Some instruments that were used for extraction and filtration are the Gemmy orbit shaker, Rota-evaporator (RE-52A), a different model of measuring flasks, beakers, and other appropriate instruments in the study such as incubator, autoclave, oven, electron balance, pH meter, test tube, taken from Wolaita Sodo University of the post-graduate microbiology laboratory.

2.5 Collection and preparation of plant material.

The fresh and healthy matured leaves and barks of *P. guajava* were collected from the Gamo zone of Kucha Woreda in February 2021. The plant was selected based on traditional medicinal values and previous history of the plant reported by many authors about the antimicrobial properties of the plant using different kinds of extracting solvents. The collected plant materials were washed with tap water and distilled water repeatedly to eliminate dust particles from the leaf surface. The washed leaves and barks of the plant air dried in the shade at room temperature for two weeks, as described in Agrios (2005).

The dried leaves and barks were ground using a grinding machine, and the powder was sieved with a 0.5mm mesh-sized sieve. Then measured amount of powder was stored at room temperature in 1000ml closed jars in the laboratory until used.

2.6 Crude extraction

A subsequent maceration method was employed to get crude extracts from leaves and barks of *Psidium guajava* using two solvents by increasing their polarity order according to the methods described in Singh (2008) and Geremew Yalemtsehay (2012) with modification. The sieved powders were macerated (the leaves with 96% ethanol and barks with 99.5% acetone by ÷ solvent ratio of 1:5 (w/v) that was 80gram of powder in 400ml ethanol and acetone solvent and the solution was inserted into Gemmy orbital rotary shaker (180 rpm) at 40°C. After 72 hrs, the macerated mixture was filtered using a double-layer filter paper (Whatman No.1), giving filtrates and residues. The residue was re-macerated for subsequent extraction in ethanol and acetone for another 72 hrs with a similar ratio, and filtration was made using filter paper (Whatman No.1), giving filtrates and residue. All filtrates were concentrated using Rota vapor to obtain crude extracts. After filtration, acetone and ethanol were evaporated from the extracts using a Rotary evaporator (RE-52A). The extracts were air-dried in the shade at room temperature and then kept in a refrigerator at 4°C until used for further experiments (Fazli *et al.*, 2012, Geremew *et al.*, (2018). Four crude extracts were collected after extracting the

leaves and bark powder of *P. guajava*. These are *P. guajava* leaf acetone, *P. guajava* leaf ethanol extract, *P. guajava* bark acetone extract, and *P. guajava* bark ethanol extract.

2.7 Fungal strains

The pure culture environmental isolate of *F. oxysporum* was obtained from Ethiopian Biodiversity Institute (EBI), Addis Ababa. The new pure culture was prepared to confirm the purity of the pathogen at the Wolaita Sodo University post-graduate Microbiology Laboratory. The purity of the culture was confirmed and stored at 4 °C in the refrigerator until used.

2.8 Standard fungicides

The following fungicide Mancozeb ($C_8H_{12}MnN_4S_8Zn$), was prepared according to the method described in Nel *et al*. (2007) and Bashir *et al*. (2017). 100mg/ml of Mancozeb ($C_8H_{12}MnN_4S_8Zn$) was used as a positive control for the antifungal susceptibility test whereas distilled water was used as a negative control.

2.9 Inoculum Preparation

According to the manufacturer's protocol, the inoculation media were prepared primarily by the potato dextrose broth (BAM Media M127). The fungus culture was incubated for four days at 28°C to verify the growth of the pathogen. Adjusting the turbidity of the inoculum spore suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the 250 ml measuring flask on the media. Then the cotton swab of potato dextrose broth was inoculated by streaking the swab over the entire sterile agar surface from the center to the rim of the agar plate Martyn et al., (1991).

2.10 Preparation of the test solution

The crude ethanol and acetone extracts of *P. guajava* leaves and barks were prepared in four different working solutions, i.e., 60mg/ml, 80mg/ml, 100mg/ml, and 120mg/ml for the antifungal activity test Neela *et al*., (2014). The first working solution was prepared by transferring 0.06g of each extract to a sterile test tube containing 1 ml of distilled water to make a 60mg/ml working solution. Similarly, the second, third, and fourth working solutions were prepared by adding 0.08g, 0.1g, and 0.12g, respectively.

2.11 Antifungal test of crude extracts.

The antifungal activity of the plant extracts was evaluated by the modified agar well diffusion method (Perez *et al*., 1990). PDA was prepared according to the manufacturing industry protocol; 1000 ml of distilled water was added to 39 g PDA and boiled to mix the powder and distilled water. The media was autoclaved at 121°C for 15 minutes and poured into sterile

Petri dishes. Once the media get solidified, a loop-full suspension of *F. oxysporum* was placed from the five days old matured broth inoculums at the plate's center and spread uniformly with a sterile cotton swab. Then six wells were prepared with the help of a sterile cork borer (6mm diameter). Each well was poured with 50µl test solution of the extract using a sterile micropipette. One well with 50µl of standard fungicides Mancozeb was taken as the positive control, and one with 50µl distilled water was taken as the negative control. Finally, the plates were incubated at 28°C for seven days. After seven days of incubation zone of inhibition was recorded by measuring the diameter of each zone (in mm). Experiments were performed in triplicate and recorded the average result (Biswas *et al*., 2013).

2.12 Determination of minimum inhibitory concentrations.

The minimum concentration of crude leaves and bark extracts to inhibit fungi growth was determined by the method described in Antara and Amla (2012) and Geremew *et al*. (2018). From the dried ethanol and acetone crude extracts, the working solution was prepared in test tubes to obtain the solution, which was serially diluted to get 12mg/ml, 11mg/ml, 10mg/ml, 9mg/ml, 8mg/ml, 7mg/ml and 6mg/ml (v/v) concentrations according to in Neela *et al*., (2014). The potato dextrose broth (PDB) medium was first prepared according to the manufacturer's protocol and sterilized by autoclaving for 15min at 121^0C. The inoculums of mycelium spore were subcultured by slant form using the sterilized inoculating loop and immersed into each test tube containing 10ml of test solution. Serially prepared ethanol and acetone crude extracts of Psidium guajava leaves and barks were transferred to the broth using a micropipette. All the test tubes were then incubated at 28 ^{0}C for five days. Following this, turbidometry measured the turbidity of the solution's suspension and recorded it. All procedures were repeated three times to confirm minimum inhibitory concentration results. The lowest concentration of extracts that inhibit the visible growth of the *F. oxysporum* was recorded as the MIC value (Antara and Amla, 2012; Geremew *et al*., 2018).

2.13 Statistical analysis

The triplicate experiment measurements' mean values and standard deviations were calculated using SPSS. Comparisons were made between the effects of crude extracts and positive control using one-way ANOVA SPSS (Turkeys). $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Antifungal activity of crude extracts

The antifungal activity of different crude extracts from *P. guajava* leaves and barks against *F. oxysporum* was evaluated at various concentrations. The results of different crude extracts are discussed below.

Antifungal activity of ethanol crude extracts of *P. guajava* leaf against *F. oxysporum*

Ethanol crude extracts of the *P. guajava* leaves were tested for their antifungal activity against the mycelium growth of *F. oxysporum*. Antifungal activity was tested by various concentration i,e., 60mg/ml, 80mg/ml, 100mg/ml, and 120mg/ml, and with the controlled groups of Mancozeb and distilled water as the positive and negative control, respectively. As seen in Table 1, the zone inhibition is directly proportional to the working concentrations. Maximum inhibition was observed at 120mg/ml concentration, where a 19.2±1.0mm zone of inhibition was noted. A minimum zone of inhibition was achieved at 60mg/ml, producing a 17.5±0.5mm zone of inhibition. The two intermediates' concentrations, i.e., 80mg/ml and 100mg/ml, showed 18.0±0.8mm and 18.5±0.8 mm zone of inhibition. The positive control mancozeb with a 23.9±0.5mm zone of inhibition was recorded (Table 1).

Table 1. Antibacterial activity of crude leaf and stem bark extract *P. guajava* against *F. oxysporum*

Plant parts	Solvent	Concentration (mg/ml)	Zone of Inhibition (mm)
Leave	Ethanol	60	17.5±0.5
		80	18.0±0.8
		100	18.5±0.8
		120	19.2±1.0
	Acetone	60	16.0±0.4
		80	16.8±0.5
		100	17.6±0.9
		120	18.5±0.5
Bark	Ethanol	60	14.0±0.3
		80	14.8±0.9
		100	15.1±0.9
		120	16.0±1.0
	Acetone	60	13.8±0.3
		80	14.4±0.4
		100	14.9±0.1
		120	16.5±1.0
Positive Control	Mancozeb	100	23.9±0.5
Negative control	Distilled water	--	0.00±00

The growth inhibitory activities of crude extracts at a concentration of 120mg/ml differ significantly from that of distilled water ($P < 0.05$) but not from the standard antifungal drugs ($P > 0.05$). There is a significant difference between the crude extract of P. guajava at 120mg/ml and the negative control (distilled water) but a non-significant difference between the extract and the positive control (Mancozeb). It indicates that the extract inhibits tested fungal pathogens, comparable to the standard antifungal drugs. Although the crude extracts of

P. guajava leave at a concentration of 60mg/ml, 80mg/ml, and 100mg/ml was significantly different from that of the standard drugs, the significant difference that exists between them and the negative control may suggest that they have some inhibitory effect against the fungal strain tested in this study (Table 1). Positive control (Mancozeb) at 100mg/ml concentration inhibits the mycelium growth of *F. oxysporum* with a mean inhibition value of 23.9±0.5mm. This indicates that positive control has the highest antifungal activity than crude extracts of P. guajava leave. The negative control used here, distilled water, showed no inhibition against tested fungal strains (Table 1).

Antifungal activity of acetone crude extracts of *P. guajava* leaf against *F. oxysporum*

The effect of acetone crude extracts of *P. guajava* leaves against the mycelium growth of F. oxysporum was studied at various concentrations, i.e., 60mg/ml, 80mg/ml, 100mg/ml, and 120mg/ml. The result highlighted in Table 1 showed that acetone crude extracts at 120mg/ml showed the highest antifungal activity with a mean inhibition value of 18.5±0.5mm. In contrast, 60mg/ml acetone leaf extracts showed the lowest antifungal activity (16.0±0.4mm) against *F. oxysporum*. Whereas 80mg/ml concentration has 16.8±0.5 mm and 100mg/ml has 17.6±0.9mm mean value of zone of inhibition compared to the positive control of Mancozeb where 23.9±0.5 mm zone of inhibition.

Antifungal activity of crude ethanol extracts of *P. guajava* bark against *F. oxysporum.*

As denoted in Table 1, ethanol extract of *P. guajava* bark produced a concentration-dependent inhibition of mycelium growth *F. oxysporum*. 120mg/ml of bark extract has produced a 16.0±1.0 mm zone of inhibition. On the other hand, 60mg/ml resulted in a 14.0±0.3mm zone of inhibition, 80mg/ml had a 14.8±0.9 mm zone of inhibition, and 100mg/ml concentration resulted from 15.1±0.9mm mean value of zone inhibition in comparison to positive control where 23.9±0.5mm zone of inhibition was recorded (Table 1).

Antifungal activity of acetone crude extracts of *P. guajava* bark against *F. oxysporum.*

As noted in Table 1, the growth inhibition was concentration dependent. The highest anti-mycelium activity was recorded at 120mg/ml, resulting in a 16.5±1.0 mm zone of inhibition value. In contrast, 60 mg/ml produced a 13.8±0.3mm zone of inhibition, 80mg/ml concentration recorded a 14.4±0.4mm zone of inhibition, and 100mg/ml concentration recorded a 14.9±0.1mm zone of inhibition in comparison to the positive control mancozeb where 23.9±0.5 mm zone of inhibition.

Minimum inhibitory concentration.

The Minimum inhibitory concentration assay was employed to evaluate the effectiveness of the crude extract of *P. guajava* in inhibiting the growth of *F. oxysporum*. The table above (Table 2) elaborates on the minimum inhibitory concentrations of the ethanol and acetone

crude extracts of *Psidium guajava* leaf and bark against *F. oxysporum* in vitro test in that the ethanol extracts of the leaf shows the lowest inhibitory concentration at 7mg/ml and acetone extracts of bark shows the highest inhibitory concentrations at 10 mg/ml while, the acetone extracts of leaf show at 8 mg/ml and ethanol extracts of bark shows at 9 mg/ml the MIC value. Therefore, the MIC value shown at the lowest concentration would be taken as a relatively low MIC value record for the leaf ethanol extracts of *P. guajava* against the test pathogens. Extracts with lower MIC scores are very effective antifungal agents.

Table 2. The minimum inhibitory concentration of crude leaf and bark extract of *Psidium guajava* against *Fusarium oxysporum*

S.No.	Crude Extracts	***Fusarium oxysporum***
		Minimum Inhibitory Concentrations (mg/ml)
1.	Ethanolic Leaf Extract	7
2.	Acetone Leaf Extract	8
3.	Ethanolic Bark Extract	9
4.	Acetone Bark Extract	10

4. Discussion

The present study focused on the antifungal activity of solvent-based plant extracts of the medicinal plant *P. guajava* against soil-borne pathogenic fungus *F. oxysporum*. In this study, the agar well-diffusion method was employed for an antifungal assay that was reported to be more sensitive than other methods like disc diffusion (Milyani, 2012). It was observed that extracts of leaves and barks of *P. guajava* had produced antifungal activity against *F. oxysporum*. The crude ethanol extracts of *P. guajava* leaves, and bark showed antifungal activity in a concentration-dependent manner. In most cases, high concentration showed better antifungal activity against the mycelium growth of *F. oxysporum*. In this test, the ethanol leaf extracts of *P. guajava* showed a significant growth inhibition against the fungal species. The ethanol crude extracts of bark illustrated less antifungal activity than ethanol leaf extracts and positive control, i.e., Mancozeb. Among these crude extracts, ethanol crude extracts of *P. guajava* leaves at a concentration of 120mg/ml showed superior antifungal activity against *F. oxysporum*. However, the effect was not higher than that of positive control of Mancozeb.

The result agrees with Enespa and Dwivedi (2014), who showed that with the increase in the concentration of the crude extracts, the greater inhibition of mycelium growth of *F oxysporum* was observed. The same result effects were observed with Enespa and Dwivedi (2014) regarding the efficacy of *P. guajava* leaf extract against the mycelium growth of *F. oxysporum*. Also, these findings resemble Joseph and Priya (2010) regarding the efficacy of *P. guajava* leaf extract against the mycelium growth of *F. oxysporum*. Moreover, findings from this study suggest that Neela *et al.* (2014), the antifungal activities in acetone and ethanol extracts of the leaf against *F. oxysporum* can be

possessed. Also, another support for these findings of the antifungal activities of medicinal plant extracts inhibits the mycelium growth of *F. oxysporum* (Padmaja and Yogesh, 2016). This study disagreed with the study conducted by Pratibha *et al.* (2016), in which the ethanol crude extract of leaves with the highest concentration recorded a low zone of inhibition.

The activities of the ethanol and acetone extracts of the two parts of the plant against the test pathogens varied. The findings of this study revealed that the plant parts extracted from 96% ethanol provided more consistent antifungal activities than those extracted using acetone. The greater activities recorded by leaf extracts in this study suggest that more bioactive ingredients are lodged in these parts (Chen and Yen, 2007; Thenmozhi and Rajan, 2015). Similarly, active ingredients such as phenols that confer broad-spectrum activities in plants were observed by Mahesh and Satish (2008) in a study of medicinal plants using ethanol extracts of fresh leaves of *P. guajava*. The positive control, Mancozeb, showed the most pronounced activity on the tested fungus *(F. oxysporum*).

The minimum inhibition concentration test was done for only the crude extracts with positive results. The MIC value of acetone and ethanol crude extracts of *P. guajava* leaves and barks against the tested fungus *F. oxysporum* ranged from (6, 7, 8, 9, 10, 11 to 12mg/ml). The minimum inhibitory concentration (MIC) assay evaluated the effectiveness of the crude extracts that showed significant antimicrobial activities in the previous tests. Among the crude extract of *P. guajava,* ethanol extracts of the leaf had the lowest MIC value, 70 mg/ml, then followed by acetone extract of the leaf at 80mg/ml, and ethanol extract of bark were 90mg/ml against the mycelium growth of *F. oxysporum.* The highest MIC value was exhibited by acetone extracts of *P. guajava* bark against the mycelium growth of *F. oxysporum,* with a MIC value of 100mg/ml. Elekwa *et al*. (2009) reported that crude ethanol extract of *P. guajava* leaves inhibits *A. niger* at MIC of 9.6 mg/ml. This report agreed with the present work in which ethanol extract of *P. guajava* leaves inhibits the mycelium growth of *F. oxysporum*. Basel *et al*. (2015) analyzed the antifungal activity of acetone extract of *P. guajava* leaves with a MIC value of 6.2 mg/ml against the mycelium growth of *C. albicans.* Basel *et al*. (2015) also reported that ethanol extract from *P. guajava* leaves inhibited the growth of *C. albicans* at a MIC value of 6.2mg/ml. According to Anikata and Kanika (2012), aqueous extract of *P. guajava* against the mycelium growth of *F. oxysporum* with MIC value (4.6mg/ml).

5. Conclusions

The present study's findings revealed that crude extracts of *P. guajava* leave and barks collected from the Gamo zone of Kucha woreda exhibited significant antifungal effects against the selected fungal strain of *F. oxysporum.* The extracts inhibited the growth of the selected fungal pathogens *(F. oxysporum)*. The present study suggested that the two solvents, such as ethanol and acetone extracts of *P. guajava* leaves and barks have a great prospect as antifungal agents against selected fungal species, *F. oxysporum*. *P. guajava* extracts can be used as an alternative medicine in treating the *Fusarium* wilt of

tomato. The antimicrobial activity and MIC assays showed promising evidence for the antifungal activity of *P. guajava* leaves and bark extracts against selected fungal pathogens. Therefore, the *P. guajava* leaves and bark extracts could be a good source of useful bio-fungicides.

Acknowledgements

The authors have expressed thanks to the Post Graduate Laboratory, Department of Biology, College of Natural and Computational Sciences, Wolaita Sodo University, Ethiopia, and Kucha Woreda farmers have helped to collect the guava and *P. guajava* plant leaves in their gardens.

Funding: No funding

Conflict of Interest: The author has confirmed that they have no conflict of interest in publishing this article.

Technical Terms: The Ethiopian people usually use technical terms, and the Government also exercises in the Official documents and reports. Woreda means District, Kebele means Village, Dega means High land, Wynedega means Mid-highland, Kolla means low land.

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ORAL PRESENTATION - FULL PAPER

CULTIVATION STUDIES FOR TAURUS SNOWDROP (*GALANTHUS ELWESII* HOOK.)

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Abstract

Galanthus elwesii Hook is one of the most important species of Amaryllidaceae family, from which bulb plants are exported. The present study design was based on *G. elwesii*, which is an important export product and was conducted in Suluova district of the city of Amasya in the growing season of 2022-2023 to cultivate its species. The study was established according to the Random Blocks Trial Design with four replications. The number of emerging plants (number), flowering rate (%), leaf length (cm), number of leaves (number), number of fruits (number), fruit setting rate (%), bulb diameter (cm), bulb weight (g), number of bulbs (number/plant), ratio of young bulbs (pieces/plant), bulb yield in the plot (g), bulb yield per decare (kg/da), phenolic compounds, and total phenolic and flavonoid contents were investigated. The number of emerging plants was found to be 299.75 ± 61.62, flowering rate 53.12% ± 17.80, leaf length 9.45 ± 0.23 cm, number of leaves 2.01 ± 0.10, number of fruits 55.5 ± 24.28, fruit setting rate 35.08% ± 10.56, bulb diameter 1.63 ± 0.07 cm, bulb weight 3.30 ± 0.39 g, number of bulbs 159.87 ± 87.27, new bulbs ratio unit/plant, and bulb yield per plot was found to be 285.98 ± 22.68 g and bulb yield per decare 1.900 kg/da. The major component of the snowdrop extract was determined as Gallic acid in the study. Total phenolic and total flavonoid contents were lowest at the beginning of the flowering period and at the root. The highest amount of total flavonoid was in the flower at the beginning of the flowering period (0.005 mg QE/g), and the total amount of phenolic was found in the bulbs (0.06214 mg GAE/g) in the post-flowering period.

Key Words: Kaempferol, plant development period, quercetin, snowdrop

1. Introduction

Galanthus elwesii Hook. is a highly valued bulbous perennial herbaceous plant with white flowers, commercially cultivated in many countries due to its economic significance. This species, along with other *Galanthus* species, contains various bioactive compounds, including Galanthamine, Lycorine, Graciline, Tazettine, and Hordenine, present in both the flowers and bulbs (Ay et al., 2018; Bozkurt et al., 2021; Ay et al., 2023). Galanthamine and Lycorine exhibit anti-inflammatory, anti-cancer, anti-bacterial, anti-malarial, acetylcholinesterase, and butyrylcholinesterase inhibitory properties (Kang et al., 2012; Cimmino et al., 2017; Ying et al., 2017; Pesaresi et al., 2022). Lycorine has shown potential in the fight against SARS-CoV-2 due to its antiviral activity (Jin et al., 2021). Additionally, Galanthamine is utilized in the treatment of neurological disorders such as Alzheimer's

disease (Kaur et al., 2022; Pesaresi et al., 2022). The available studies on G. elwesii are limited and mainly focused on the qualitative and quantitative determination of its bioactive compounds through collection from nature (Latvala et al., 1995; Berkov et al., 2004, 2007, 2008; Berkov et al., 2011; Bozkurt et al., 2017; Bulduk and Karafakıoğlu, 2019; Mahomoodally et al., 2021). Only a few studies have been conducted on its biological activities, such as acetylcholinesterase inhibitory activity, alkaloid accumulation, and effectiveness against microorganisms (El Tahchy et al., 2011; Ay et al., 2018; Ay et al., 2023). Snowdrops can be propagated through bulbs or seeds, but the process takes a significant amount of time for the plant to mature and flower. The *Galanthus* species, which includes snowdrops, faces several threats such as habitat destruction, illegal harvesting, and climate change. If the current exploitation of wild stocks continues, these species may become extinct within a decade, and their genetic diversity may also diminish. So, it is crucial to cultivate endemic geophytes like *Galanthus* species. Additionally, bulbous plants like snowdrops can be significantly impacted by environmental factors like the growth medium and water availability. Hence, scientific studies should be conducted to understand the growth requirements of these species. All this information reveals to us that the available data on the breeding of this species is quite limited and more satisfactory information should be obtained through research. Therefore, the phenological observation of the plant with this study will enable the data to be enriched and provide important contributions to researchers in future studies.

2. Material and Methods

2.1. Plant Material and Trial Plan

The present study was conducted in Suluova, Amasya, Turkey (40°50'39.3"N~40°50'40" N, 35°37'57.3"E~35°37'58" E, altitude 510m) in the 2022-2023 growing season. The bulbs of *G. elwesii* snowdrop species that were larger than 4 cm were used as the study material. The bulbs were obtained from commercial companies exporting natural flower bulbs. The study was established according to the Random Blocks Trial Design with four replications.

2.2. The Characteristics Examined in the Study

Plant emergence (pieces): Obtained by counting the plants that emerged in January.
Leaf length (cm): The leaves of 10 plants were measured from each plot after the plants set fruit.
Flowering rate (%): It was obtained by proportioning the number of plants to the number of blooming flowers.
Number of leaves (pieces/plant): It was obtained by taking the total number of plant stems to be formed in 10 bulbs selected from each plot.
Number of fruits (pieces): The plants that set fruit were counted.
Fruit setting rate: It was found by proportioning the number of plants that set fruit to the number of flowers that bloomed.
Bulb diameter (cm): The measurements were made in 10 bulbs selected from each plot with a caliper from the place where the circular circumference of the bulb was the largest.
Bulb weight (gram): It was determined by measuring the weight of 10 rootless and stemless seeds that were selected from each plot.
Number of bulbs (number): It was obtained by counting the number of bulbs.
Cormen ratio (pieces/plant): The bulbs with a circumference of less than 4 cm were weighed in each plot and calculated by proportioning them to the total plot yield.
Bulb yield in the plot: After every five rows were harvested, the soil and roots were cleaned, weighed, and the bulb yield was found in the plot.

Bulb yield per decare: The bulbs that were harvested in each plot were weighed in ***g*** and averaged, and yield per decare was calculated in this way.

2.3. Plant Extraction

All samples were pulverized in the mill after the drying process of the harvested plants and were taken into 50 mL falcons and stored at room temperature. In the extraction of powdered plant samples, 5 g each was weighed on the balance and transferred into glass jars with lids, and 200 ml of methanol was added. Then, the mixtures were macerated for 3 days (72 hours) by using a shaker, and the obtained plant suspensions were filtered through filter paper, the liquid part was taken and the pulp was stored. The liquid extracts were passed through the Rotary Evaporator Device and the Methanol was removed from the environment.

2.4. Determination of Total Phenolic Substance

The total phenolic content of the snowdrop extracts was determined according to the method suggested by Slinkard and Singleton (1977) by using Folin-Ciocalteu Reagent linked to phenolic standard Gallic acid. After 1 mL of plant extract was taken into a test tube, 4.5 mL water, and 0.1 mL Folin-Ciocalteu Reagent were added. The solution was kept in the dark for 5 minutes and 0.3 mL of sodium carbonate (2%) was added. The tubes were covered with parafilm and kept in the dark for 1 hour. The measurements were made at 765 nm in a spectrophotometer and a comparison was made with the Gallic acid calibration curve. The results are expressed as mg Gallic acid/g (mg GAE/g) in the dried sample.

2.5. Determination of Total Flavonoid Substance

The total flavonoid content was determined with the Quercetin Standard Solution by using the methods suggested by Park et al. (2008) and 1 mL plant extract was placed in test tubes, followed by 2 mL distilled water, 0.15 mL 0.5M $NaNO_2$ and 0.15 mL of 0.3 M $AlCl_3$ reagent. Five minutes after this, 1 mL NaOH was added and the absorption was measured at 510 nm with a spectrophotometer and compared with the Quercetin Calibration Curve. Total flavonoids were defined as mg equivalents of Quercetin (mg QE/g) per g of the dried fraction.

2.6. Determination of Alkaloid Components by HPLC

HPLC working conditions and gradient elution program were used for the quantitative determination of alkaloid compounds of the extracted plants. The components in the samples were determined according to the retention times of the components in the system. Firstly, the standards were read individually and the retention times were determined for quantification. Then mixed solutions of the standards were prepared at different concentrations and it was determined whether it caused changes in the retention times of Galanthamine and Lycorine.

3. Results and Discussion

The measurements of plant emergence, flowering rate, leaf length, leaf number, fruit number, fruit setting rate, bulb diameter, bulb weight, bulb number, Cormen ratio, and bulb yield in the plot of *G. elwesii* grown in the culture medium are given in Table 1. The number of plants that emerged was found to be 299.75±61.62 (pieces), the flowering rate was 53.12%±17.80, the leaf length was 9.45±0.23 cm, the number of leaves was 2.01±0.10 (pieces), the number of fruits was 55.5±24.28

(pieces), the fruit setting rate was 35.08%±10.56, bulb diameter was 1.63±0.07 cm, bulb weight was 3.30±0.39 g, bulb number was 159.87±87.27 (pieces/plant), Cormen ratio was 1 (piece/plant), and bulb yield per plot was found to be 285.98±22.68 g. In a study that was conducted to determine the effects of different growing mediums on the bulb performance of *G. elwesii*, it was reported that the effects of different growing mediums on the bulb diameter were statistically significant and the bulb circumference ranged between 3.6 and 5.1 cm (Kahraman and Özzambak, 2015). In the study in which Uysal and Kaya (2013) applied different nitrogen doses to the Taurus Snowdrop, it was determined that the bulb circumference was between 32.03 and 35.64 mm. Kahraman and Özzambak (2015) reported that the effects of growing medium on bulb weight were significant in Taurus Snowdrop and the highest bulb weight was obtained from coconut peat (2.2 g) and peat (2.0 g) media and the lowest bulb weight was obtained from sawdust (0.9 g) medium. The bulb diameter values obtained from this study coincide with the values reported in the literature. Arslan et al. (1998) reported that the leaf length varied between 9.46-12.58 cm by showing differences according to the regions, and the leaf length was between 10.25 and 11.89 cm according to the bulb length. Arslan et al. (2002) reported that there were differences in leaf lengths of snowdrops that were collected from different regions, and determined that the leaf length varied between 9.85 and 15.3 cm in bulbs larger than 4.5 cm in circumference and between 10.02 and 16.89 cm in bulbs smaller than 4.5 cm in circumference. Kahraman and Özzambak (2015) reported that the effects of different growing media on plant height were statistically significant in snowdrops, and in the same statistical group, coconut peat followed by 22.7 cm plant height, perlite and soil (21.9 cm) by 21.9 cm, and the shortest plant height was in the sawdust medium with 12.8 cm. When the results obtained from this study were compared with the studies in the literature, some values were found to be lower and some were similar. This difference might have occurred because of the different bulb growing media, the circumference size of the bulb, and the area where the bulbs were harvested, which affect the leaf length.

The total phenolic and total flavonoid substance amounts of Taurus Snowdrops grown in the culture medium according to the different development periods (flowering beginning, end of the flowering, and fruit ripening period) are given in Table 2. The highest total phenolic substance content was determined at the end of the flowering period in 0.06214 mg GAE/g bulb, and the lowest total phenolic substance content was determined at the beginning of the flowering period in 0.00995 mg GAE/g root. The highest amount of total flavonoid substance was determined at 0.005 mg QE/g at the beginning of flowering, and the lowest amount of total flavonoid substance was at 0.0002 mg QE/g at the beginning of flowering in the root. Ay et al. (2018) reported that the highest total phenolic substance content was in the bulb during the fruit ripening period in *Galanthus elwesii* extracts, and the lowest total phenolic substance content was in the root during the fruit ripening period. Similarly, it was also reported in the same study that the highest amount of total flavonoid substance was in the bulb during the fruit ripening period, and the lowest amount of total flavonoid substance was in the root during the fruit ripening period. Similar to our study, some previous studies reported that the highest total phenolic substance and total flavonoid substance contents were in the bulb, and the lowest substance amounts were in the root, which shows us that the snowdrop bulb is richer in terms of secondary metabolite source.

It was determined that the major phenolic component of the snowdrop extracts grown in the culture medium was Gallic acid 4.9 ppm/1mg extract, and the lowest phenolic component was Kaempferol 1.56 ppm/1mg extract. Kaempferol was found as 1.56 ppm/1mg extract. Caffeic acid, Myricetin, and Formononetin could not be detected in the extracts. Ay et al. (2018) reported that the phenolic components changed in snowdrop extracts according to the plant growing period and the plant organ, and the highest phenolic compound was Quercetin in the leaves.

Table 1. Plant emergence, flowering rate, leaf length, number of leaves, number of fruits, fruit setting rate, bulb diameter, bulb weight, number of bulbs, Cormen ratio, and bulb yield in the plot of Taurus Snowdrop grown in culture medium

Block	Number of Emerging Plants (piece)	Flowering Rate (%)	Leaf Length (cm)	Number of Leaves (pcs)	Number of Fruits (pieces)	Fruit Setting Rate (%)	Bulb diameter (cm)	Bulb Weight (g)	Number of Bulbs (pieces/plant)	Cormen Ratio (piece/plant)	Bulb Yield on Plot (g)
1st Block	375	26.66	9.72	2.17	21	21.00	1.54	2.80	290	1	295.60
2nd Block	315	64.44	9.22	2.00	78	38.42	1.71	3.68	120	1	252.55
3rd Block	281	62.63	9.57	1.92	61	34.65	1.60	3.17	126	1	292.95
4th Block	228	58.77	9.30 am	1.97	62	46.26	1.67	3.56	103.5	1	302.85
Average	**299.75±61.62**	**53.12±17.80**	**9.45±0.23**	**2.01±0.10**	**55.5±24.28**	**35.08±10.56**	**1.63±0.07**	**3.30±0.39**	**159.87 ±87.27**	**1**	**285.98 ±22.68**

Table 2. Total phenolic and total flavonoid substance amounts according to different development periods (beginning of flowering, end of flowering, and fruit ripening period) in Taurus Snowdrop grown in culture medium

Total Phenolic Substance Amount (mg GAE/g)				Total Flavonoid Substance Amount (mg QE/g)			
	Sample Collection Periods				Sample Collection Periods		
Plant Part	**Beginning of Flowering**	**End of Flowering**	**Fruit Ripening Period**	**Plant Part**	**Beginning of Flowering**	**End of Flowering**	**Fruit Ripening Period**
Leaf	0.01014	0.04519	0.01876	**Leaf**	0.002	0.003	0.004
Bulb	0.02609	0.06214	0.01923	**Bulb**	0.003	0.0034	0.00337
Root	0.00995	0.02957	0.01476	**Root**	0.0002	0.003	0.00310
Flower	0.02195			**Flower**	0.005		

3. Conclusion

In the export of natural flower bulbs, large bulbs are generally preferred and bulbs with a circumference of over 4 cm are in demand in Taurus Snowdrop bulbs. Sufficient bulb perimeter size could not be achieved because the study was conducted for one year in field conditions. However, a two-year growing period without the dislocation of *G. elwesii* bulbs will allow for larger exportable bulbs. Also, the highest total phenolic substance content was found in the bulb, and the highest total flavonoid substance content was determined in the flower. It was also seen that the lowest total phenolic substance and total flavonoid substance amounts were in the root and at the beginning of flowering. Gallic acid was found to be the major phenolic component of the snowdrop extracts. The results show that the total amount of phenolic and flavonoid substances in snowdrop extracts may vary depending on the plant organs and plant growth stages, and the root contains a lower amount of substance compared to other plant organs.

Acknowledgments

We are thankful to the Scientific Research Projects Unit (BAP) of Amasya University for providing support to this research, with the number of FMB-BAP 22-0574 BAP Project.

Conflict of Interest

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

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ORAL PRESENTATION - FULL PAPER

PHENOLIC PROFILE, ANTIOXIDANT AND CYTOTOXIC POTENTIAL OF ETHANOLIC EXTRACTS OF *FERULAGO HUMILIS*

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Abstract

Ferulago genus (Apiaceae) is represented by about 50 species in the world, 35 of which are naturally distributed in Türkiye. Various studies have been carried out on the biological activities of many species belonging to this genus until today. *Ferulago* species have been used since ancient times as a digestive, carminative and analgesic in the treatment of various gastrointestinal disorders and haemorrhoids. According to the literature survey, *Ferulago* species are used in our country for bronchitis, various skin diseases and depression, to increase body resistance and as an immune enhancement. *F. humilis* is an endemic species naturally distributed in Türkiye. In our study, the phenolic contents, antioxidant capacities and cytotoxic effects of the extracts obtained from the *F. humilis* species by two different techniques were evaluated. In line with the data obtained at the end of the study, 11 phenolic components in the extracts were determined, and it was determined that the extracts had the antioxidant capacity and showed dose- and time-dependent cytotoxic effects in the HL60 cell line. After this preliminary screening, it is aimed to carry out further studies to determine the compounds responsible for the activity and to experimentally confirm the observed activity.

Key Words: Endemic, Kılkuyruk, Medicinal plants.

1. Introduction

When the burden of disease increases in society and the current treatments are insufficient, people have to turn to different and alternative treatment methods. Traditional medicine has its roots in the usage of plants, which have long been valued as valuable sources of medicine. Such traditional medicinal plants are essential to addressing the current and future needs of the global healthcare system (Leonti and Casu, 2013). The use of medicinal plant extracts for the treatment of human ailments has significantly expanded over the past few decades due to the negative side effects of chemical medications.

Today, various phytochemicals or extracts obtained from plants used as alternative and/or complementary in the treatment of many diseases are of great importance. Thousands of medicinal plants have been used for several diseases such as cancer, diabetes, epilepsy, malaria and depression etc. Natural products' structural diversity has a significant impact on the development of many contemporary medications (Hong, 2011). Because they have the potential to benefit society in many ways, particularly for medicinal purposes, medicinal plants are now receiving more attention than ever. These plants have medicinal properties because of their phytochemicals, which have specific physiological effects on people. In this context, it is essential to evaluate plants that have not been studied before. The *Ferulago* W. Koch genus, which is a member of the Apiaceae family comprises

about 50 species distributed all over the world (Akalın-Uruşak and Kızılarslan, 2013). This genus is common throughout the Mediterranean area, particularly in Turkey, Greece, Iraq, Western Iran, Macedonia and Serbia. Türkiye is home to 35 *Ferulago* species, 18 of which are endemic (Akalın-Uruşak and Kızılarslan, 2013). In Turkey, the *Ferulago* species is commonly referred to as "Çakşırotu"(Özhatay and Akalın, 2000; Akalın and Koçyiğit, 2010). In conventional pharmaceuticals, a few species of *Ferulago* have been utilized for the treatment of different ailments. Broad thinks about have been conducted to assess the ethnomedicinal employments of Ferulago species such as antibacterial, antioxidant, anti-diabetics, anti-malaria, anti-coagulant, and sexual enhancer impacts (Rahimpour et al., 2021). According to the literature survey, *Ferulago* species are used in our country for bronchitis, various skin diseases and depression, to increase body resistance and as an immune enhancement. *Ferulago humilis* is an endemic species naturally distributed in Türkiye. As far as we know there is a limited study about this species. The antioxidant and antimicrobial potential of *F. humilis* extracts were studied and this study differs from our study with extraction technique and solvent (Süzgeç-Selçuk et al., 2020). And the other study on the cytotoxic and apoptotic effects of this species applied on four different cell lines and reported as more effective on PC-3 cells (Gürbüz et al., 2023). Although there were some studies in the literature, further studies on the biological activities of the genus are needed. The aim of this study, is to determine the phenolic contents, antioxidant capacities and cytotoxic effects of the extracts obtained from the *F. humilis* species by two different techniques.

2. Material and Methods

2.1. Plant Material

The plant material was collected from its natural habitat from Muğla, Türkiye and brought to our laboratory, then the necessary cleaning processes were made and left to dry in an environment that does not receive directly sunlight. After the plant samples were completely dried, they were ground into powder in the mill. They were stored in a dry and cool environment.

2.2. Extraction

The dried plant samples were extracted with two techniques, namely maceration and ultrasonication. In the maceration technique, it was treated with ethanol for 48 hours, then the plant material was separated by filtration and the solvent was removed by evaporation. In ultrasonic extraction, the plant material was kept in an ultrasonic bath at 45 °C for 20 minutes, and then the extract was obtained by following the series for maceration. The maceration extract was coded as FHM and the ultrasonic extract as FUAE, respectively.

2.3. HPLC

HPLC analyses were performed at the Sargem Laboratory of the Konya Food and Agriculture University. 12 phenolic standards were used to determine the phenolic content in the extracts. The results are given in the table.

2.4. DPPH Assay

The radical scavenging activity of the extracts was measured using the DPPH test. DPPH analysis was carried out according to the Chu method but with minor modifications (Chu et al., 2000; Ahmed et al., 2015). Absorbance was measured at 490 nm, and radical scavenging activity (RSA) was calculated as 50 % inhibition (IC_{50}) values for each sample. Also, ascorbic acid was used as the reference compound, and the IC_{50} value was determined.

2.5. MTT Assay

Human leukaemia cell line HL60 was used for cytotoxicity assignment. Cells were maintained in RPMI1640 medium (Sigma) supplemented with 10 % fetal calf serum at 37°C in a humidified atmosphere of 5 % CO_2. The prepared extracts were applied to the HL60 cell line at various concentrations and two-time intervals. At the end of the incubation period, MTT solution

was added (Mosmann, 1983). Plates were read on an ELISA reader at 540 nm wavelength. The effect of the extracts on cell viability was calculated by comparing the absorbance values obtained from the control group (no treatment). Analyses were done in triplicate, with at least two replicates per plate. Mean values for cell viability values were taken into account.

3. Results and Discussion

3.1. HPLC

The amounts of the standards used in HPLC analysis and the phenolic substances screened in the extracts are given in Table 1.

According to Saibabu et al. (2015) and Shahidi and Yeo (2018), phenolic compounds, such as phenolic acids, have the potential to treat a variety of diseases by acting as antioxidants, anti-inflammatory agents, cancer preventatives, and treatments for Alzheimer's disease. In this work, the phenolic acids present in various extracts of *F. humilis* were investigated. HPLC analysis was performed on 12 common phenolic acids reagents for this purpose. When we examined the HPLC results, it was determined that the amount of coumaric acid was the highest in both extracts in accordance with the literature (Hazrati et al., 2019). Then, while the highest amount of benzoic acid and chlorogenic acid were detected in the extracts, gallic acid was not detected. The antioxidant, anticancer, anti-inflammatory, hepatoprotective, cardiovascular, anti-diabetic, anti-lipidemic and renoprotective activities of chlorogenic acid have been described before (Zhao et al., 2010; Maalik et al., 2016). Benzoic acid, another abundant phenolic acid, also has antimicrobial, antifungal, and anticancer properties (Anantharaju et al., 2017; Synowiec et al., 2021). Although there is no study on the detailed phenolic content directly related to the *F. humilis*, our results are compatible with studies on other *Ferulago* species. When the extraction methods and phenolic content were evaluated together, it was seen that the extraction technique did not make a significant difference in terms of the screened phenolic compounds.

Table 1. Phenolic substances and their amounts screened in extracts according to HPLC analysis

		FHM	**FUAE**
	Phenolic compound	**Calculated amount**	
1	4-OH-Benzoic acid	5.703	6.501
2	Chlorogenic acid	1.980	2.480
3	Vanillic acid	0.026	0.029
4	Cafeic acid	0.213	0.214
5	Syringic acid	0.135	0.368
6	Coumaric acid	9.778	11.235
7	Rutin	0.501	0.702
8	Benzoic acid	2.163	2.638
9	Cinnamic acid	0.110	0.153
10	Rosmarinic acid	0.317	0.322
11	Quercetin	0.268	0.425
12	Gallic acid	ND	ND

3.2. DPPH

The antioxidant activity was tested using via DPPH assay. For comparison, Table 2 presents the results of the antioxidant activities, expressed as IC_{50}.

It was determined that both extracts had antioxidant potential and as can be seen from the IC_{50} values, the maceration extract of the plant showed a higher scavenging effect on DPPH than ultrasonic extraction. In the study conducted by Süzgeç-Selçuk et al., (2020), it was reported that the above-ground methanolic extracts of *F. humilis* have higher antioxidant capacity than rhizome methanol extracts.

When the studies revealing the antioxidant capacities of other *Ferulago* species are examined in recent years; Süzgeç-Selçuk et al. (2017) reported that above-ground methanol extracts of the plant had higher antioxidant capacity in their study to determine the antioxidant capacity of *F. trojana*. Kızıltaş et al., (2017) reported flower methanol extract of *F. angulata* has antioxidant potential and its IC_{50} value is 67.34 ± 4.14 µg/mL. Karakaya et al., (2018) reported the $CHCl_3$ fractions of roots from *F. isaurica* and *F. syriaca* (8.78 and 9.99µg/mL, respectively) showed the highest radical scavenging effect when compared to the studied phenolic standards. Mohammed et al (2020) reported the antioxidant potential of *F. plathycarpa* and dedicated it could be used as a natural antioxidant source. As a general assessment *Ferulago* species prepared with different solvents and /or extraction methods have antioxidant capacity at various levels.

Table 2. Antioxidant capacities and extraction yields of extracts

	FHM	**FUAE**
DPPH IC_{50} (ug/ml)	154,41± 0,50	333,3±1,15
Extraction yield (%)	4,2	4,7

3.3. MTT Assay

To determine the cytotoxic potential of *F. humilis* extracts on the HL-60 cell line, the MTT assay was performed. According to the results of the MTT test, the extract shows a cytotoxic effect dose and time-dependent manner. Also the cytotoxic effects of the extracts are close to each other. The % viability graphs for two time intervals were given in Figure 1. In the study conducted by Gürbüz et al. (2023) the cytotoxic and apoptotic effects of extracts obtained from five *Ferulago* species, including *F. humilis*, were investigated. The cytotoxic effect was evaluated by the MTT test applied to the extracts MCF-7, A549, SW480 and PC3 cell lines. The IC_{50} values of *F. humilis* extract on cell lines were 0.655, 1.755, 0.519 and 0.552 mg/ml, respectively. According to the IC_{50} values, it can be said that the extract is more effective on the PC-3 cell line than on the other lines. Although the cell line used in our study is different from the lines used in this study, the IC_{50} dose of our extract is lower.

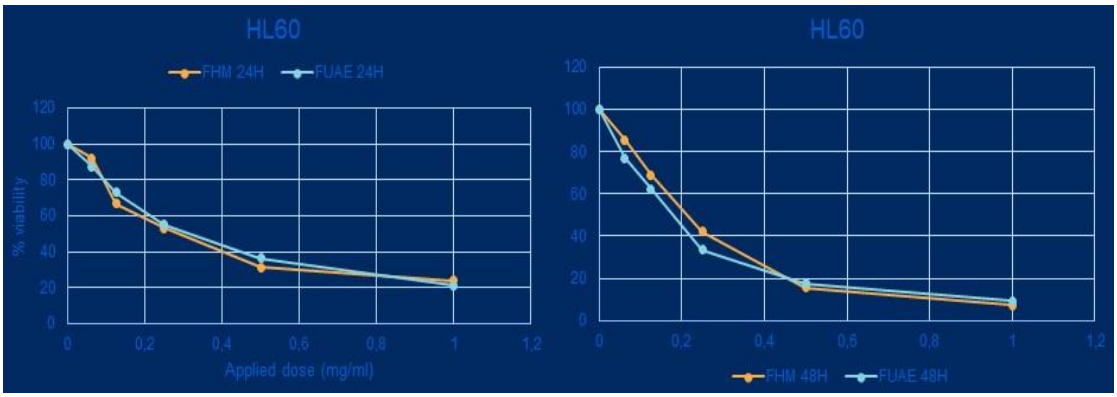

Figure 1. MTT Assay graphs of the extracts for two time intervals

Ferulago species showed anticancer efficacy when tested against a variety of tumor cell lines. Cytotoxic effect in studies on other species belonging to the genus *Ferulago*;
The cytotoxic effects of *Ferulago tirifida* extracts were studied on three cancerous cell lines (HT-29, A-549 and MCF-7) and the IC_{50}= 42.55, 25.0 and 22.0 µg/ml respectively (Tavakoli et al., 2017). Rezaei Dezaki et al., (2019) studied the antiproliferative effects of *Ferulago angulata* extracts on human promyelocytic leukaemia cell line (HL-60) and they reported that *F.angulata* decreased cell viability in a concentration of more than 500 µg/ml. Bakar-Ateş et al., (2020) studied five *Ferulago* species' cytotoxic potentials on different cell lines such as MCF-7, A549, SW480 and PC3 and they reported that all examined species except *F. setifolia* inhibited cell viability in SW480 and PC3 cells. In future studies, we intend to evaluate the cytotoxic effect on different cancer cell lines and healthy cell lines.

4. Conclusion

In this study, phenolic content, antioxidant capacity and cytotoxic effects on the HL60 cell line of extracts obtained from *Ferulago humilis* by two different techniques were reported for the first time. Such studies, which reveal the effects of extracts and phytochemicals obtained from plants, are very important for future studies. After this preliminary screening, it is aimed to carry out further studies to determine the compounds responsible for the activity and to experimentally confirm the observed activity.

Conflict of Interest

There is no conflict of interest.

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ORAL PRESENTATION - FULL PAPER

CAROB (*CERATONIA SILIQUA* L.) SEED EFFECT ON SEMEN QUALITY AND BLOOD PARAMETER IN LORI-BAKHTIARI RAM

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Abstract
Ceratonia silique L., is one of the herbal medicines which contains more than one flavonoid and carotenoid, is widely used for antioxidant purposes. Carob is good for sexual and blood metabolites functions. This study was conducted to investigate the effect of adding hydro-alcoholic extracts of carob seeds to the diet on semen quality and blood parameter in Lori-Bakhtiari Ram. In this study, carob seeds were extracted by percolation method. Thirty Lori-Bakhtiari rams used in current study were divided in control group (no carob extract), treated group (containing 150 mg of carob seed extract in diet). This experiment was performed in December and March. Sperm was collected from treated and control groups during the experimental month using the artificial vaginal. Sperm motility, were evaluated by the CASA software. Furthermore, Blood samples were collected for evaluation of Fasting Blood Sugar (FBS), Cholesterol (CH) and Triglyceride (TG). After using 150 mg/kg of carob extract in diet during experiment the motility of sperms was significantly increased compared to the control group while, the level of t FBS, CH and TG were significantly decreased ($P<0.05$).In general, it is concluded that the amount of 150 mg/kg of carob extract in the diet of Lori-Bakhtiari ram has a positive effect on sperm quality and blood parameters.

Key word: Carob, Semen Parameter, Blood parameter, Ram

1. Introduction

For increasing the pregnancy rates of domestic animals, requires sperm with high quality. Artificial insemination is used to fertilize domestic animals. (Davis et al., 2020). Today, for this purpose, sperm collection centers collect, produce, freeze, package sperm, and provide it to livestock centers. (Rodriguez-Martinez, 2007). Infertility is one of the important problems in domestic animal and some medicinal plants such as carob can have positive effects on increasing fertility (Mokhtari et al., 2012, Aghajani et al., 2019).

Ceratonia siliqua, contains flavonoid and carotenoid, phenolic compounds, and antioxidant. Furthermore, it contains a lot of minerals such as calcium, potassium, sodium, iron, phosphorus and vitamins. (Youssef et al., 2013, Najafi et al., 2017). Various parts of plants, such as fruit peel and seed, demonstrated the various chemical compounds (Ziya Motalebipour and Pirestani 2022). The effects of *Ceratonia siliqua* seed extract on diet have not been made known in ram. Therefore, the objective of this study was to determine the effects of adding hydro-alcoholic extract of carob (*Ceratonia siliqua*) seed to the diet on semen and blood parameters in ram.

2. Materials and Methods

1. Semen and blood collection

Semen samples were collected from thirty mature (50 ± 5 kg) Lori-Bakhtiari rams (3 to 4 years old). They were divided equally into 2 groups: control group and treated group with 150 mg/kg of carob seed extract in diet. Semen samples were collected from the rams using the artificial vagina (*imv*, Frances). It was performed in 2 breeding season in December and March. Semen collection was done twice a week and two times for each ram. However, at the same time of semen sampling, blood samples were collected from a jugular vein and their serum were separated in a laboratory for evaluation of Fasting Blood Sugar (FBS), Cholesterol (CH) and Triglyceride (TG).

2. Extract preparation

Percolation method were used to accomplish the *Ceratonia siliqua* extract (prepared in GOLDARU, Pharmaceutical Co. He and Hubbell, 2005).

3. Sperm motility

Sperm motility was evaluated using computer automated semen analysis (CASA analyzer, video sperm test 2.1), an Olympus BX40 microscope under 100× magnifications on a warm stage with 37 ˚C (Joshi et al. 2003, Kumar et al. 2007). Percentage of progressive motility (PM %) and percentage of total motility (TM %) were analyzed.

4. Statistical analysis

The data analysis was performed using the SPSS ver. 21 software package. Statistical analysis was carried out using the ANOVA procedure and the mean comparison was conducted by LSD test and considering a p-value < 5% as the statistically significance level.

3. Results and Discussion

3.1. Sperm motility

In this study, adding hydro-alcoholic extract of carob (*Ceratonia siliqua*) seed in diet was evaluated on sperm motility, in pre-freeze and post-thaw (Table 1). Percentage of progressive motility (PM %) and percentage of total motility (TM %) in treatment group (diet with 150 mg/kg) were significantly greater than the control group ($p < 0.05$) in both pre-freezing and post-thawing evaluation. Antioxidant protected proteins and enzymes against free radical attack or oxidation (Inouce et al., 1994; Osaretin and Gabriel, 2008, Pirestani and Ziya motalebipour, 2022). Therefore, this result is a good reason for increasing total motility of sperm in treatment group. Carob extract have an important role in semen quality recovery with regard to sperm concentration.

Table 1. Comparison of total and progressive motility of sperm of ram in two different times.

		Total Motility		Progressive Motility	
	Sampling time	**Control**	**Treatment**	**Control**	**Treatment**
Pre- freezing	December	52.0± 2.5	54.4± 2.8	29.0± 1.8	37.8± 2.7*
	March	48.0± 1.8	98.7± 3.5*	34.4± 1.5	43.9± 1.6*
Post-Thawing.	December	50.6± 1.5	48.78± 1.3	29.0± 1.3	37.8± 1.8*
	March	48.7± 1.7	69.8± 1.6*	27.9± 2.4	41.0± 1.9*

*indicated significant difference between control and treatment group at $P<0.05$.

3.2. Blood parameters

The effect of carob extract in diet were investigate on blood parameters in this study (Table 2). Carob significantly decreased fasting blood sugar (FBS), cholesterol (CH) and triglyceride (TG).

Table 2. Blood parameter in different experimental group.

No	**Blood Parameters**	**Sampling time**	**Control**	**Treatment**
1	**FBS (g/dL)[1]**	December	76±1.9	68±1.8*
		March	71±1.9	58±1.9*
2	**TG (mg/L)[2]**	December	20±1.6	14±1.7*
		March	25±1.3	19±1.9*
3	**CH (mg/dL)[3]**	December	48±1.6	45±1.6
		March	49±1.3	43±1.6*

*indicated significant difference between control and treatment group at $P<0.05$.
[1] FBS: Fasting Blood Sugar
[2]TG: Triglyceride
[3] CH: Cholesterol

Regarding the blood sugar factor, a significant difference ($p<0.05$) was observed between treatment group and control group. There was a statistically significant difference ($p<0.05$) in the level of cholesterol (CH) between treatment and control group. The amount of triglyceride in treatment group had a statistically significant difference ($p<0.05$) with control. Recent studies found carob as a significant and positive impact on blood parameter of animal (El-Manfaloty and Ali, 2014). And similar results were observed by Macho-Gonzalez et al., (2019) and Rtibi et al., (2021). Carob extraction in diet significantly decreases levels of FBS, TG and CH. which can explain by the pathways of hepatic metabolism directly.

Conclusion

According to current results, the increased semen quality were observed by adding the carob extraction in diet during four month usage. Extracts concentration was one of the main factors which effected on the sperm parameters. The usage of 150 mg/kg of carob extract for 4 month in Lori-Bakhtiari ram diet improved sperm quality and increase total and progressive motility of sperms before and after a freezing-thawing process. Carob treatment may be effective in infertility of Lori-Bakhtiari ram in reproduction age. These results confirm the traditional use of carob for increasing the possibility of fertility and improve the blood parameter.

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MESMAP – 9
ABSTRACTS & PROCEEDINGS BOOK
03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION - FULL PAPER

AMINO ACID DERIVED ELECTROSPUNNED ELECTRODES FOR SUPERCAPACITOR APPLICATIONS

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Abstract

In this study, Histidine decorated GO-PANI ternary composite was synthesized on acid-activated carbon cloth (CC) through electrospining processes. This electrode material exhibits good electrochemical behavior with specific capacitance reaching 55.6 $mFcm^{-2}$, as well as the highest areal power (12.6 Wm^{-2}) and energy (0.32Whm^{-2}) densities.

Furthermore, an asymmetric hybrid supercapacitor (AHS) was designed using HGO-PANI@CC as the positive electrode, graphite plate (G) as the negative electrode and 0.5 M Na_2SO_4 aqueous solution as the supporting electrolyte. The supercapacitor shows maximum power density of 662 mWm^{-2} and energy density of 16.2 $mWhkg^{-1}$ at the current density of 1 $mAcm^{-2}$ with a operational voltage window (1.0 V) and a good capacitance retention. The results show that the HGO-PANI@CC electrodes are promising materials for the future generation of biocompatible supercapacitors.

Key Words: Biosupercapacitor, biocompatibility, energy storage, wearable electronics, Atomic Force microscopy, electrochemistry

1. Introduction

Portable, wearable energy storage devices with high safety and unlimited cycle life have gained increasing attention for the development of next-generation personal electronics. The application areas of these systems range from implantable medical devices to electrochemical sensors, from solar energy conversion to stimulators and actuators. One of the most important types of energy storage systems is supercapacitors. Their main advantages of them are high power–energy densities, robust cycle life, and rapid charging capabilities. Their scalable dimensions make them attractive for a variety of applications in biological systems such as electronic skins, capsule endoscopies, deep brain

stimulators, cardiac pacemakers, transcorneal electrical stimulators, etc. [1-3]. The main parameters affect that the performance of supercapacitors are capacitance, energy-power densities, and operational potential windows.

According to the literature review, it is reported that the specific capacitance increases proportionally with the increase of the surface area [4-7]. Thus, in the present study, histidine amino acid-loaded PANI-GO nanomembranes (HGO-PANI) were fabricated on the conductive carbon fabric by the electrospinning method. Nanomembranes with an increased surface area were produced by this nanotechnological production obtained via the electrospinning technique [8-9]. This technique is aimed to increase the supercapacitor performance and to produce a biocompatible nanomembrane electrode material by amino acid loading.

Biofriendly, amino acid-based energy storage devices that successfully work body fluids of living organisms as electrolytes and electrode materials are nontoxic to biosystems. Thus, their performance is highly promising for future biosupercapacitor applications. To prepare a biocompatible supercapacitor, histidine amino acid-functionalized graphene oxide (GO) and polyaniline (PANI) ternary nanocomposites were used as the active material of the electrode for energy storage applications.

Polyaniline shows several useful properties as a conductive polymer, such as good reversibility and electro-conductivity that could be served good pseudocapacitive behavior. However, their mechanical degradation due to the doping-undoping steps limits the cycle stability in charge-discharge processes. Thus, the modification or doping of PANI using carbon-derived substances such as active carbons, carbon aerogels, carbon nanotubes, graphene, and graphene oxides, etc. provide conductivity to the composite. The most preferred carbon-derived materials using this aim are graphene oxides (GO). They have several advantages in terms of chemical, optical and electronic properties that allow them to be considered as an independent nanomaterial with a wide range of applications [10].

The electrospinning technique was carried out to coating of the current collector surface. The ink was prepared in DMF as the solvent. The fiber morphology and chemical characterization of electrospunned film was investigated by atomic force microscopy (AFM) and Scanning Electron Microscopy (SEM) techniques, respectively.

Electrochemical behavior of the prepared films was studied by the electrochemical workstation. Cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS) techniques were applied for the evaluation of electrochemical performance. The electrode performance was evaluated by a three-electrode system, while the supercapacitor prototype was investigated by two-electrode system.

2. Material and Methods

2.1. Reagents and chemicals

$KMnO_4$ (≥99%), $NaNO_3$, H_2O_2 (30%), H_2SO_4, HNO_3, graphite powder, $(NH_4)_2S_2O_8$ were supplied from Merck Chemical Co. (Darmstadt). Aniline, DMF, histidine were purchased from Sigma-Aldrich. They were used as received without further purification.

2.2. Preparation of graphene oxide

Synthesis of GO was performed by Modified Hummer's Method. According to this procedure[11]. 2 g powdered graphite, 2 g sodium nitrate, 50 mL 98% sulfuric acid were mixed in an ice bath and stirred for 2 hours. 6 g of $KMnO_4$ was added to the mixture. During the process, the temperature was kept at 35 °C for 24 hours. Then 100 mL of DI water was added to the solution. The 30 % H_2O_2 was dropped into the mixture and was mixed for 2 hours. At this stage, the color of the mixture changed from black to brown. The precipitate was washed throughly with 5 mL of HCl and 10 mL DI water mixture until the pH balance was reached. The product was dried at room temperature for 24 hours and GO was obtained in a powder form.

2.3. Preparation of HGO-PANI@CC electrodes

For the synthesis of PANI, 0.4 ml of aniline was mixed with 10 ml of 0.1 M APS in an ice bath. Then, to prepare the ink solution, histidine solution in methanol and aniline were mixed in the molar ratios 1:10 and 20 ml of DMF was added to this solution. After mixing throughly, the GO was added to the mixture. The activated carbon cloth electrodes were decorated by electrospinning method with the prepared ink solution. The obtained electrode was named as HGO-PANI@CC.

2.4. Characterization techniques

Atomic force microscopy (AFM) and Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFT) techniques were applied for the characterization of active electrode materials. Electrochemical behavior of the electrospunned films was studied using cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS) techniques.

Conventional three-electrode system was used for the evaluation of electrochemical performance of electrodes. HGO-PANI@CC was used as the working electrode (WE), Ag/AgCl was the reference electrode (RE) and Pt plate (~0.4 cm^{-2}) was utilized as the counter electrode (CE). Electrochemical performance was carried out by Gamry Interface 1010 E potansiostat/galvanostat combined with Echem Analyst Software interfaced with a PC. To prevent Ag^+ and Cl^- ions leaking into the WE compartment double bridged design was adopted for RE. During experiments uncompensated resistance between the RE and WE was corrected by IR compensation mode. All experiments were performed at room temperature (25 ºC) and atmospheric pressure.

DRIFT analysis was done with a Bruker Alpha DRIFT spectrometer with a universal module. This included two sample cups. All of the spectra were recorded by averaging 100 scans at a resolution of 4 cm^{-1}. Background spectrum was obtained from pure CC, and the spectra of the HGO-PANI@CC electrodes corrected against the background. The spectra were corrected for the negative effects of atmospheric CO_2 and humidity. OPUS 6.5 version was used as the software (Bruker Optics, Inc.).

Scanning electron microscopy combined with energy dispersive X-ray spectroscopy (QUANTA brand FEI FEG 450 Model Field Emission Gun Scanning Electron Microscopy (FEGSEM) was used to investigate the morphology and distribution of the synthesized graphene oxide. Images were recorded at 20 kV.

Prior to electrospinning, the viscosity of the polymer solution was determined with a digital viscometer (DV-E, Brookfield AMETEK, USA). Density was measured using a 10 mL specific gravity flask. All measurements were made at room temperature (25 °C).

HGO-PANI nanomembranes were prepared using a laboratory scale electrospinning unit (NS24, Inovenso Co., Turkey). The stainless steel needle (ID: 0.3 mm and OD: 0.8 mm) was used to control the flow while the pump was connected to a syringe controlled by a syringe pump (NE-300, New Era Pump Systems, Inc., USA) grade with a needle. At the beginning of the spinning process, the polymer solution was placed in the syringe and then a high voltage was applied between the needle and the collector. In order to collect the nanofibers during the spinning process, the circular collector was covered with wax paper and the distance of the needle tip to the collector was set as 45mm. Electrospinning was carried out at an applied voltage of 22-30 kV and a flow rate of 1-3 mL/h.

The nanostructural properties and height asymmetries of the HGO-PANI@CC examined with an AFM instrument, which was produced by Nanomagnetics Instruments. The dynamic mode was used for the operation at room temperature with aluminumcoated silicon probes (PPP–NCLR nanosensors). The samples were scanned at a 5 ms-1 scanning rate and a 256x256 pixel resolution to obtain a view with a 5x5 cm2 area. The statistical parameters were calculated from the AFM images with the NMI Viewer 2.0.7 version Image Analyzer Software.

3. Results and Discussion

3.1. Characterization of HGO-PANI@CC Electrode

Structural and electrochemical characterization of HGO-PANI@CC was performed AFM and electrochemical techniques, respectively. Furthermore, the GO prepared by Hummer's Method was investigated using SEM technique.

3.1.1. Scanning Electron Microscopy (SEM) Studies

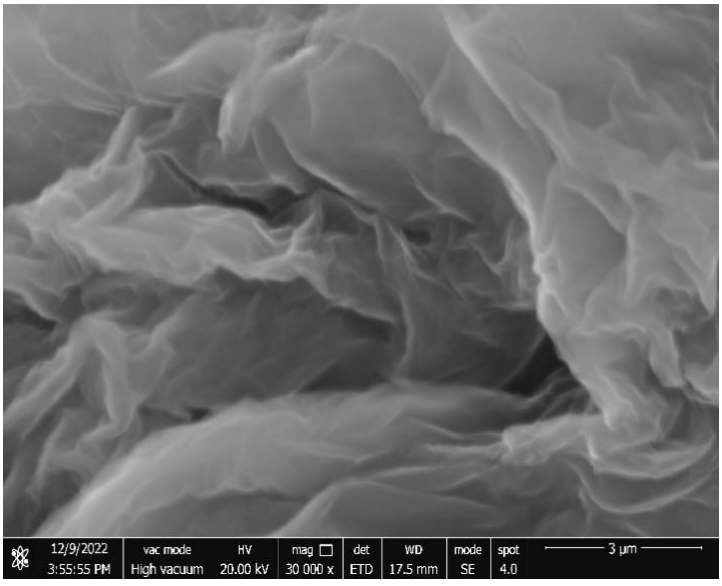

Figure 1. The scanning electron microscopy image of GO produced by Modified Hummer's method.

According to the Fig. 1, the SEM images of GO shows characteristic 3D-stacked layers. The flakes are so prominent on the surface. Images of layered GO structures were obtained by zooming in to 3 µm (30.00kx) with the SEM device (Figure 1). SEM of the layered GO structures seen in the photographs

sometimes with several layers in line with the literature that there are scattered overlapping GO structures observed. [10,12].

3.1.2. Atomic Force Microscopy (AFM) Studies

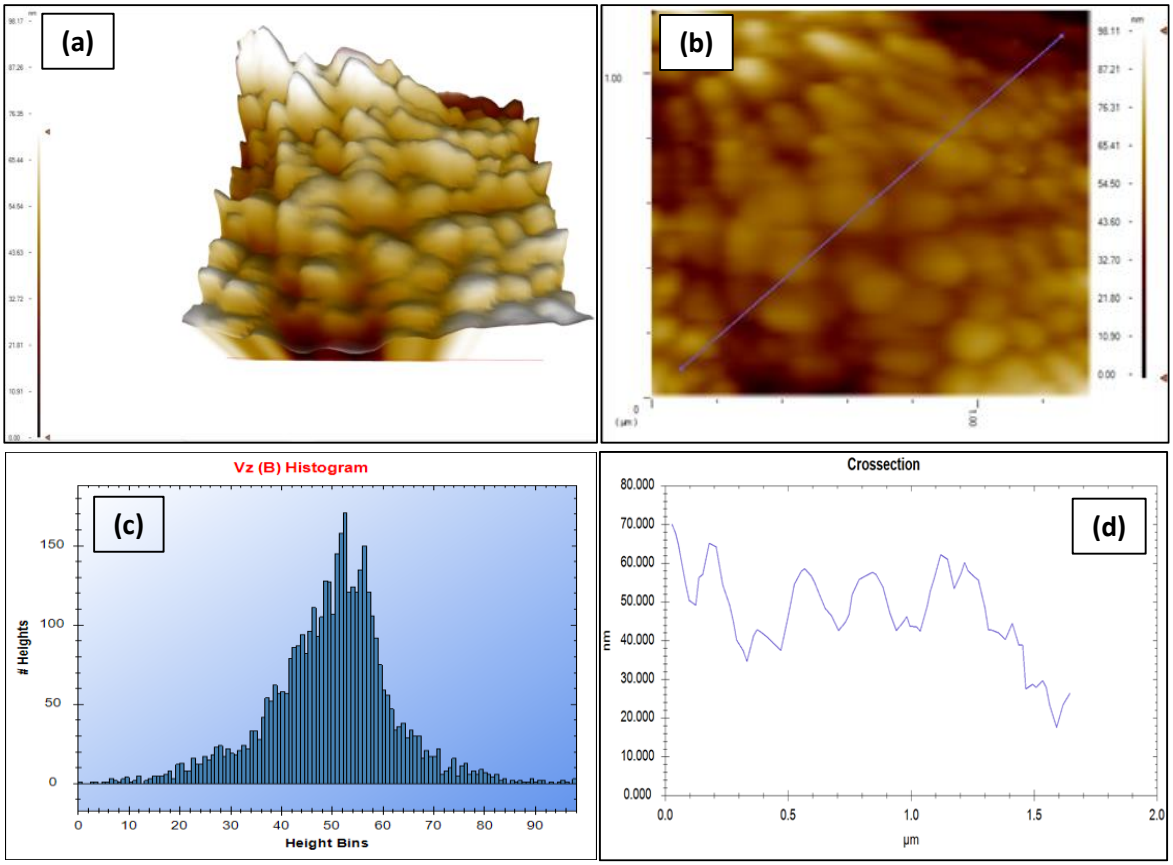

Figure 2. AFM height images a) 3D view, b) 2D view, c) height distribution, d) the height profile through crossectional line shown in Fig. 2.b.

The morphological images of the electrode surfaces provide valuable information about the roughness, heterogenity and size distribution of the electrode surface. Fig.2. shows three-dimensional (3D), two-dimensional AFM images (2D), height distribution histograms and line crosssection profile of HGO-PANI@CC electrode. From the image, it was revealed that peaks and valleys like formation. This characteristic structure causes high values of roughness parameters. The AFM images were used to estimate the height asymmetry and distributions. Table 1 shows the values of average roughness, RMS roughness, Skewness (Ssk) and Kurtosis (Sku) statistical parameters of the HGO-PANI@CC electrode surface. The RMS and the average roughness were found as 9.09 nm and 12.23 nm, indicating a rough and nano scaled surface. According to the surface kurtosis and Skewness values it was revealed that the surface has more valleys than the peaks with respect to the mean surface.

Table 1. Statistical parameters obtained from AFM images of HGO-PANI@CC electrode surface.

Coefficient	Value	Unit	Description
sa	9.0894	nm	Average
sq	12.2338	nm	Root Mean Square
ssk	-0.2242	nm	Surface Skewness
sku	4.3483	nm	Surface Kurtosis

3.2. Electrochemical Performance

The electrochemical performance of HGO-PANI@CC electrode was investigated by CV, GCD and EIS techniques (Fig.3).

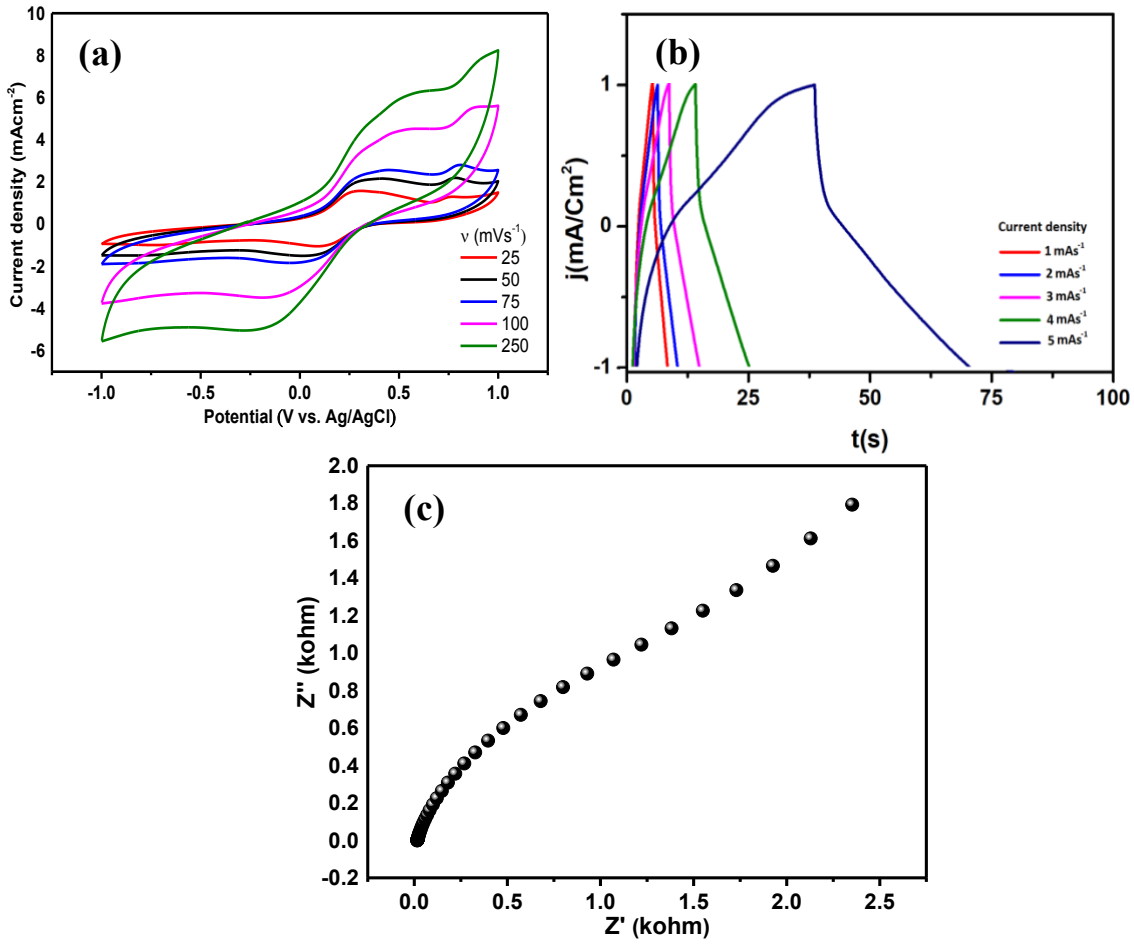

Figure 3. a) CV voltammograms of HGO-PANI@CC electrode as a function of various scan rates ranging between 25-250 mVs^{-1}, b) GCD curves in different current densities, c) EIS spectrum in the frequency range between 0.01 Hz-100 kHz and 10 mV amplitude.

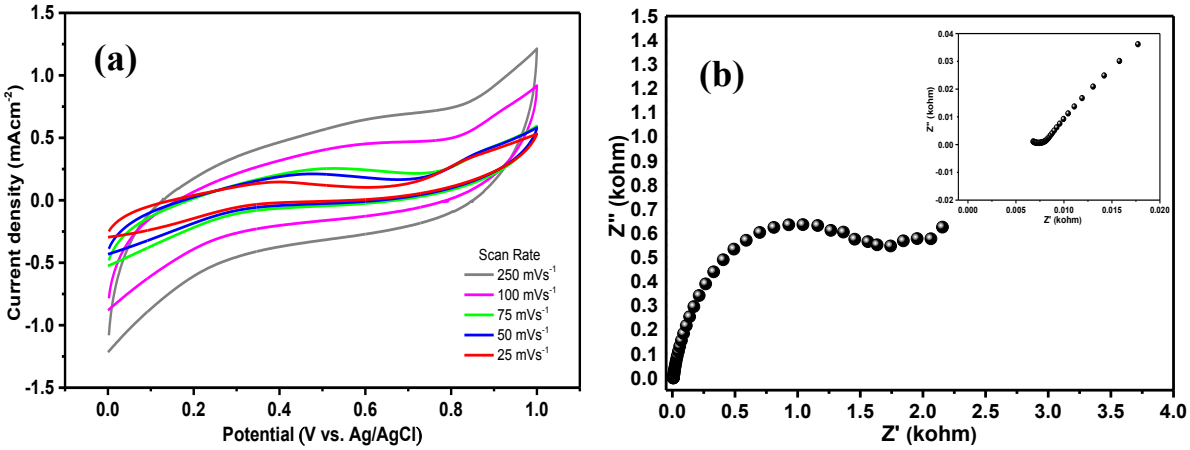

Figure 4. a) CV voltammograms as a function of various scan rates ranging between 25-250 mVs^{-1}, b) EIS spectrum (in the frequency range between 0.01 Hz-100 kHz and 10 mV amplitude) of HGO-PANI@CC//Graphite supercapacitor device.

The areal capacitance was calculated from the the CV curves according to following equations:

$$C_{CV} = \frac{\int_{Va}^{Vc} I dV}{A\vartheta(V_c - V_a)}$$

where C is the areal capacitance in Fcm^{-2}, A (cm^2) is the area of the active electrode surface, ϑ is the scan rate, (Vs^{-1}) is the $\int_{Va}^{Vc} I dV$ integrated area under the CV curve, (Vc-Va) is the potential window (V) and Δt is the discharge time (sec).
The energy density (E) and the power density (P) are determined from the following equations:

$$E = \frac{1}{2} C(\Delta V)^2$$

$$P = \frac{E}{\Delta t}$$

where C is the areal capacitance ($mFcm^{-2}$), ΔV is the potential window (V), P represents the power density ($Whcm^{-2}$) and Δt is the discharge time (sec).

4. Conclusion

In this study, histidine loaded GO-PANI ternary composite produced for modification of acid activated carbon cloth in order to obtain biofriendly supercapacitor prototype. The modification process was performed electrospinning technique to provide increasing surface area on the electrode. The porous nanomembranes was utilized for supercapacitor positive electrode. The structural characterization of the electrode was performed by SEM and AFM techniques, while the electrochemical characterization of the electrode was carried out using CV, GCD and EIS techniques.

As a future prospect, biocompatible nanohybrid supercapacitors can be developed via amino-acid derived nanocomposite materials to increase the cell voltage (V), active surface area, energy and power densities.

Conflict of Interest

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

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ORAL PRESENTATION - FULL PAPER

BIO-INSPIRED ELECTRODE FABRICATION FOR HIGH PERFORMANCE ENERGY STORAGE SYSTEMS

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Abstract

In the present study, biocompatible electrode active materials were synthesized for energy storage applications. Histidine and L-proline amino acids were used to produce ternary nanocomposites that contained graphene oxide (GO) and polyaniline (PANI) moieties. The hydrothermal synthesis was utilized to fabricate wearable electrode materials.

The fabricated supercapacitor devices exhibit good electrochemical performances with a maximum areal capacitance of 0.8 $mFcm^{-2}$ and 1.8 $mFcm^{-2}$ for HGO-PANI@CC/Graphite and PGO-PANI@CC/Graphite, respectively. The highest areal power was calculated as 439.65 mWm^{-2} and the energy density was 1.1 Whm^{-2} for histidine derived supercapacitor device while 1404.8 mWm^{-2} energy density and 2.5 Whm^{-2} power density were obtained from the PGO-PANI@CC/Graphite supercapacitor device.

Key Words: Bio-inspired supercapacitor, wearable, energy storage, L-proline, histidine, carbonaceous material.

1. Introduction

Increasing energy consumption and worldwide environmental problems lead to high interest in renewable energy resources. Recently, energy storage and conversion systems such as batteries, supercapacitors, photovoltaic devices, fuel cells, etc., are at the forefront of technological progress. For this perspective, researchers have focused on the fabrication of novel electrodes and electrolyte systems.

Among the energy storage devices, supercapacitors are a favorite since they have unique properties such as high power density, rapid charge and discharge capability, long cycle life, and excellent security features. Creating high-performance electrode materials is vital to obtain effective supercapacitor devices. The core components of supercapacitors are the anode, cathode, electrolyte, and separator units. Thus, the electrode material is one of the major factors affecting the electrochemical performance of supercapacitors.

Lately, bio-inspired energy storage devices gain immense attention due to their biocompatibility, abundant resources, low cost, availability, and low toxicity [1-2]. For this aim, one of the interesting approaches to obtaining biocompatible devices is the use of natural materials such as

amino acids, peptides, enzymes, etc., in active electrode materials design. These materials have excellent advantages such as biocompatibility, natural abundance, availability, and containing active functional groups.

In the present work, the electrode materials composed of biological components were prepared for energy storage devices. Amino acids such as histidine and L-proline were used to fabricate electrode materials. L-proline which is a natural non-essential amino acid and histidine that is an N-rich amino acid containing an amino group, an imidazole side chain, and a carboxylic group in its structure were used as a substance for active electrode preparation. These functional groups allow efficient faradaic electron transfers [3]. The other components of active electrode materials were chosen in carbon-derived samples and polyaniline conducting polymers. Among numerous synthesis procedures of electrode materials, surface modification with carbon-derived substances plays an important role to obtain a high specific surface area, good electrical conductivity, eco-friendliness, and low cost [4].

In this study, polyaniline and graphene oxide were used to design electrode active materials. Amino acid-derived GO-PANI ternary nanocomposites were synthesized through a hydrothermal process. The products were named PGO-PANI@CC and HGO-PANI@CC. The electrochemical performances of the two electrodes were compared.

The electrochemistry of the prepared electrodes was evaluated by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and galvanostatic charge-discharge (GCD) techniques. The experimental process for electrode characterization involved a three-electrode system, while two-electrode systems were used for the evaluation of the supercapacitor device.

The structural characterization of electrode materials was performed atomic force microscopy (AFM) technique.

2. Material and Methods

2.1. Reagents and chemicals

Aniline, DMF, and histidine were purchased from Sigma-Aldrich. Potassium permanganate ($KMnO_4$) (≥99%), sodium nitrate ($NaNO_3$), hydrogen peroxide (H_2O_2 (30%), sulfuric acid (H_2SO_4), nitric acid (HNO_3) graphite powder, ammonium peroxy disulfate $(NH_4)_2S_2O_8$ were supplied from Merck Chemical Company from Darmstadt. They were used as received without further purification.

2.2. Preparation of GO

Synthesis of GO was performed by Modified Hummer's Method. According to this procedure, (Chen et al. (2013), Song et al., (2014), Sun and Fugetsu (2013)), 2 g graphite powder, 2 g sodium nitrate, 50 mL 98% sulfuric acid were added together in an ice bath and stirred vigorously for 2 hours. 6 g of $KMnO_4$ was added to the mixture. During the process, the temperature was kept at 35 °C for 24 hours. Then 100 mL of DI water was added to the solution. The 30 % H_2O_2 was dropped into the mixture and was stirred for 2 hours. At this step, the mixture color changed from black to brownish. The precipitated product was washed vigorously with HCl and DI water mixture (v/v 1:2) until the pH balance was kept stable. Then the product was dried at room temperature for 24 hours.

2.3. Preparation of HGO-PANI@CC and PGO-PANI@CC electrodes

For the PANI synthesis, 0.4 mL of aniline was mixed with 10 mL of 0.1 M ammonium persulfate in an ice bath. Then, to prepare the nanocomposite, the solution of histidine or L-proline in methanol and aniline were mixed in the molar ratios 1:10. After mixing throughly, the GO addition was followed the mixing procedure. The conductive carbon textile electrodes were decorated by hydrothermal method. For this aim, the solution was prepared and a piece of carbon textile put in a Teflon lined otoclave

reactor and it was placed into the muffle furnace for 12 hours at 120 °C. The obtained electrodes were named as HGO-PANI@CC and PGO-PANI@CC.

2.4. Characterization Studies

DRIFT Spectroscopy and AFM techniques were utilized for the chemical and structural characterization of active electrode materials. Electrochemical behavior of the electrodes was investigated using cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS) techniques.

The three-electrode system was applied for the investigation of electrochemical performance of electrodes. HGO-PANI@CC and PGO-PANI@CC were used as the working electrodes (WE), Ag/AgCl was utilized reference electrode (RE) and Pt plate (~0.4 cm^{-2}) was operated as the counter electrode (CE). Electrochemical performance was performed by Gamry Interface 1010E potansiostat/galvanostat with Echem Analyst Software interfaced combined with a PC. During experiments, the uncompensated resistance was corrected by IR compensation mode. All experiments were carried out at room temperature (25 °C) and ambient pressure.

DRIFT analysis was performed with a Bruker Alpha DRIFT spectrometer combined with a universal module. All of the spectra were recorded by averaging 100 scans at a resolution of 4 cm^{-1}. Background spectra were taken from pure CC, and the spectra of the electrodes corrected against the background. The spectra were corrected for the negative effects of atmospheric CO_2 and humidity. OPUS 6.5 version was used as the software (Bruker Optics, Inc.).

Scanning electron microscopy combined with energy dispersive X-ray spectroscopy (SEM-EDX, SEM, MA-EVO10, ZEISS) was used to investigate the morphology and distribution of the synthesized graphene oxide. Images were recorded at 20 kV.

The nanostructural properties and height asymmetries of the electrodes investigated with an Ambient AFM instrument, which was produced by Nanomagnetics Instruments. The dynamic mode was used for the operation at room temperature with aluminumcoated silicon probes (PPP–NCLR nanosensors). The samples were scanned at a 5 ms^{-1} scanning rate and a 256x256 pixel resolution to obtain a view with a 5x5 cm^2 area. The statistical parameters were calculated from the AFM images with the NMI Viewer 2.0.7 version Image Analyser Software.

3. Results and Discussion

3.1. Characterization of HGO-PANI@CC and PGO-PANI@CC Electrode

Structural, chemical and electrochemical characterization of electrodes were realized by AFM, DRIFT spectroscopy and electrochemical techniques, respectively. Furthermore, SEM technique was used for the morphological characterization, also.

3.1.1. Scanning Electron Microscopy (SEM) Studies

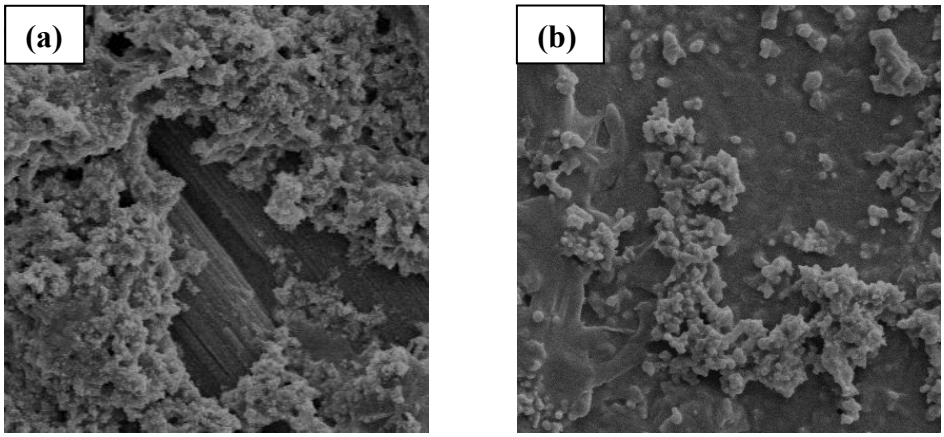


Figure 1. The scanning electron microscopy images of a) PGO-PANI@CC, b) HGO-PANI@CC electrodes.

According to the Fig. 1, the SEM images of electrodes show high surface area. When compared proline-modified electrode with the histidine derived electrode, it was revealed that the first one has more porous structure. HGO-PANI@CC surface has randomly distributed granular shape particles. Besides, it was clearly observed the sticky and flat formation on the surface.

3.1.2. Atomic Force Microscopy (AFM) Studies

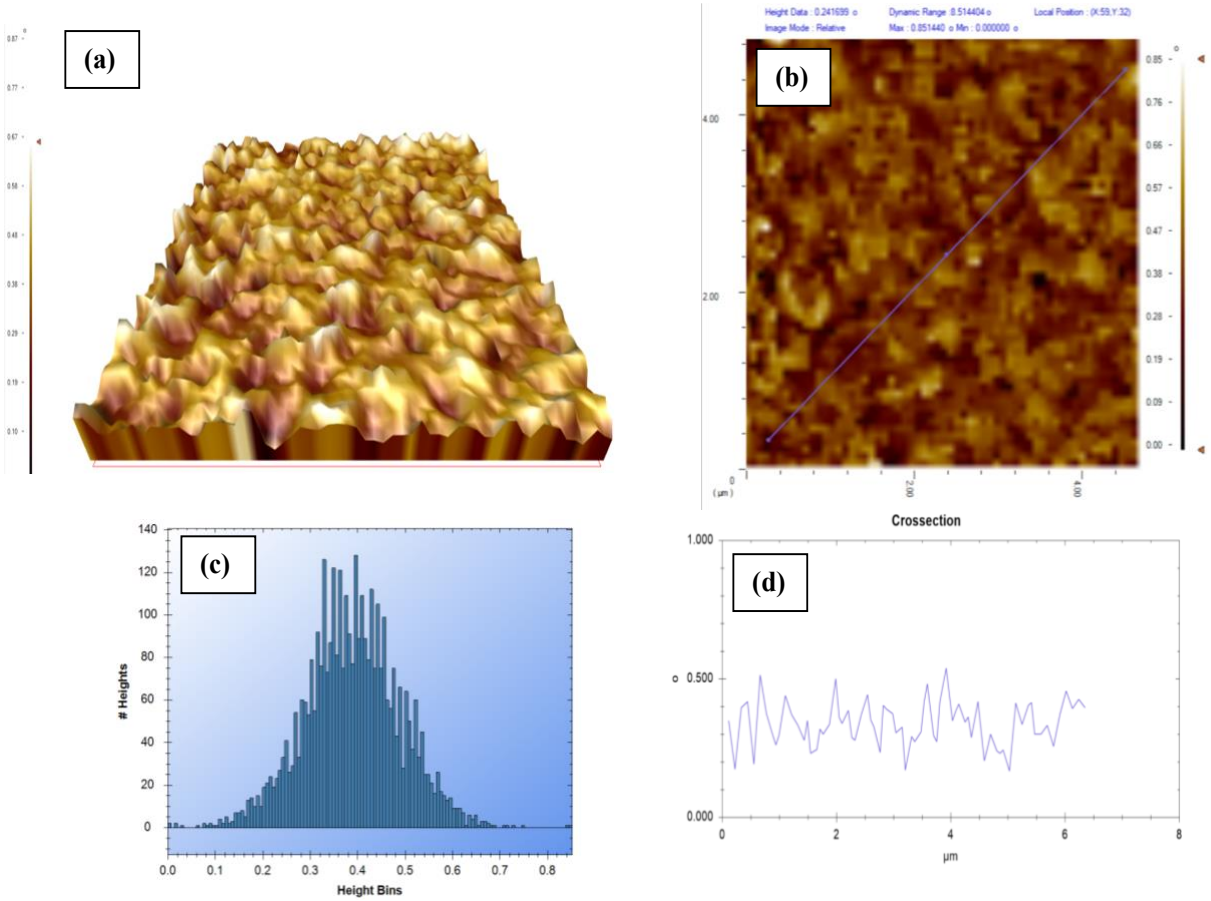


Figure 2. AFM images of PGO-PANI@CC electrode. a) 3D view, b) 2D view, c) phase distribution histogram and d) crossectional line profile.

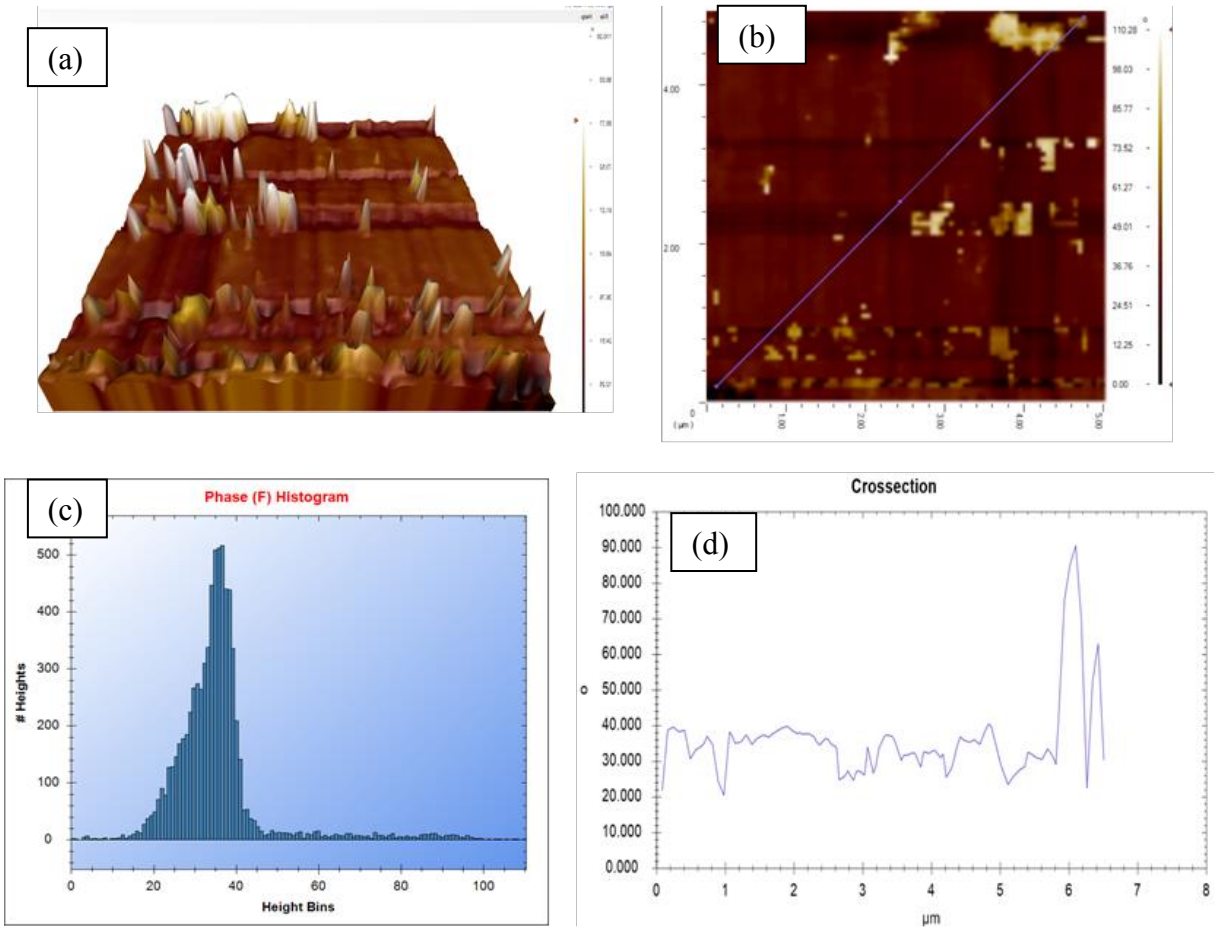


Figure 3. AFM images of HGO-PANI@CC electrode, a) 3D view, b) 2D view, c) phase distribution histogram and d) crossectional curve showing phase changes.

The morphological characterization of the electrode can give useful information about the roughness, size distribution and homogeneity of the electrode surface. Fig. 2 and Fig. 3 exhibit three-dimensional (3D), two-dimensional AFM images (2D), height distribution histograms and line crosssection profile of PGO-PANI@CC and HGO-PANI@CC electrodes, respectively. From the figure, it was clearly observed peaks and valleys like formation on the surface. When compared HGO-PANI@CC, PGO-PANI@CC has more dense and peaked structure. This characteristic structures causes to increase roughness parameters. The AFM images were also used to evaluate the height asymmetry and distributions.

3.1.3. DRIFT Spectroscopy

The characteristic peaks corresponding to the PANI moiety observed at 1216 cm^{-1}, 1434 cm^{-1}, 1539 cm^{-1} were attributed to C-O stretching band, C=C stretching vibrations of quinonoid and benzenoid rings in the PANI structure. The shift observed from ~1600 cm^{-1} to 1539 cm^{-1} indicates that π-π interactions between GO – PANI structure. The N-H bands were observed at about 3100-3200 cm^{-1}.

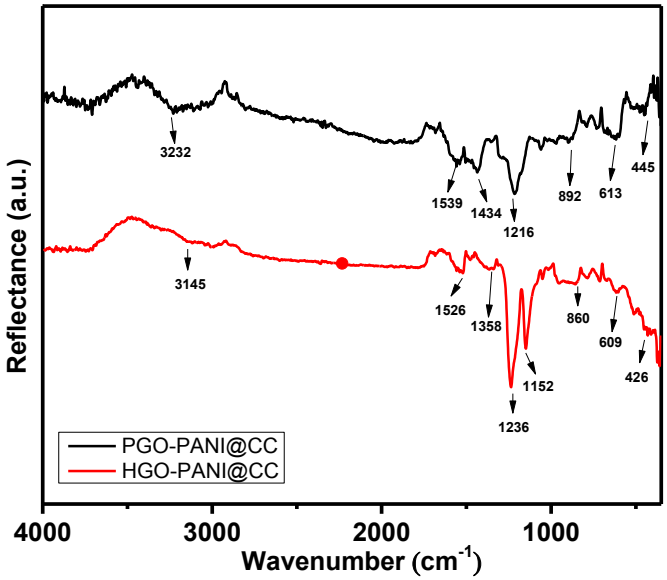
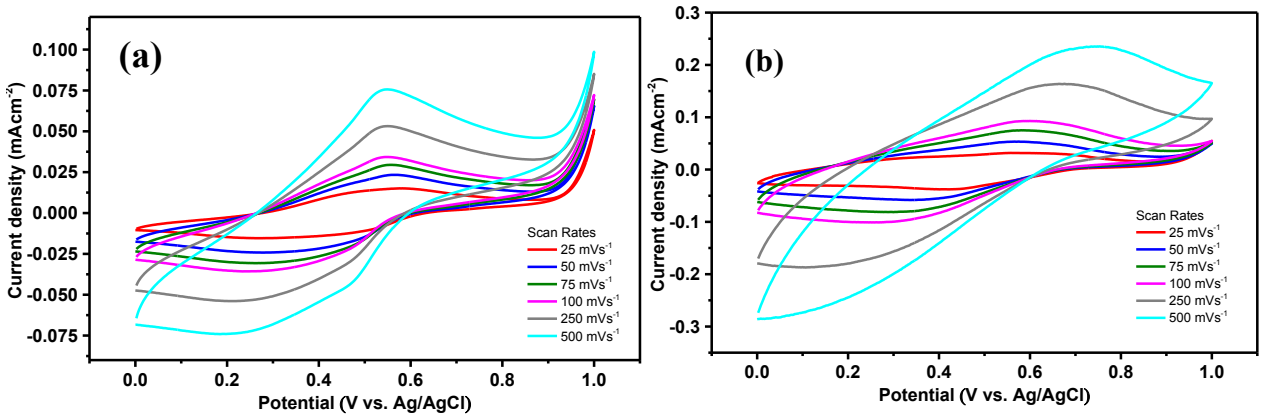


Figure 4. DRIFT spectra of HGO-PANI@CC and PGO-PANI@CC electrodes.

3.2. Electrochemical Studies

The electrochemical performance of HGO-PANI@CC/Graphite and PGO-PANI@CC/Graphite supercapacitor device were investigated by CV and EIS techniques (Fig. 5).



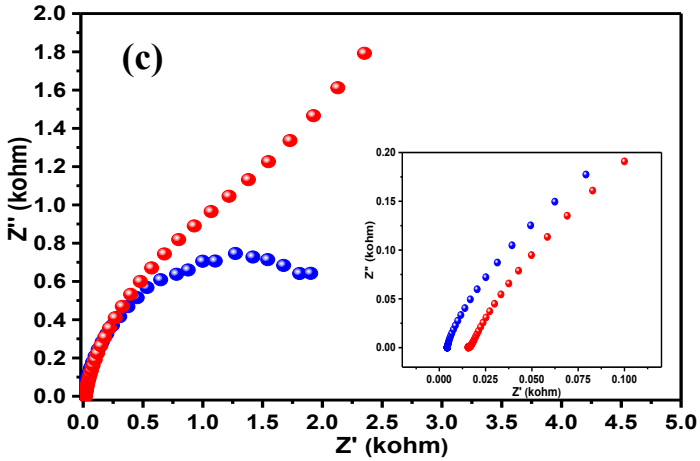


Figure 5. a) CV voltammograms of HGO-PANI@CC/Graphite as a function of various scan rates ranging between 25-250 mVs^{-1},
b) CV voltammograms of PGO-PANI@CC/Graphite as a function of various scan rates ranging between 25-250 mVs^{-1}, c) EIS spectra (in the frequency range between 0.01 Hz-100 kHz and 10 mV amplitude) of HGO-PANI@CC/Graphite and PGO-PANI@CC/Graphite supercapacitor devices.

The areal capacitance were calculated using CV curves according to the following equations:

$$C_{CV} = \frac{\int_{Va}^{Vc} IdV}{A\vartheta(V_c - V_a)}$$

where C is the areal capacitance in Fcm^{-2}, A (cm^2) is the area of the active electrode surface, ϑ is the scan rate, (Vs^{-1}) is the $\int_{Va}^{Vc} IdV$ integrated area under the CV curve, (Vc-Va) is the potential window (V) and Δt is the discharge time (sec).
The energy density (E) and the power density (P) are determined from the equations (3) and (4), respectively.

$$E = \frac{1}{2} C(\Delta V)^2$$

,

$$P = \frac{E}{\Delta t}$$

where C is the areal capacitance ($mFcm^{-2}$), ΔV is the potential window (V), P represents the power density ($Whcm^{-2}$) and Δt is the discharge time (sec) [5].

According to the equations above, the electrochemical performances of supercapacitor devices were given in the following table.

Table 1. The maximum capacitance, energy and power densities of supercapacitor devices

Supercapacitor	Max. Capacitance ($mFcm^{-2}$)	Max. Energy Density ($mWhm^{-2}$)	Max. Power Density (mWm^{-2})
HGO-PANI@CC/Graphite	0.8	1.1	439.65
PGO-PANI@CC/Graphite	1.8	2.5	1404.8

4. Conclusion

In the present study, HGO-PANI and PGO-PANI ternary nanocomposites were synthesized via a hydrothermal deposition process on carbon textile surfaces. The porous electrodes were utilized for supercapacitor-positive electrodes. The morphological and structural characterization of the electrode was performed by SEM and AFM techniques, while the electrochemical characterization of the electrode was performed using CV and EIS techniques.

The results obtained from experimental processes show that PGO-PANI@CC/Graphite supercapacitor device has higher energy and power densities. Its capacitance was found greater than that of histidine-derived supercapacitor devices. The results were in good agreement with the AFM and SEM findings.

Conflict of Interest

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

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ORAL PRESENTATION - FULL PAPER

TOTAL PHENOLIC CONTENT, ANTIBACTERIAL AND ANTIOXIDANT INVESTIGATIONS OF *CYSTOSEIRA FOENICULACEA* BROWN MACROALGAE COLLECTED FROM THE MEDITERRANEAN (ANTALYA)

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Abstract

Cystoseira foeniculacea macroalgae contains several medicinal traditionally used and pharmacologically explored. However, *C.foeniculacea* has not been well valorized.
So, the aim of the present study was to evaluate the antibacterial and antioxidant activities, total phenolic (TPC) and flavonoid (TFC) contents of *C.foeniculacea* different extracts. To achieve the objectives of this study, ethanol, methanol and aqueous extracts of *C.foeniculacea* were prepared. Then, antibacterial activity was evaluated against gram positive bacteria *Staphylococcus aureus* ATTC 43300, *Bacillus cereus* ATTC 11778, *Sarcina lutea* ATTC 9341 and gram negative bacteria *Escherichia coli* ATTC 25922, *Pseudomonas aeruginosa* ATTC 27853, *Klebsiella pneumoniae* ATTC 70603, *Salmonella enteritidis* ATTC 13076 by broth microdilution methods. The antioxidant activity was evaluated by DPPH scavenging assay, ABTS, CUPRAC and metal chelating activity.
TPC and TFC of the extracts were calculated as 14.38±1.22- 37.75±1.05µg GAEs/mg extract and 8.43±0.60–30.13±1.87µg QEs/mg extract, respectively. BHT (Butyryl hydroxy toluene) and BHA (Butyryl hydroxy anisole) and EDTA were used as standard in the total antioxidant activity determination methods we applied to *C.foeniculacea* extracts and the highest ABTS radical scavenging activity in ethanol extract of *C.foeniculacea* (IC_{50}: 37,91±0,73µg/ml), metal chelating activity values of *C.foeniculacea* in ethanol extract (IC_{50}: 138,86±0,71µg/mL) ml). CUPRAC activity in ethanol extract($A_{0.50}$:150,0±0,12 µg/mL).DPPH scavenging assay did not show any significant activity in any extract of *C.foeniculacea*.
The highest antimicrobial effect against the tested pathogens was seen in the methanol extract of the *C.foeniculacea*, and the most effective strain was determined as gram (-) bacteria Pseudomonas aeruginosa (ATTC 27853) (MIC: 1.562 mg/ml). The water extract did not show antimicrobial activity against any test pathogen.
Therefore, *C.foeniculacea* could be a good source for the identification of antioxidant and antibacterial drugs. In addition, the observed findings could open new horizons on the ethnobotanical usages of *C.foeniculacea*. But, further investigations are required to identify and isolate bioactive compounds from this macroalgae as well the investigations of their biological effects.

Key Words: Antibacterial, antioxidant, macroalgae, Mediterranean.

1. Introduction

Macroalgae have been shown to produce an array of compounds provided of interesting biological properties including antioxidant activity (Balboa et al., 2013). In particular, brown algae have been the richest algal source of antioxidant natural products, mainly meroterpenoids and phlorotannins. The use of antioxidants in functional foods, nutraceuticals, cosmetics, and pharmaceuticals has been greatly extended during the last years. Moreover, the growing preference of consumers for compounds from natural sources over synthetics, has led to intense research on the antioxidant capacity of natural products. Brown algae of the genus Cystoseira (family Sargassaceae) are widely distributed in the Mediterranean Sea The genus Cystoseira (Cystoseiraceae, Phaeophyta) contains about 50 species around the world (De Los Reyes, 2013) is widespread in Atlantic intertidal zones and Mediterranean, and is relevant for its biochemical and medical use. *Cystoseira foeniculacea* (Linnaeus) Greville is a marine brown alga which is distributed extensively, as most of the Cystoseira species, in the Mediterranean Sea and to a lesser extent, along the northwestern coasts of the Atlantic Ocean (Guiry and Guiry, 2013)(Fig 1.)

Figure 1. Morphological view of *C.foeniculacea* collected from Antalya.

In this study, we were interested in the beneficial important properties of " *C.foeniculacea* (Linnaeus) Greville 1830 ", a brown macroalgae collected from the Antaltya in Mediterrenaean. The aims of this study are to determine total phenolic (TPC) and flavonoid (TFC) contents of *C.foeniculacea* different extracts and to investigate antibacterial (using minimum inhibition concentration method) and antioxidant four in vitro assays DPPH• (2,2′-diphenyl-1-picrylhydrazyl) radical scavenging activity ABTS•+ (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) CUPRAC (cupric reducing antioxidant capacity) and metal chelating activities.

2. Material and Methods

2.1. Macroalgae Sample Collection

Samples of *C.foeniculacea* (Linnaeus) Greville 1830 was collected from a sampling as deep as 1-2 m, station in Antalya and the coordinates are 36°27'40.47 "N - 30°32'38.18 "E as follows in Fig 2.
C.foeniculacea belong to the genus Phaeophyta. The systematic classification of the algae types used in our study is as follows in Table 1 (AlgaeBase).

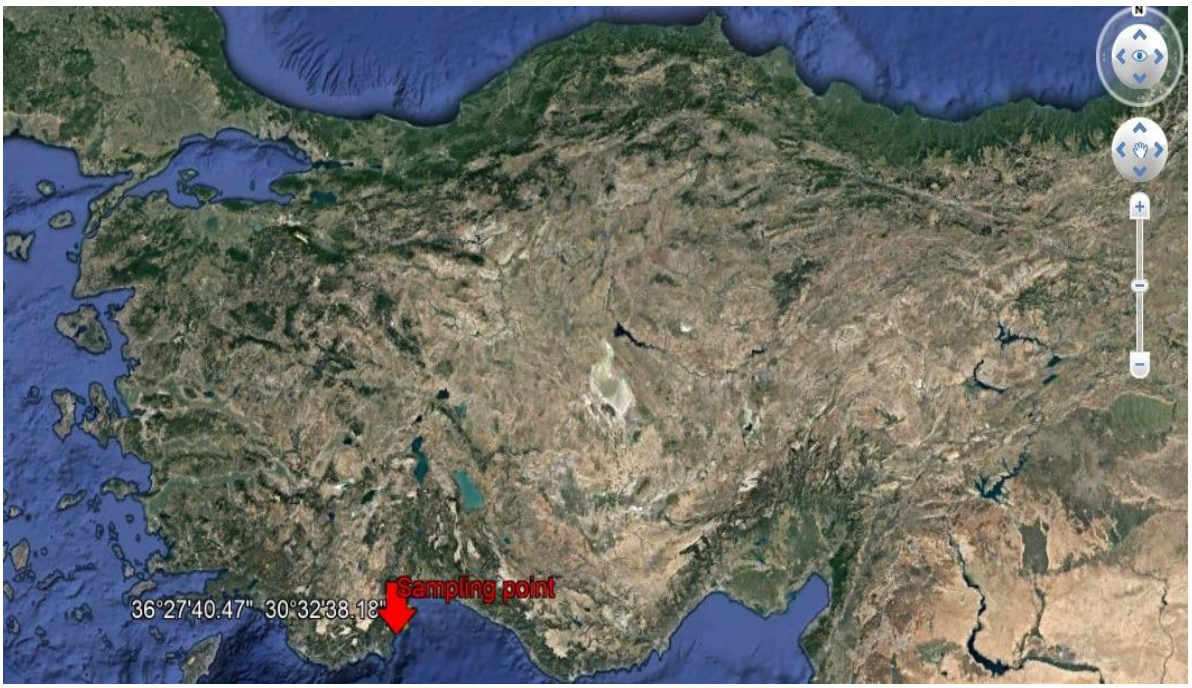

Figure 2. Map of sampling point from Antalya (Mediterranean).

The collected macroalgae samples were washed with water for remove foreign substances. After macroalgae samples were placed in sterile polyethylene bags. They were washed with distilled water in the laboratory to remove epiphytic creatures and necrotic particles from the samples.
To accelerate the drying process of the marine macroalgae that drained the water and after this process, the algae were placed in an oven set at 40ºC to prevent the phytochemical compounds from being damaged, and pre-drying was carried out by keeping it for 17 h. Marine macroalgae were dried correctly, and ground with hand homogenizer's help was kept airtight at room temperature until extraction.

Table 1. The systematic classification of *C.foeniculacea* (AlgaeBase).

Species	Kingdom	Phylum	Class	Subclass	Order	Family	Genus
C.foeniculacea	Chromista	Ochrophyta	*Phaeophyceae*	*Fucophycidae*	*Fucales*	*Sargassaceae*	Cystoseira

2.2. Extraction process of macroalgae

The soxhlet extraction method was applied to the grinded marine macroalgae samples to obtain extracts. Macroalgae samples were extracted with various solvents according to their increasing polarity: methanol, ethanol and water for 6 h by using the soxhlet apparatus. The methanol and ethanol were evaporatedunder a vacuum by anevaporator to obtain the all extracts. The water was lyophilized to get the water extract by using a freeze-drier. All macroalgal extracts were stored at +4°C until analysis.

2.3. Total phenolic (TPC) and total flavonoid contents (TFC)

TPC of the macroalgae extracts was measured according to the Folin Ciocalteu method (Slinkard&Singleton,1977). Results were calculated using the following equation obtained from the standard gallic acid graph:

Absorbance=0.0104 [gallic acid (µg)] - 0.0263, (r^2, 0.9924)

TFC of the marine macroalgae extracts was measured according to the aluminum nitrate method (Park et al., 1997). Results were calculated using the following equation obtained from the standard quercetin graph:

Absorbance=0.0158 [quercetin (µg)] - 0.0306 (r^2, 0.9993)

2.4. Bioactivity assays

2.4.1. Antibacterial activity testing

Minimum inhibitor concentration (MIC) tests were performed in accordance with the M27-A8 CLSI (Clinical Laboratory Standards Institute) criteria for bacteria (CLSI). The broth microdilution method reported by Alsenani et al. (2020) was used to determine the antimicrobial activities of the marine macroalgae extracts. The antimicrobial test was performed by determining minimum inhibitory concentration (MIC) values of different marine macroalgae extracts (0.0061-6.25 mg/mL) against fungus, gram-positive, and gram-negative bacterial strains. In addition, we used negative growth control DMSO (100%) and positive growth control contained gentamisin (0.1 mg/mL). The lowest concentration values for bacterial inhibition were calculated and reported as a MIC.

2.4.2. Antioxidant activity testing

DPPH• (2,2′-diphenyl-1-picrylhydrazil) Free Radical Scavenging Activity Method

The free radical scavenging activities of the extracts were determined using the DPPH• free radical (Blois, 1958). 160 µL of 0.4 mM DPPH solution was added to 40 µL of sample at different concentrations. 40 µL of methanol was used as a control. At room temperature, 30 min in the dark. The absorbance was measured at 517 nm using a 96-well microplate reader. The free radical scavenging activity was calculated using the equation in (1):

$$\text{DPPH scavenging activity (\% İnhibition)} = \frac{A_{Kontrol} - A_{Örnek}}{A_{Kontrol}} \times 100 \quad (1)$$

$A_{Control}$ is the absorbance of the control, A_{sample} is the absorbance of the sample.

ABTS•+ (2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) Cation Radical Scavenging Activity Method

ABTS used cation radical to determine the free radical scavenging activities of the extracts (Re et al., 1999). 160 µL of ABTS•+ solution was added to 40 µL of sample at different concentrations. 10 min at room temperature. were incubated and their absorbance was measured using a 96-well microplate reader at 734 nm. He used 40 µL of methanol as a control. ABTS cation radical scavenging activity was calculated using the equation in (2):

$$\text{ABTS}^{\cdot+} \text{ scavenging activity (\% İnhibition)} = \frac{A_{Kontrol} - A_{Örnek}}{A_{Kontrol}} \times 100 \quad (2)$$

CUPRAC (Cupric Reducing Antioxidant Capacity; Cu(II) Ion Reducing Antioxidant Capacity) Method

The reducing power of the extracts was determined using the copper (II) ion reducing antioxidant capacity method (Apak et al., 2004). 50 µL of 10 mM Cu (II), 50 µL of 7.5 mM neocuproin and 60 µL of ammonium acetate (NH4OAc) buffer 1 M, pH=7 solutions were added to 40 µL of algae sample of different concentrations and mixed for 1 hour incubation and then incubated with 96 wells. Absorbance was measured at 450 nm using a microplate.

Metal Chelating Activity

The chelating activities of the extracts were measured using the method developed by Decker and Welch (1990). The reaction was started by adding 40 µL of ethanol, 40 µL of 0.2 mM FeCl2 solution and 80 µL of 0.5 mM ferrin to 40 µL of sample solutions of different concentrations. Mix 10 min. kept at room temperature and 10 min. Finally, absorbance was measured at 593 nm. EDTA was used as standard. The metal binding activity was calculated using the equation in (3):

$$\text{Metal chelating activity (\% İnhibition)} = \frac{A_{Kontrol} - A_{Örnek}}{A_{Kontrol}} \times 100 \quad (3)$$

2.5. Statistical analysis

The analysis of the obtained data was performed using SPSS 22.0. All analysis were made for continuous variables and the arithmetic mean ± standard deviation (SD) values of the variables were given. The error was kept at the 0.05 level in the interpretation of the analysis results, and the results were reflected at the 95% confidence level.

3. Results and Discussion

Total phenolic (TPC) and total flavonoid contents (TFC)

As a result of the increase in the tendency to products with natural ingredients, scientific studies have been turned in this direction. Thus, the fact that the phenolic compounds in algae are related to many bioactive properties makes the identification of phenolic compounds valuable (Cotas et al. 2020).TPC and TFC results of the methanol, ethanol, acetone and water extracts of *C.foeniculacea* were shown in Table 2. Data are expressed in mean ± SEM (n =3).

TPC of the extracts was ranged between 14.38±1.22 and 37.75±1.05µg GAEs/mg extract. The highest concentration of TPC was found in the ethanol extract of *C.foeniculacea* (37.75±1.05 µg GAEs/mg extract). TFC of the extracts were ranged between 8.43±0.60 and 30.13±1.87µg QEs/mg extract. The highest concentration of TFC was found in the ethanol extract of *C.foeniculacea* (30.13±1.87µg µg QEs/mg extract). In a previous study, TPC of the acetone and water extracts of *C.foeniculacea* were reported as 90.61 and 57.34 mg catechin/g extract, respectively (Leão et al. 2017). TPC of *C.foeniculacea* extracts were decreased in the order of pressurized hot water (1.17±0.08 mg GAE/g dry wt.)> ethanol (1.13±0.02 mg GAE/g dry wt.)> boiling water (0.80±0.02 mg GAE/g dry wt.)> cold water (0.67±0.01 mg GAE/g dry wt.) in the study of El-Shazoly and Fawzy (2018)

Table 2. Total phenolic (TPC) and flavonoid (TFC) contents of PP and ZT extracts[a]

Extracts	Total phenolic content (TPC) (µg GAEs/mg extract[b])	Total flavonoid content (TFC) (µg QEs/mg extract[c])
CFE	37.75±1.05	30.13±1.87
CFM	15.63±1.63	30.01±1.87
CFW	14.38±1.22	8.43±0.60

[a]: The results are given as a mean ±SD of three parallel measurements. CFE: CF Ethanol Extract, ,CFM: CF Methanol Extract, CFW: CF Water Extract.
[b] GAEs, gallic acid equivalent, y=0,0104x-0,263 r^2=0,9924
[c]QEs, quercetin equivalent, y=0,0158x-0,0306 r^2=0,9993.

Antibacterial activity

The broth microdilution method reported by Alsenani et al. (2020) was used to determine the antimicrobial activities of the marine macroalgae extracts. The antimicrobial test was performed by determining minimum inhibitory concentration (MIC) values of different marine macroalgae extracts (0.0061-6.25 mg/mL) against fungus, gram-positive, and gram-negative bacterial strains. In addition, we used negative growth control DMSO (100%) and positive growth control contained gentamisin (0.1 mg/mL). The lowest concentration values for bacterial inhibition were calculated and reported as a MIC. The highest antibacterial effect against the tested pathogens was seen in the methanol extract of the *C.foeniculacea*, and the most effective strain was determined as gram (-) bacteria Pseudomonas aeruginosa (ATTC 27853) (MIC: 1.562 mg/ml). The water extract did not show antibacterial activity against any test pathogen (Table 3). According to this results the antibacterial capacity of macroalgae extracts changes depending on different parameters such as type of macroalgae, solvent, extraction method, extract concentration, and type of microorganism (Keskinkaya et al, 2022). Bacterial resistance epidemic that has developed against antibiotics that are currently in use; has led to the exploration of new antibacterial agents of natural origin without side effects. In this regard, studies on the discovery of new agents from terrestrial and marine sources are gaining momentum (Shannon and Abu-Ghannam 2016)

Table 3. Minimum inhibitory concentration (MIC) of CF extracts

Test Microorganism	MIC (mg/ml)				
	CFE	CFM	CFW	Gentamycin (0.1 mg/ml)	DMSO
Escherichia coli	6.25	3.125	3.125	<0.02	%12.5
Pseudomonas aeruginosa	NA*	1.562	NA*	<0.02	%12.5
Klebsiella pneumoniae	6.25	NA*	NA*	0.78	%12.5
Staphylococcus aureus	NA*	3.125	3.125	<0.02	%25
Salmonella enteritidis	NA*	3.125	3.125	0.04	%12.5
Sarcina lutea	NA*	1.562	1.562	<0.02	%12.5
Bacillus cereus	NA*	NA*	NA*	<0.02	%12.5

CFE: CF Ethanol Extract, ,CFM: CF Methanol Extract, CFW: CF Water Extract.

Antioxidant activity

Since antioxidants have different action mechanisms, more than one method is preferred to determine the antioxidant activity rather than a single method.Antioxidant activities of CF extracts were investigated by using $DPPH^{\bullet}$ radical scavenging, $ABTS^{\bullet+}$ radical scavenging, CUPRAC activity and metal chelating assays. The results were given in Table 4. BHT (Butyryl hydroxy toluene) and BHA (Butyryl hydroxy anisole) and EDTA were used as standard in the total antioxidant activity determination methods we applied to *C.foeniculacea* extracts and the highest ABTS radical scavenging activity in ethanol extract of *C.foeniculacea* (IC_{50}: 37,91±0,73µg/ml), metal chelating activity values of *C.foeniculacea* in ethanol extract (IC_{50}: 138,86±0,71µg/mL) ml). CUPRAC activity in ethanol extract($A_{0.50}$:150,0±0,12 µg/mL). DPPH scavenging assay did not show any significant activity in any extract of *C.foeniculacea.* Antioxidant activities of various extracts of *C.foeniculacea* were reported in earlier studies. Antioxidant activity of the hexane, dichloromethane, and methanol extracts of *C.foeniculacea* was investigated by β-carotene bleaching and hydroxyl radical scavenging assays with inhibition values of ~50-80% and ~50-70% respectively at 40 mg/mL concentration (Koz et al., 2009). Surget et al. (2017) studied antioxidant activity of the ethanol extract and ethyl acetate and water fractions of *C.foeniculacea* according to DPPH• and reducing power assays. Among the studied extract and fractions, the ethyl acetate fractions were found as the best active in DPPH• (IC50: 0.303±0.002 g/L) and reducing power (IC50: 5.478±0.891 g/L) assays. In the study of Heffernan et al. (2015), antioxidant activity of ethanol (80%), methanol (70%), hot water, and cold water extracts of *C.foeniculacea* was tested by DPPH• (IC50: 0.13±0.01-0.56±0.01 mg/mL) and FRAP (0.94±0.03-32.70±0.10 µg Tr equivalents mg-1 sample) assays. Our results agree with previous studies. Numerous studies have described that algae extracts rich in phenolic compounds had higher antioxidant activity (Jimenez-Lopez et al. 2021). Apart from the study, Yuan et al. (2018) also reported that the highest antioxidant activity of the ethanol extracts of macro algae species can be associated with the highest amounts of TPC and TFC.

Antioxidant Activity

		DPPH• assay		ABTS•+ assay		CUPRAC assay		Metal Chelating assay	
		Inhibition (%)[a]	IC_{50} (µg /mL)[b]	Inhibition (%)	IC_{50} (µg/mL)[b]	Absorbance[c]	$A_{0.50}$ (µg/mL)[d]	Inhibition (%)[a]	IC_{50} (µg /mL)[b]
Extracts	**CFE**	-	>400	89,59±0,11	37,91±0,73	1,06±0,01	150,0±0,12	75,06±0,49	138,86±0,71
	CFM	-	>400	87,42±0,15	112,2±0,43	0,38±0,04	602,83±0,06	58,60±1,10	254,45±0,89
	CFS	-	>400	61,90±0,29	301,16±0,74	-	>400	-	>400
Standards	**BHT**	86,64±0,19	23,90±0,14	85,62±0,32	12,75±0,63	2,98±0,03	28,21±0,01		
	BHA	88,18±0,10	22,80±0,59	87,54±0,74	12,05±0,97	3,22±0,01	26,54±0,02		
	EDTA							90,21±0,24	4,29±0,06

[a]: Inhibition values % of 400 µg/mL concentration of the extracts are given as a mean ±SD of three parallel measurements.
[b]: IC_{50} values are given as a mean ±SD of three parallel measurements.
[c]: Absorbance values of 400 µg/mL concentration of the extracts are given as a mean ±SD of three parallel measurements.
[d]: $A_{0.50}$ values are given as a mean ±SD of three parallel measurements.

4. Conclusion

The marine flora and fauna need exploration as they contain diverse variety and lack scientific validation. In this direction, a new scan was made for updating and validation with this study. In this study, cytotoxic, antibacterial, and antioxidant activities of the various extracts of *C.foeniculacea* was investigated with TPC and TFC. The ethanol extracts obtained from this marine macroalgae with the highest TPC and TFC demonstrated close or higher antioxidant and antibacterial activities than the standards. The findings of this study emphasized that *C.foeniculacea* ,which is considered as the food of the future, could serve to discover promising, new and natural antioxidant, antibacterial and anticancer agents that are critically important for a wide variety of industries such as medicine, food, and cosmetics. However, it is necessary to perform isolation studies to determine the biological active compounds apart from phenolic compounds that may cause these bioactive properties.

Conflict of Interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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ABSTRACTS & PROCEEDINGS BOOK
03-05 May 2023, Ankara-Türkiye
www.mesmap.com

ORAL PRESENTATION - FULL PAPER

COMPARISON OF THE EFFECTS OF QUERCETIN AND QUERCETIN-LOADED CYCLODEXTRIN FORMULATIONS ON ACUTE PAIN

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Abstract

Quercetin is in the class of flavanols that its chemical formula is 3.3', 4', 5,7-pentahydroxyflavone. It has been demonstrated to possess diverse pharmacological benefits, such as serving as an antioxidant, having antiviral properties, and exhibiting anti-cancer effects. Quercetin has also anti-inflammatory and antinociceptive/analgesic properties. However, the low solubility and bioavailability of quercetin adversely affect its effectiveness. Thus, in order to increase the bioavailability and solubility of quercetin, it is aimed to examine the change in the analgesic effect of quercetin by preparing a biologically compatible cyclodextrin formulation that can be targeted to the tissues in this study. Quercetin and quercetin-β-cyclodextrin (β-CD) complex were administered intraperitoneally at doses of 3, 5, 10, and 20 mg/kg in mice. Time-dependent analgesic effects of pure quercetin and quercetin-β-CD were compared in hot-plate and tail-immersion tests, acute pain models tested supraspinal and spinal organization of pain respectively. Quercetin at the doses of 5, 10 and 20 mg/kg was found to increase pain thresholds against to thermal stimuli in tail immersion and hot plate tests in 30-180 min. time interval. Only the effect of 20 mg/kg quercetin weakened at 180 min. The effect at 3 mg/kg was observed only in hot-plate test at 120 min. β-CD complex prepared with 3 mg/kg quercetin showed significant analgesic effect in 30-180 min time intervals in both two tests. Additionally, the analgesic effect of the β-CD complex prepared with 20 mg/kg quercetin was not weakened in both tests like pure 20 mg/kg quercetin at 180 minutes. Thereby, it can be concluded that application of quercetin in β-CD complex is provided to improve pharmacokinetic properties such as bioavailability of quercetin. Thus, the efficacy and duration of action of quercetin were enhanced. This study displays the analgesic potential of quercetin by spinal and supraspinal regulations and the advantages of application in β-CD formulation for pain relief.

Key Words: Cyclodextrin, β-Cyclodextrin, quercetin, pain, tail-immersion, hot-plate.

1. Introduction

Quercetin is a plant-derived flavonoid that belongs to the class of flavonols. Its chemical formula is 3.3', 4', 5,7-pentahydroxyflavone (Fischer et al., 1997). It is found abundantly in various fruits and vegetables, such as apples, berries, onions, and green leafy vegetables. Quercetin has gained considerable attention in recent years due to its potential health benefits. It has been shown to have a variety of pharmacological effects, including antioxidant, anti-inflammatory, antiviral, and anticancer properties (Mien and Mohammed, 2001; Grawaltney-Brant, 2006; Johari et al., 2012). One of the primary pharmacological effects of quercetin is its antioxidant activity. It can scavenge free radicals and prevent oxidative damage to cells and tissues. This may help chronic diseases such as cancer, heart disease, and neurodegenerative disorders. Quercetin has also anti-inflammatory activity (Boots et al., 2008; Lesjak et al., 2018). It can inhibit the production of inflammatory cytokines and enzymes, which may help to reduce inflammation in the body. It has been studied for its potential health benefits, including its analgesic effects (Lee vd., 2005; Filho vd., 2008; Chunmei vd., 2017). Quercetin may relieve pain is by blocking the transmission of pain signals in the nervous system (Ferraz et al., 2020; Ye et al., 2021).

Analgesics are drug or substances that relieve pain without causing loss of consciousness. Studies have shown that quercetin has analgesic effect in both animal and human models. In human studies, quercetin has been shown to reduce pain associated with conditions such as osteoarthritis and migraine headaches (Samadi et al., 2022). The analgesic effects of quercetin in experimental animals were first demonstrated by Rylski et al., (1979) and later by Picq et al., (1991). In different analgesia tests, quercetin has been shown to have an antinociceptive effect at doses of 10–60 mg/kg (i.p.) and 100–500 mg/kg, (p.o.) (Filho et al., 2008). It has been reported that cytokine inhibition and oxidative stress inhibition contribute to the analgesic effect of quercetin (Valério et al., 2009)

It is known that flavonoids are converted into conjugation forms such as glucuronides, sulfates and methylated derivatives after their absorption. It has been reported that these conjugates contribute to the therapeutic properties of flavonoids (Viskupicova et al., 2008). However, the bioavailability of quercetin can vary depending on several factors. One of them is quercetin's metabolism. Quercetin undergoes extensive metabolism in the liver and intestines before it can be absorbed into bloodstream. This can reduce its bioavailability and limit its effectiveness. Bioavailability refers to the amount of a substance that is absorbed by the body when consumed in food or supplements. To improve solubility and absorption, quercetin supplements can be often formulated with other compounds such as phospholipids or nanoparticles (Jaganath, et al., 2006; McClements vd., 2010).

Various drug delivery systems, such as lipid-based carriers, polymer nanoparticles, cyclodextrins, etc., are used to improve conditions such as solubility and bioavailability that limit the biological effect (Mainardes & Silva, 2004; Tiwari, et al., 2012). Cyclodextrins are substances in oligosaccharide structure that can change the physicochemical structures of the substances they form complexes with, through their lipophilic outer surfaces and hydrophilic interior spaces (Del Valle, 2004). Within the scope of this study; in order to increase the bioavailability and solubility of quercetin, it is aimed to examine the changes in the analgesic effect of quercetin by preparing a biologically compatible cyclodextrin formulation that can be targeted to the tissues.

2. Material and Methods

2.1. Materials

Quercetin and β-cyclodextrin (β-CD) were procured from Sigma-Aldrich (Steinheim, Germany), while all other chemicals utilized were of analytical grade. Deionized and filtered water was employed throughout all experiments (Milli-Q Academic, Millipore, Molsheim, France).

2.2. Experimental animals

Balb-c female mice weighing 25-35 grams were used in our experiments. Animals were obtained from Anadolu University Experimental Animals Research and Application Unit. Mice were housed in well-ventilated rooms with day/night lighting for 12 hours before the experiments. The animals were allowed to freely feed and drink water. Analgesia tests were conducted during the hours of 11:00 AM to 5:00 PM. Our experimental procedure was approved by the local ethics committe of Anadolu University, Eskisehir, Turkey (11.12.2019 - 2019-57).

2.3. Drug administration

Quercetin was dissolved in 10% DMSO and injected into (ip) mice 30 minutes before the experiment at doses of 3, 5, 10 and 20 mg/kg in a volume of 0.1 ml. The control group was given 10% DMSO in a volume of 0.1 ml.

2.4. Formulations

In this study, the formulation production technique of solvent evaporation was chosen. (Jantarat et al., 2014; Öztürk et al., 2023). According to Higuchi and Connors' phase solubility experiments, a quercetin-containing β-CD complex was made using the solvent evaporation method in an ethanol:water (1:1, v/v) solvent system. Firstly, the mixtures were strongly shaken at 300 shake / min−1 during the equilibrium period as being protected from direct sunlight. After the shaking period, the mixtures were dried at a controlled temperature and pressure with Büchi R-205 (Switzerland) rotary evaporator. In this case, the temperature was set to 50°C ± 1°C and the pressure was set to 100 mbar .

Table 1. Lists the ingredients in the made formulation.

Formulation code	Quercetin	β-CD	Ethanol	Water
Q- β-CD	10 mg	37.58 mg	32 mL	32 mL

2.4.1. Characterization of formulation

HPLC was used to determine the concentration of quercetin in the complex. To carry out the analysis, an accurately weighed formulation of 1 mg is dissolved in 1 mL of a mobile phase and then HPLC analyses were performed. Encapsulation efficiency was expressed in terms of associated drug percentage (Öztürk et al., 2023, Perret et al., 2013).
At 25 °C ± 2 °C in distilled water, zeta potential studies were carried out using the Zetasizer Nano ZS (Malvern, UK) (Tilki et al.,2023; Baghirova, et al., 2023).

2.4. Experimental methods

2.4.1. Hot plate test

The hot plate test is used to assess the response of experimental animals to thermal painful stimuli (Eddy and Leimbach, 1953; Uzbay, 2004). This test is related to higher brain functions and supraspinal organized response (Dzoyem et al., 2017). Animals were handled gently and placed on the heated surface of the hot plate apparatus (No. 7280, Ugo Basile Instruments, Comerio, Italy) set to 56 degrees. The latency to hind paw licking, hind paw flicking or jumping, was recorded, and at this point the animal was immediately removed from the hot plate. After baseline measurement of animals' pain

response, post-drug latency was measured after the administration of the test compounds in experimental groups, and vehicle in the control group. Time intervals of the measurements after administration of the compounds were chosen as 30, 60, 120, and 180 min. The cut-off time was determined at 20 seconds to prevent hind paw damage (Eddy and Leimback, 1953; Langfor and Mogil, 2008).

2.4.2. Tail-immersion test

The tail-immersion test is a commonly used experimental procedure for assessing thermal nociception in experimental animals. In this test, approximately 3 cm from the distal end of the animals' tails were immersed in a water bath heated to 52.6°C to determine the nociceptive response. In the tail-immersion test, the time it takes for the animal to withdraw its tail from the thermal stimulus is measured as the reaction time (Aydın et al., 2002; Gawel et al., 2018). After baseline measurement of animals' pain response, post-drug latency was measured after the administration of the test compounds in experimental groups, and vehicle in the control group. Time intervals of the measurements after administration of the compounds were chosen as 30, 60, 120, and 180 min.. Time intervals of the measurements after administration of the compounds were chosen as 30, 60, 120, and 180 min The cut-off time of 15 s was set to prevent harm to tail tissue (Ramabadran et al., 1989; Aydın et al., 2002).

2.5. Statistical analysis

Hot plate and tail immersion test results were analysed by use of Two-way ANOVA test in the GraphPad Prism version 5 statistical programe. Values are given mean±SEM and $p < 0.05$ was considered statistically significant.
The hot-plate and tail-immersion tests produced results that were represented as a percentage of the maximum possible effect (MPE %). The MPE % was determined by calculating the response latencies to thermal stimuli and using that value as the basis for the percentage calculation:

$$MPE\% = [(Postdrug\ latency) - (Predrug\ latency)]/\ [(Cutoff\ time) - (Predrug\ latency)] \times 100$$

3. Results and Discussion

3.1. Formulation

The sum of the masses of the dried complex and the amount of quercetin and β-CD used was calculated as the complexation performance. The Q-β-CD coded formulation was produced with a yield of 65.5% ± 4.9%. The aqueous solubility and encapsulation efficiency of quercetin were obtained as 27.5 $\mu g.mL^{-1}$ and 84.0 ± 4.2%, respectively. The aqueous solubility of quercetin is 1.5 $\mu g.mL^{-1}$, and it has been determined that it increases considerably when complexed with β-CD (Marques et al., 2019). Because greater loading results in a lower quantity of formulation for a given dose of treatment, the drug encapsulation efficiency of the formulation is a crucial factor in formulations. The result of 84.0 ± 4.2% obtained in this study is quite ideal (Ekinci et al., 2022). The zeta potential of the Q-β-CD -coded formulation was obtained as −19.5 ± 0.2 mV. In colloidal systems, the particles resist one another if the zeta potential is strongly positive or negatively charged. If a colloidal dispersion has a zeta potential of ± 30 mV when dispersed in a liquid, it is assumed to be a stable formulation. Zeta potential values between 5.0 and -15.0 mV are considered in the limited flocculation zone, while zeta values between -5.0 and -3.0 mV are considered the maximum flocculation zone. When the study's findings were investigated, it was discovered that the zeta potential values were higher than the flocculation limit. This demonstrates the formulation's stability (Öztürk et al., 2023). When all these results are evaluated, especially the increase in water solubility of quercetin due to the complex will increase the bioavailability of quercetin (Carnerio et al.,2019).

3.2. In vivo Experiments

The objective of this study was to evaluate and compare the analgesic properties of pure quercetin and a Q-β-CD formulation. It was hypothesized that the use of cyclodextrin could enhance the pain-relieving effect of quercetin by improving its solubility and increasing its bioavailability. Antinociceptive effect of pure quercetin has been investigated dose dependently (3, 5, 10, 20 mg/kg) on hot plate and tail immersion tests. Q-β-CD formulation was studied at the same dose range on the hot plate and tail immersion tests.

In the hot plate test, which is a behavioral model of nociception, paw licking, mustache movements and jumping are observed, which are controlled by supraspinal mechanisms. In this test, a significant antinociceptive effect was observed that the quercetin at doses of 5, 10, 20 mg/kg compared to the control group at all time intervals (30-180 min.) ($^{c}p< 0.001$, Figure 1). No analgesic effect was observed at a dose of 3 mg/kg of pure quercetin. However, it was determined that the effect of 20 mg/kg dose, where the highest effect was seen, is reduced by half in the 180th minute. Despite the decrease in dose to 20 mg/kg, the antinociceptive effect was still present when compared to the control group.

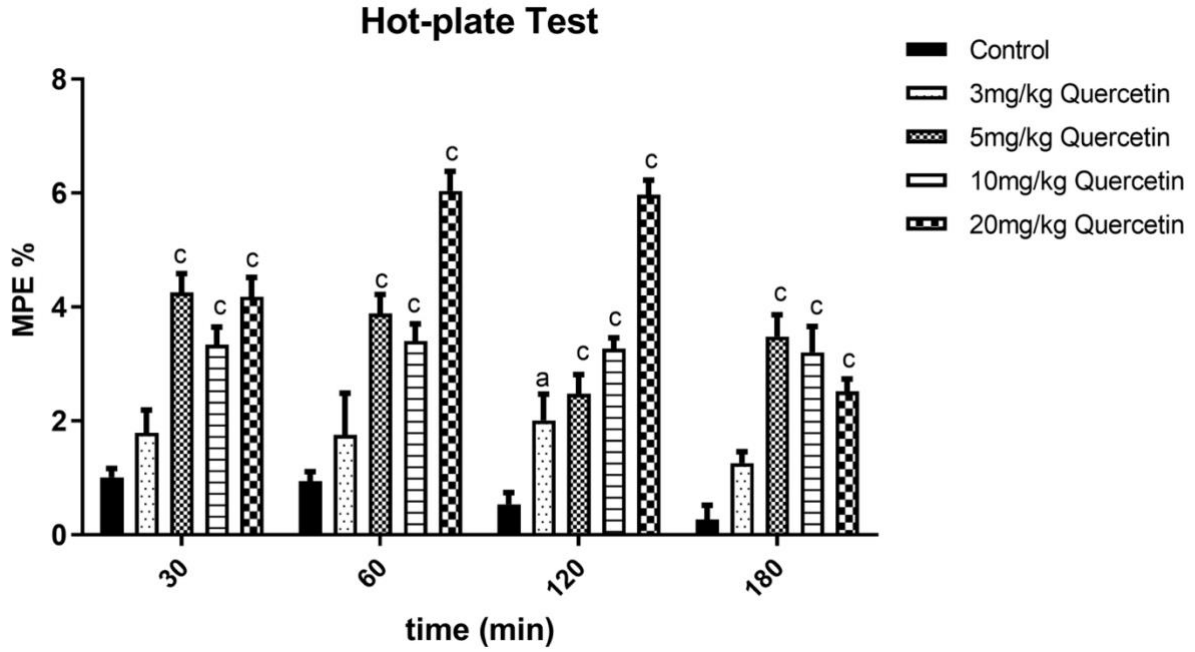

Figure 1. Antinociceptive effect of quercetin in hot-plate test. Compared with the control group: $^{a}p< 0.05$., $^{c}p< 0.001$. Values are expressed as the mean ± S.E.M. (n = 8)

At the same doses of quercetin was found to increase pain thresholds against to thermal stimuli in tail immersion test in 30-180 min time interval ($^{c}p< 0.001$, Figure 2). Similar to the hot plate test results, the antinociceptive effect obtained at all doses decreased at the 180th minute.

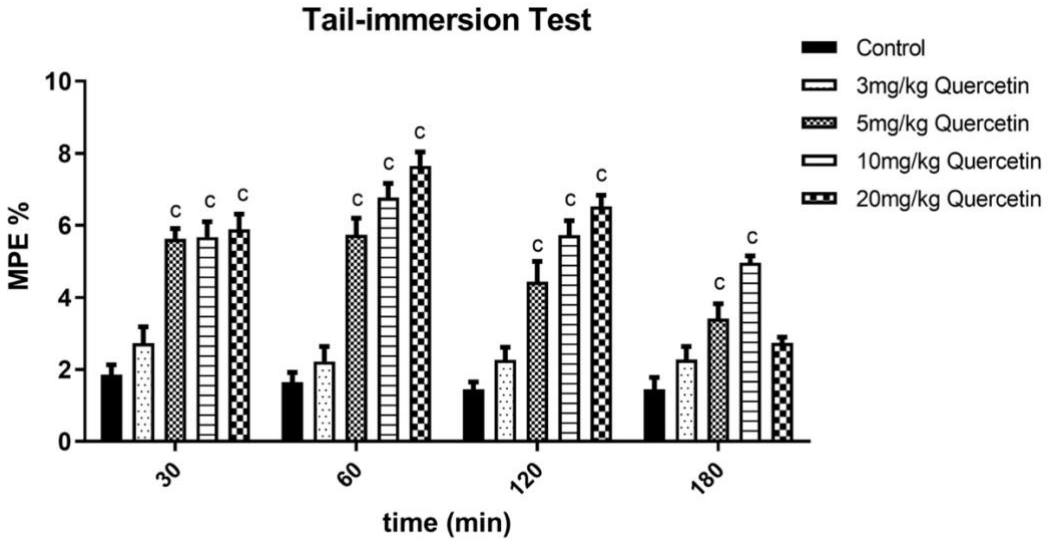

Figure 2. Antinociceptive effect of quercetin in tail-immersion test. Compared with the control group: $^{c}p< 0.001$. Values are expressed as the mean ± S.E.M. (n = 8)

When pure quercetin and the Q-β-CD coded formulation was compared, it was determined that the 3 mg/kg Q-β-CD coded formulation increased the analgesic efficacy between 30-180 minutes in the hot plate test (Figure 3). The analgesic activity of the 3 mg/kg Q-β-CD formulation has significant difference compared to the pure 3 mg/kg quercetin in the hot plate test ($^{*}p< 0.05$ and $^{***}p< 0.001$, Figure 3). Similarly, it was observed that the analgesic effect of 20 mg/kg Q-β-CD formulation continued at the same time interval and also significantly higher compared to pure quercetin ($^{\&\&}p< 0.01$ and $^{\&\&\&}p< 0.001$, Figure 3). Comparing analgesic activities of 5 and 10 mg/kg quercetin with their Q-β-CD formulation showed no significant difference at all time intervals. Thus, application of quercetin in formulation provides an advantage to supraspinal controlled analgesic effect of quercetin.

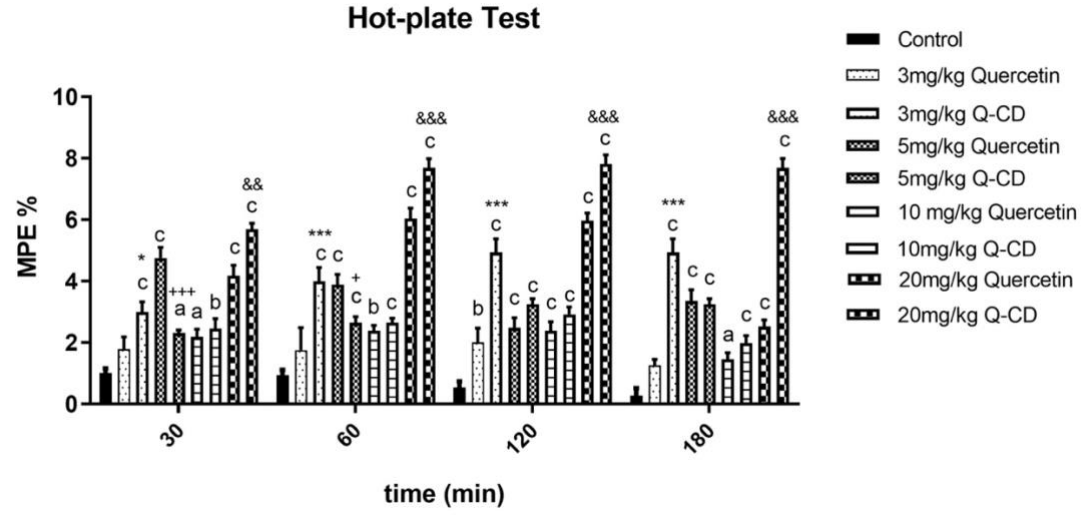

Figure 3. Comparison of pure quercetin and cyclodextrin-quercetin formulation application in hot plate test. Significant difference compared to the control group: $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$. Comperison with pure quercetin and Q-CD formulation-3mg/kg : $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; Comperison with pure quercetin and Q-CD formulation 20mg/kg: $^{\&}p<0.05$, $^{\&\&}p<0.01$, $^{\&\&\&}p<0.001$. Values are expressed as the mean ± S.E.M. (n = 8) (Q-CD: Quercetin-β Cyclodextrin formulation)

In the tail immersion test, when the antinociceptive effect of pure quercetin and Q- β-CD formulation is compared with each other at 3 mg/kg doses, it was seen that the formulated groups have more significant analgesic effects between 60-180 minutes ($^{*}p< 0.05$, $^{**}p< 0.01$; Figure 4). At the dose of 20 mg/kg, the formulation provided a significant increase in analgesic effect, especially when compared to the reduced pure quercetin effect at the 180th minute ($^{\&\&\&}p< 0.001$, Figure 4). The cyclodextrin formulation did not provide a significant difference in the analgesic effect of 5 and 10 mg/kg quercetin. In both analgesia tests, only the analgesic effect of 5 mg/kg Q-β-CD formulation showed significant reduction (Figure 3-4). However, it can be said that application of quercetin in complex folmulation provides an advantage to spinally controlled analgesic effect of quercetin.

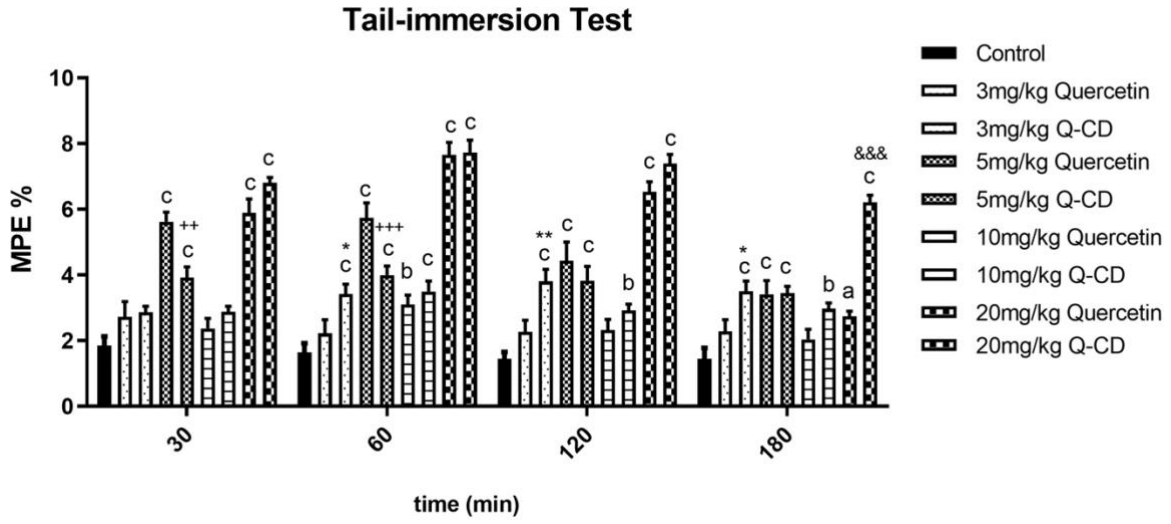

Figure 4. Comparison of pure quercetin and cyclodextrin-quercetin formulation application in tail immersion test. Significant difference compared to the control group: $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$. Comparison with pure quercetin and Q-CD formulation-3mg/kg : $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; Comparison with pure quercetin and Q-CD formulation 20mg/kg: $^{\&}p<0.05$, $^{\&\&}p<0.01$, $^{\&\&\&}p<0.001$. Values are expressed as the mean ± S.E.M. (n = 8) (Q-CD: Quercetin-β Cyclodextrin formulation)

As stated earlier, quercetin is a bioactive substance with many important pharmacological effects. However, its pharmacokinetic properties, which cause low bioavailability, set the limits for its use as a drug (Cai, et al., 2013). Various strategies could be employed to enhance the bioavailability of quercetin, including the utilization of advanced drug delivery systems. These systems have shown promising results in improving the solubility and bioavailability of quercetin (Cai, et al., 2013).

Drug delivery systems are designed to deliver the necessary amount of medication to the target area effectively and accurately over a required period. Additionally, suitable carrier materials are used to design advanced dosage forms and improve the undesirable properties of drug molecules (Loftsson et al., 2005; Adepu and Ramakrishna, 2021). Cyclodextrins are an oligosaccharide carrier system widely used in drug development to solve problems such as absorption, stability and bioavailability. Cyclodextrins facilitate the formulation of drugs by providing a more stable and homogeneous distribution of drugs. In this way, they enable drugs to affect the targeted tissues more easily. Cyclodextrins are water-soluble drug carriers with a hydrophobic core and a hydrophilic outer surface, which make them suitable for delivering hydrophobic injectable drugs (Uekama, et al., 1998). Additionally, it has been demonstrated that this carrier system provides advantages to biological effects of various flavonoids by developing pharmacokinethic properties (Rezende et al., 2009;

Tommasini, et al., 2004). So, cyclodextrin carrier system was choosen for improve bioavalibility of quercetin in this study and successful results were obtained.

In our results, while 3 mg/kg pure quercetin has no analgesic effect, Q-β-CD formulation increase the quercetin analgesic effect at the lowest dose (3 mg/kg) and the formulation allowed this effect to continue until the 180th minute. At the same time, the decreasing effect of 20 mg/kg quercetin at 180 minutes was enhanced by the cyclodextrin complex and mediated the increase of this effect at all time intervals. Briefly, cyclodextrin provided that demonsrate analgesic effect of quercetin at low doses, with an early onset and prolonged duration of action.

When all findings are considered, it was determined that the quercetin β-CD complex increased the analgesic effect of quercetin and prolonged its duration of action. The cyclodextrin carrier system is thought to facilitate a controlled release of quercetin, enabling it to more efficiently reach the target tissues and thereby enhance its efficacy.

4. Conclusion

Recently, quercetin, a notable flavonoid, was loaded into the cyclodextrin carrier system to increase its efficiency and bioavailability. Results of the present study indicated that the cyclodextrin complex enhanced the analgesic effect of quercetin and prolonged its duration of action.

Acknowledgements
There are no acknowledgements.

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

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ORAL PRESENTATION - FULL PAPER

AN EFFICIENT GREEN METHOD FOR RECOVERY OF POLYPHENOLS FROM OLIVE TREE (OLEA EUROPAEA) LEAVES BY MEANS OF LACTIC ACID-BASED DEEP EUTECTIC SOLVENT

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Abstract

Biowastes generated during the processing of industrial crops and products are often rich in bioactive compounds like polyphenols. Using biowastes to obtain natural antioxidants is a sustainable and environmentally friendly approach that can promote human health while reducing waste. Olive leaf, which is one of the by-products of the olive oil industry, was used as a raw material in this study due to its rich bioactive content.

The current study is aimed at establishing an efficient green media, using an eco-friendly lactic acid-based deep eutectic solvent (DES) system, consisting of a hydrogen bond donor-HBD (glycerol) and hydrogen bond acceptor-HBA (lactic acic). The molar ratio of lactic acid-glycerol mixture was selected 1:1 depending on our previous study [1]. Ultrasound-assisted extraction (UAE) method, a green extraction method, has been used to recover natural antioxidants from olive leaves. The extraction time and amplitude were determined for ultrasound-assisted extraction of bioactive-rich extract from olive leaves prior to routine studies. The Box–Behnken design type of the response surface method (RSM) was employed to investigate the effects of independent parameters (particle size, solid mass and water content in DES) on the total phenolic content (TPC). Bioactive properties of the olive leaf extracts were also assessed depending on total flavanoid content (TFC) and antioxidant activity measured by 2 different assays such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) methods.

Key Words: Natural antioxidants, biowaste, deep eutectic solvent, green extraction, Box-Behnken-RSM.

1. Introduction

By-products from the olive oil industry, such as crude olive cake, vegetation water, twigs, leaves and olive leaf (traditionally used as animal feed), collectively account for approximately 10% of the total olive weight [2]. Olive leaf extract has potential as a source of valuable bioactive compounds for the cosmetic, therapeutic, and food industries, as well as a treatment for conditions such as hypertension, atherosclerosis, inflammation, high blood sugar, and high cholesterol [3].

Extraction is a powerful tool for the recovery of valuable compounds from biowastes [4]. Green extraction aims to minimize solvents, energy, wastes, and pollution while maximizing yields through safe and cost-effective methods, including novel technologies that reduce energy costs and greenhouse gas emissions [5]. Ultrasound is a promising technology that can contribute to achieving the goal of sustainable "green" chemistry and extraction. Ultrasonic technology is a green extraction method that offers advantages such as lower costs, reduced operation time, lower energy consumption, and higher yield, and is commonly used in the food industry to accelerate chemical reactions. The

ultrasonic technology leads to structural changes in food matrices, leading to the release of bioactive compounds from plant matrices after cell disruption [6]. This is achieved through the production of cavitation bubbles in the biological matrix, resulting in high yields and extraction rates [5].

Green Chemistry aims to develop safer and more efficient chemical processes with minimal impact on health and the environment, using sustainable solvents such as deep eutectic solvents (DESs) as alternatives to conventional solvents. DESs are formed by combining a eutectic mixture of Lewis or Brønsted acids and bases, which can include various cationic and/or anionic species [7]. DESs have several advantages over conventional solvents, including low toxicity, low volatility, high thermal stability, and biodegradability. These properties make them ideal for use in a wide range of applications, including as a solvent in the extraction of high-added value bioactive compounds for food and pharmaceutical applications. [8]. In this study, a recently developed and quickly growing type of ionic liquid known as deep eutectic solvent (DES) was utilized. A mixture of lactic acid and glycerol in a 1:1 molar ratio was prepared as a DES for the purpose of extracting high-value compounds from olive leaves using ultrasound-assisted extraction (UAE), based on our previous study [1]. The use of lactic acid and glycerol as components of the DES was chosen based on their availability, low toxicity, and potential for biodegradability.

To achieve successful UAE, it is crucial to take into account several process variables, such as ultrasonic power, frequency, output amplitude, extraction time, solvent-sample interaction and particle size [9]. Thus, the main objective of this study was to examine how particle size, solid mass, and water content in the DES system affect the UAE process, using the optimal amplitude and extraction time determined through preliminary trials. Furthermore, the bioactive properties of the olive leaf extracts were evaluated by quantifying the total flavonoid content (TFC) and assessing their antioxidant activity through two in vitro assays: the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method. In addition, various kinetic models were employed to evaluate the kinetic behavior of lactic acid-based deep eutectic solvent integrated into UAE system, providing a comprehensive understanding of the kinetics of the extraction process.

2. Material and Methods

2.1. Plant material and chemicals

During the olive harvest season olive leaf samples, which are by-products of olive oil, were collected from Bursa in Turkey. After being dried in the dark, the olive leaves were ground and passed through a sieve system (Endecotts Test Sieves Ltd, London, UK) to classify the particles into a size range of 0.5-2 mm. All chemicals were provided from Merck (Darmstadt, Germany).

2.2. Preparation of deep eutectic solvent

In this study, a lactic acid-based DES was prepared using lactic acid as the hydrogen bond acceptor (HBA) and glycerol as the hydrogen bond donor (HBD) in a 1:1 molar ratio. The preparation process involved stirring the mixture at 80°C using a magnetic stirrer until a homogenous liquid was obtained. By mixing deionized water with lactic acid-based deep eutectic solvents (DESs), solutions with different concentrations were prepared, containing 20%, 50%, and 80% water (v/v) respectively.

2.3. Ultrasound-assisted extraction

The ultrasonic treatment was carried out using an ultrasonicator (Bandelin Sonopuls Mini 20, Bandelin Electronic, Berlin, Germany) with a pulse mode (3/7, on/off) to prevent overheating (Figure 1). The frequency of the ultrasonic treatment was 20 kHz, and the treatment lasted for 3 seconds with a 7-second pause during extraction. The amplitude and extraction time were determined through preliminary trials, and an output amplitude of 30% and an extraction time of 45 minutes produced the best results. Consequently, the extraction proceeded under these specific conditions. After extraction, the extracts were filtered using a 0.45 µm injection filter.

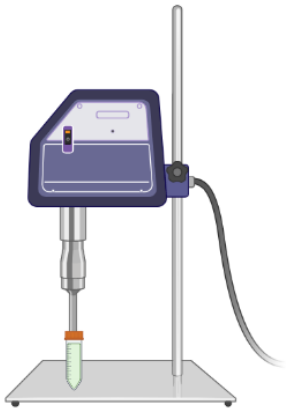


Figure 1. Ultrasound-assisted extraction system

2.4. Measurement of bioactive compounds

The bioactive compounds in the extracts were determined using a UV-visible spectrometer (PG Instruments, T60/Leicestershire, England). The total phenolic content (TPC) was measured at 765 nm using the Folin-Ciocalteau method, while the TFC was measured using the aluminum chloride method at 510 nm [10]. Gallic acid and catechin solutions were used to create calibration curves for determining the TPC and TFC concentrations of the extracts, respectively. The TPC values were expressed as mg-GAE/g-DM in gallic acid equivalent (GAE) per gram dried material (DM), while the TFC values were reported as mg of catechin equivalent (CE) per gram of DM.

2.5. Measurement of antioxidant activity

The antioxidant activity of the extracts was determined by performing DPPH and ABTS assays at 517 nm [11] and 734 nm [10], respectively. Trolox solution was used to create calibration curves. The results were expressed as milligram trolox equivalent antioxidant capacity per gram of dried matter (mg-TEAC/g-DM).

2.6. Kinetic study

Four different mathematical models were used to analyse the kinetic behaviour of system, including the pseudo-first-order model, pseudo-second-order model, film theory and Peleg's model. The equations for each model are provided in Table 1. The kinetic parameters of the models were determined using the TPC values obtained from preliminary trials conducted at three different amplitudes (10%, 30%, 50%). Film theory operates on a two-stage extraction mechanism, with the initial stage being the washing stage and the subsequent stage being the slow extraction stage. These stages are defined by two parameters within the model (Table 1).

Table 1. Application of kinetic model equations to for ultrasound-assisted extraction of olive leaves

Kinetic model	Equation	Model term
Pseudo first-order	$\ln\left(\frac{C_e}{C_e - C_t}\right) = k_1 t + \ln\left(\frac{C_e}{C_e - C_o}\right)$	C_0: initial TPC concentration (mg-GAE L^{-1}) C_e: TPC concentration at equilibrium (mg-GAE L^{-1}) C_t : TPC concentration at time t (mg-GAE L^{-1}) t: time (min)
Pseudo second-order	$\frac{t}{C_t} = \frac{1}{k_2 C_e^2} + \frac{t}{C_e}$	k_1:first order rate constant (min^{-1})
Film Theory	$\ln\left(1 - \frac{C_t}{C_e}\right) = \ln(1-b) - k_3 t$	k_2: second order rate constant (L mg^{-1} min^{-1}) k_3:slow extraction coefficient (min^{-1}) b: washing coefficient
Peleg's model	$C_t = \frac{t}{k_4 + k_5 t}$	k_4: Peleg's rate constant at the beginning (mingmg^{-1} GAE) k_5: Peleg's capacity constant (mg GAE g^{-1} min^{-1})

2.7. Box-Behnken design

The Box-Behnken design (BBD) is a type of response surface methodology (RSM) used in experimental design to identify the relationship between several independent variables and their effect on a response variable. It is a statistical tool that helps to optimize the process and find the optimal conditions for a given response. In this study, BBD was utilized to optimize the UAE system for extracting high-added value products from olive leaves. RSM was implemented using Design-Expert software version 12.0.1.0 from StatEase Inc., USA. The independent variables, along with their respective units and symbols, used in the UAE method are presented in Table 2. TPC findings were considered the dependent variables.

Table 2. Independent variables, units, symbol, and coded levels

Independent Variable	Units	Symbol	Coded levels		
			−1	**0**	**1**
Particle size	mm	A	0.5	1.25	2
Solid mass	g	B	0.1	0.3	0.5
Water content	(%, v/v)	C	20	50	80

3. Results and Discussion

3.1. Experimental plan, variance analysis and model fitting

Table 3 shows the experimental plan for UAE of bioactive substances from olive leaf extract using BBD of RSM, with 5 central points (1.25 mm particle size, 0.3 grams sample, and 50% water content in DES), and their corresponding TPC findings. As presented in Table 3, the TPC findings ranged from 9.02 to 71.19 mg-GAE per gram of dried matter (mg-GAE/g-DM).

Table 3. Experimental plan generated by BBD for UAE of olive leaf extract and TPC findings depending on independent parameters

Run	A: Particle size (mm)	B: Solid mass (g)	C: Water content (%, v/v)	TPC (mg-GAE/g-DM)
1	1.25	0.3	50	66.31 ± 0.002
2	1.25	0.3	50	71.19 ± 0.004
3	1.25	0.5	20	20.49 ± 0.004
4	1.25	0.3	50	66.89 ± 0.005
5	2	0.3	80	27.11 ± 0.002
6	1.25	0.3	50	68.21 ± 0.002
7	2	0.1	50	24.85 ± 0.003
8	1.25	0.5	80	31.65 ± 0.001
9	0.5	0.1	50	44.51 ± 0.003
10	1.25	0.1	20	22.02 ± 0.004
11	2	0.3	20	9.02 ± 0.005
12	2	0.5	50	19.74 ± 0.002
13	0.5	0.5	50	31.81 ± 0.002
14	1.25	0.1	80	36.36 ± 0.003
15	0.5	0.3	80	28.39 ± 0.001
16	1.25	0.3	50	67.09 ± 0.001
17	0.5	0.3	20	29.45 ± 0.002

* Data are given as the mean (n=3) ± standard deviation

Table 4 summarizes the analysis of variance (ANOVA) test results for TPC findings. The quadratic polynomial model (Eq. 1) proposed by Design-Expert software for TPC responses in terms of coded factors was determined to be statistically significant at $p<0.0001$, as shown in Table 4.

$$Y_{TPC} = 67.94 - 6.68\,A - 3.01\,B + 5.32\,C + 1.90\,AB + 4.79\,AC - 0.7950\,BC - 20.92\,A^2 - 16.79\,B^2 - 23.52\,C^2 \quad (1)$$

All of the selected independent variables (A, B and C) and their second power effects (A^2, B^2 and C^2) were found to have a statistically important effect based on their p-values ($p<0.05$). The second power effect of water content in DES (C^2) was found to be the most significant independent variable on TPC response, followed by the second power effect of particle size (A^2) and the second power effect of solid mass (B^2) ($p<0.0001$). It was followed by particle size (A), water content in DES (C), the interaction between particle size and water content in DES (AC) and solid mass (B) at $p<0.05$. As seen in Table 4, the interactions between the independent variables were not found to be statistically significant, except for the interaction between particle size and water content in DES (AC) ($p<0.05$).

Table 4 provides a comprehensive set of evaluation metrics that can be used to assess the goodness of fit of a model. These metrics include the lack of fit, coefficient of variance (C.V.), R^2, predicted R^2 and adjusted R^2 values. As seen in Table 4, a non-significant lack of fit value at $p>0.05$ is favorable because it indicates that the models fit the experimental data well. Additionally, the coefficient of determination values (R^2, adjusted R^2, and predicted R^2) are close to 1, which implies that the model can account for a substantial amount of the variation in the dependent variable.

Moreover, a coefficient of variation (C.V.) value less than 10% indicates a low degree of variation in the data relative to the mean, which suggests that the data is relatively consistent and reliable.

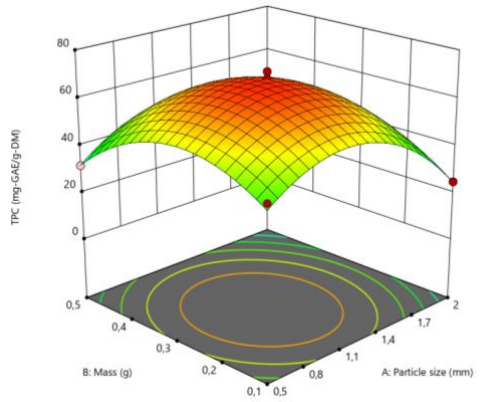
Table 4. Analysis of variance test results on TPC findings of olive leaf extracts obtained through UAE based on BBD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6737.64	9	748.63	98.19	< 0.0001	significant
A- Particle size	356.98	1	356.98	46.82	0.0002	
B- Solid mass	72.30	1	72.30	9.48	0.0178	
C- Water content	226.10	1	226.10	29.66	0.0010	
AB	14.40	1	14.40	1.89	0.2117	
AC	91.68	1	91.68	12.03	0.0104	
BC	2.53	1	2.53	0.3316	0.5828	
A^2	1843.43	1	1843.43	241.79	< 0.0001	
B^2	1186.47	1	1186.47	155.62	< 0.0001	
C^2	2329.52	1	2329.52	305.55	< 0.0001	
Residual	53.37	7	7.62			
Lack of Fit	38.25	3	12.75	3.37	0.1354	not significant
Pure Error	15.12	4	3.78			
Cor Total	6791.01	16				
C.V.: 7.06%		*R^2=0.9921*	*Adjusted R^2=0.9820*		*Predicted R^2=0.9064*	

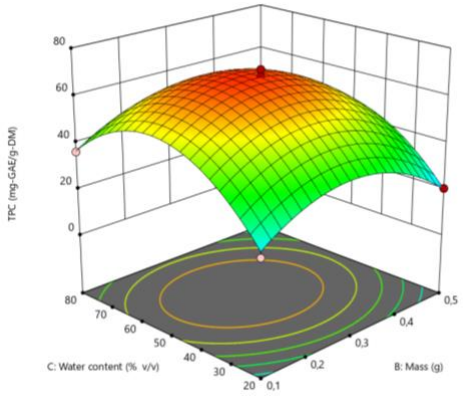
3.3. Effect of independent variables

Figure 2 shows 3D (three-dimensional) surface plots generated with Design-Expert software to visually depict how changes in independent variables affect the outcome (dependent variable). Figure 3 shows the Box-Behnken design cube with 17 experimental runs including 5 center points with 3 factors and 3 level.

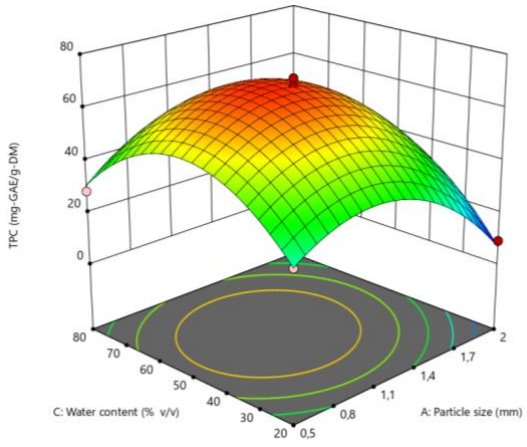
Figure 2a illustrates the impact of particle size on total phenolic content (TPC) yield. Initially, increasing the particle size resulted in higher yields. This situation may be attributed to the larger particle sizes can reduce agglomeration typically associated with smaller powders. However, after a certain point, increasing the particle size led to a decrease in TPC yield. This may be attributed to the lower surface area-to-volume ratio of larger particles, which could impede solvent penetration and extraction of phenolic compounds from the plant material [10]. Figure 2b indicates that the total phenolic content increases with an increase in solid mass until a certain point, after which it starts to decrease. After optimal point is reached, as the sample mass increases, the available surface area for solvent penetration and dissolution of phenolic compounds decreases. This results in reduced extraction efficiency and lower yields, since the solvent is unable to fully solubilize the target components. [12]. As shown in Figure 2c, initially, the addition of water to the solvent system increased the TPC yield due to a decrease in surface tension and viscosity, as well as an increase in polarity. However, after optimal concentration of water, the total phenolic yield decreased because excessive water concentration can have a negative impact on the interaction between DES and target compounds, leading to a reduction in extraction efficiency [13].



a



b



c

Figure 2. Effect of particle size to sample mass (**a**), sample mass to water content in DES (**b**), and particle size to water content in DES (**c**) on the total phenolic content

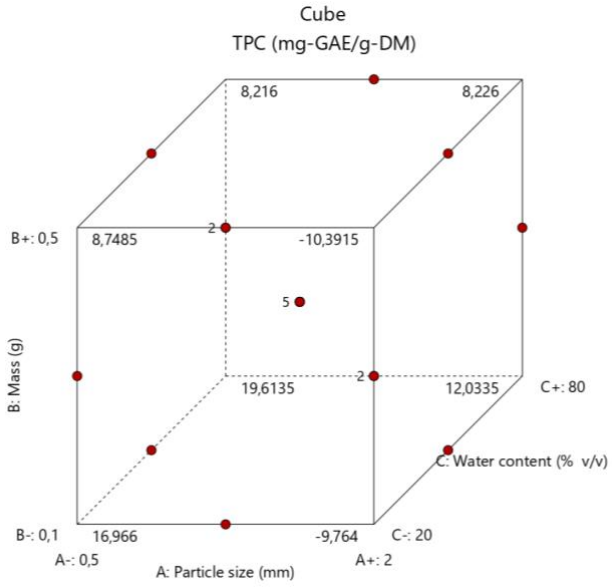


Figure 3. Box-Behnken design cube

3.3. Optimization study

The optimization study determined that the highest TPC yield (68.863 mg-GAE/g-DM) was achieved under the following optimum conditions: 1.135 mm particle size, 0.280 g sample mass, and 52.964% (v/v) water content in the DES system. The accuracy of the quadratic model equation based on TPC findings generated by RSM was confirmed with the validation study. Figure 4 illustrates the compatibility between the actual and estimated values.

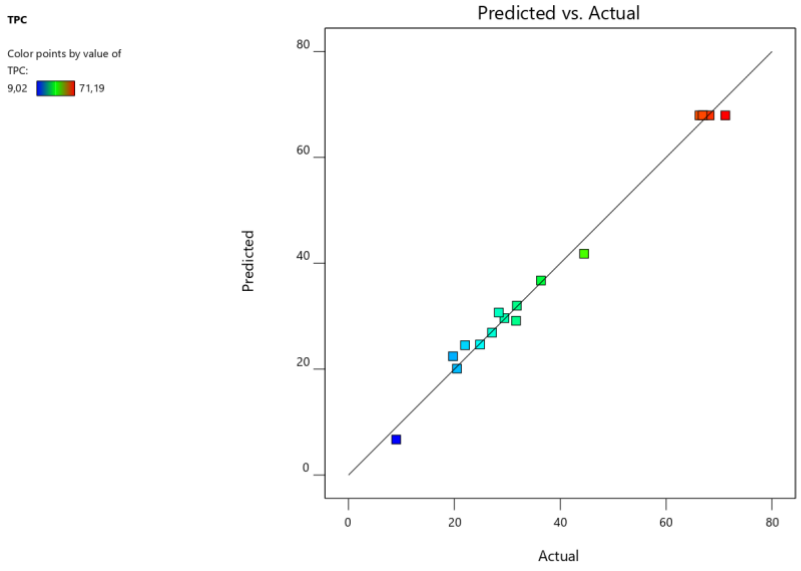


Figure 4. Comparison between actual and predicted TPC findings

3.3. Bioactive content of the extract

Table 5 reports the findings of two different tests used to evaluate the antioxidant activity of olive leaf extracts such as DPPH and ABTS assays, as well as the total flavonoid content (TFC). To better understand the antioxidant activity mechanism of the extracts, two different antioxidant activity tests, such as DPPH and ABTS assays, were performed. The results of these tests were found to be strongly correlated with each other, with a correlation coefficient of r>0.83. ABTS and DPPH assays showed a strong and positive correlation with TFC, with correlation coefficients of >0.84 and >0.81, respectively. These results suggest that the extracts have significant antioxidant potential, as indicated by the high correlation between TFC and both antioxidant activity tests. Additionaly, this relationship is thought to be due to the ability of flavonoids to act as effective scavengers of free radicals and other reactive species, thereby reducing oxidative stress and protecting cells from damage.

Table 5. Bioactive content of olive leaf extract

No	TFC (mg-CE/g-DM)	DPPH (mg-TEAC/g-DM)	ABTS (mg-TEAC/g-DM)
1	54.38 ± 0.002	19.61 ± 0.006	17.87 ± 0.001
2	55.69 ± 0.003	20.66 ± 0.005	18.52 ± 0.003
3	59.19 ± 0.004	14.07 ± 0.005	12.15 ± 0.003
4	61.15 ± 0.002	21.26 ± 0.006	18.61 ± 0.004
5	65.94 ± 0.001	20.08 ± 0.002	16.91 ± 0.004
6	60.11 ± 0.004	19.74 ± 0.004	18.30 ± 0.003
7	64.38 ± 0.002	22.25 ± 0.004	15.29 ± 0.003
8	59.31 ± 0.002	24.01 ± 0.003	17.66 ± 0.002
9	93.33 ± 0.003	34.83 ± 0.002	27.50 ± 0.002
10	58.68 ± 0.002	17.58 ± 0.002	14.64 ± 0.005
11	31.25 ± 0.005	8.99 ± 0.003	6.73 ± 0.003
12	51.46 ± 0.004	10.80 ± 0.004	10.36 ± 0.002
13	37.65 ± 0.002	14.93 ± 0.004	9.24 ± 0.002
14	111.19 ± 0.002	39.93 ± 0.006	28.82 ± 0.001
15	59.11 ± 0.001	22.38 ± 0.003	16.60 ± 0.001
16	58.06 ± 0.003	20.47 ± 0.003	18.53 ± 0.004
17	47.57 ± 0.003	19.41± 0.002	8.57 ± 0.005

* Data are given as the mean (n=3) ± standard deviation

3.4. Kinetic Analysis

Figure 4 shows the TPC variation over time at different amplitudes. The results revealed that after 75 minutes of ultrasound treatment at three different amplitudes (10%, 30%, and 50%), the TPC yield of the extraction process decreased significantly for all amplitudes after 45 minutes. Based on the findings, the optimal extraction conditions were identified to be an output amplitude of 30% and an extraction time of 45 minutes, and the extraction process was carried out under these conditions. Table 1 lists the kinetic models applied in the study, such as the pseudo-first-order model, pseudo-second-order model, film theory, and Peleg's model. The calculated kinetic parameters and correlation coefficients of determination (R^2) for these models using the kinetic data obtained from Figure 4 are presented in Table 6. Furthermore, the R^2 values obtained from the kinetic models suggest that the kinetic data is in agreement with the proposed kinetic equations (R^2 = 0.9081–0.9960). According to film theory, the extraction process occurs in two stages, with the fast extraction stage being followed by a slow diffusion stage, and the washing coefficient for optimum amplitude (30%) was found to be higher than the slow extraction coefficient, as indicated in Table 6 [14]. As seen in Table 6, an

increase in extraction rate constants was observed for all kinetic models as the amplitude was increased from 10% to 30%. This can be attributed to the enhanced cavitation and mechanical effects of ultrasound [15]. Furthermore, high amplitude levels in ultrasonication can also generate excessive heat, which can lead to thermal degradation of the phenolic compounds. Therefore, it is important to optimize the ultrasonication parameters, including the amplitude, to avoid excessive damage and ensure that the maximum phenolic content yield is achieved [16].

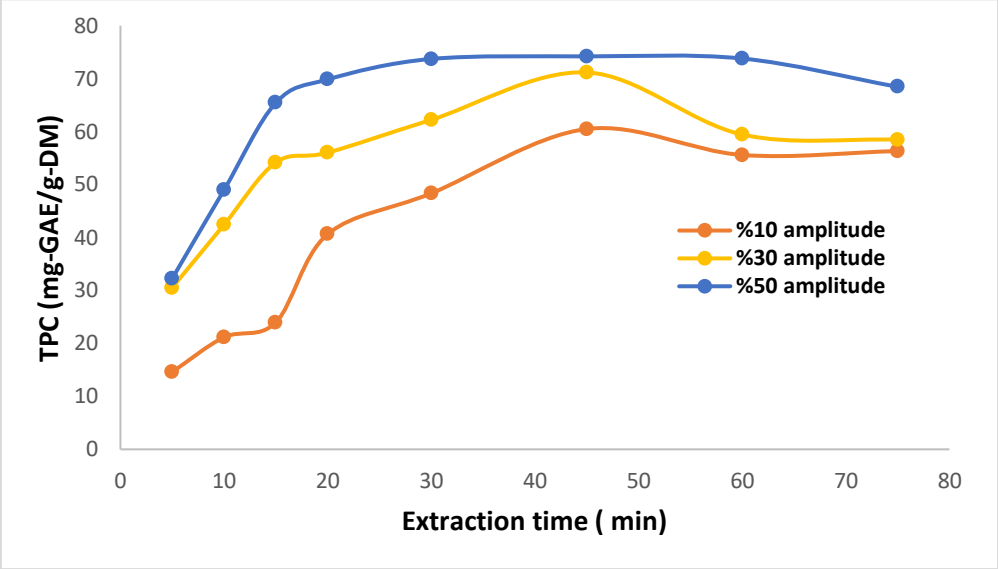


Figure 4. Effect of amplitude on TPC yield over time

Table 6. Analysis of kinetic model parameters for ultrasonic extraction of total phenolic compounds from olive leaves

Model	**Parameters**			
Pseudo first-order	**Output amplitude (%)**	**k_1 (min^{-1})**		**R^2**
	10	0.0528		0.9437
	30	0.0563		0.9854
	50	0.114		0.9960
Pseudo second-order	**Output amplitude (%)**	**k_2 (L mg^{-1} min-1)**		**R^2**
	10	$3.193*10^{-5}$		0.9081
	30	$2.063*10^{-4}$		0.9839
	50	$1.655*10^{-4}$		0.9918
Film-theory	**Output amplitude (%)**	**b**	**k_3 (min^{-1})**	**R^2**
	10	-	0.0528	0.9437
	30	0.1949	0.0563	0.9854
	50	-	0.114	0.9960
Peleg's model	**Output amplitude (%)**	**k_4 (min g mg^{-1})**	**k_5 (g mg^{-1})**	**R^2**
	10	0.2913	0.0136	0.9434
	30	0.0928	0.0138	0.9461
	50	0.0944	0.0111	0.9455

4. Conclusions

In vitro analyses have revealed that olive leaf extract contains potent bioactive components. To extract these components from dried olive leaves, a method combining ultrasound-assisted extraction with a lactic acid-based deep eutectic solvent was successfully employed. The most influential factor on total phenolic content response was found to be the second power effect of water content in DES (C^2). The optimal parameters for the ultrasound-assisted extraction process were determined to be a particle size of 1.135 mm, a sample mass of 0.280 g, and a water content of 52.964% (v/v) in the lactic acid-based deep eutectic solvent system, according to the results of the optimization study. The statistical indicators from the analysis of variance suggest that the quadratic model equation used to relate to the total phenolic content findings was a good fit for the experimental data. The total flavonoid content has been found to exhibit a strong correlation with antioxidant activity tests, with a correlation coefficient greater than 0.80. This correlation is believed to be attributed to the capability of flavonoids to function as potent scavengers of free radicals and other reactive species and diminishing oxidative stress. The all kinetic models used in the study are appropriate for describing the kinetic behavior of the olive leaf extracts obtained by ultrasound-assisted extraction depending on the high correlation coefficients (R^2 >0.90). Additionally, the use of a green solvent in the optimization process may have important implications for sustainability and reducing the environmental impact of the extraction process. Overall, this method could provide a potential new approach for the extraction of bioactive compounds from olive leaves, which could have various applications in the food, pharmaceutical, and nutraceutical industries.

Conflict of Interest

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

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ORAL PRESENTATION - FULL PAPER

TRAGOPOGON DSHIMILENSIS K. KOCH EXTRACT INDUCES WOUND CLOSURE IN DIABETIC RATS

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Abstract
Wound healing agents aid the body's natural healing process and promote wound closure. Members of the *Tragopogon* L. family are Turkish medicinal plants used to treat wounds. In this study, we explore the time-dependent effect of *T. dshimilensis* K. KOCH extract on wound closure in diabetic wound repair. Using a Soxhlet apparatus, plant specimens were extracted with methanol. Using a rotary evaporator, the methanol solvent was evaporated after extraction. There were 36 Wistar rats used in the experiments. Animals were separated into three major groups: non-diabetic (NDM), diabetic (DM), and *T. dshimilensis* (ETD). A single intraperitoneal injection of streptozotocin induced diabetes. On all animals, full-thickness dorsal skin excisions were made. In the ETD group, wounds were treated with 50 mg/kg *T. dshimilensis* methanolic extract. There was no treatment administered to the NDM and DM groups. Throughout the process of wound healing, wound areas were photographed and computed. On day seven, the wound areas in the ETD-treated group lowered significantly ($P<0.001$) when compared with the DM group. *T. dshimilensis* extract accelerates wound closure in diabetes-induced rats, and can be used to develop novel medications for diabetic wounds.

Key Words: diabetes, wound closure, wound healing, Tragopogon dshimilensis

1. Introduction

Wounds may occur in various parts of the body as a result of damage caused by many reasons (cutting-piercing tools, firearms, heat, electricity, bites, etc.) throughout the life of living organisms. The word "wound" is defined as a disruption of tissue integrity as a result of damage. The process of wound healing, which is an innate ability, begins following injury and continues under the control of growth factors and cytokines. This process, which consists of various physiological and biological events, is usually analyzed under 3 intertwined phases: inflammatory, proliferation and remodeling (Werner & Grose, 2003; Lee et al., 2012; Kaltalioglu & Coskun-Cevher, 2015).

Many local and systemic factors such as diabetes, infection, aging, oxidative stress, etc. can delay or inhibit the process by affecting wound healing (Guo & Dipietro, 2010). Diabetes mellitus (DM) is a metabolic disease that affects hundreds of millions of people worldwide (Guo & Dipietro, 2010). It severely damages most body systems, especially blood vessels and nerves (De Sousa & Batista, 2016). Given that there is currently no complete cure for diabetes, it is important to treat the complications caused by diabetes in the most effective way to improve the declining quality of life of diabetics. Wounds that do not heal or heal late are one of the major factors that reduce the quality of life of people with diabetes. It is believed that infection, neuropathy, abnormal cellular activities, peripheral vascular diseases, an impaired oxidative balance, and irregularities in the production and

degradation of growth factors are the underlying causes of the problem (Li et al., 2008; Peplow & Baxter, 2012; Berk et al., 2015).

In such cases, some chemicals or materials are exogenously applied to the wound site to improve or accelerate healing. These therapeutic products may be synthetic substances produced in the laboratory as a result of researches or folkloric plants used for this purpose from past to present. Nature has been a source for medical treatments for thousands of years (Maver et al., 2015).

The genus *Tragopogon* L., which includes species containing milk secretion, is in the Asteraceae family. Members of the genus *Tragopogon*, which are annual, biennial or perennial herbaceous plants, generally spread in steppe-like and semi-arid areas. The stem is simple or branched and has a cylindrical taproot. It has tongue-tied flowers in yellow or purple. Fruits usually have an elongated and prominent beak (Coşkunçelebi et al., 2017). This genus, which is represented by approximately 150 species in the world, has 21 species in Türkiye. The *T. dshimilensis* species used in our study is a European-Siberian element plant and it is distributed in Trabzon, Gümüşhane, Rize, Giresun, Artvin, Erzurum and Ardahan regions in Türkiye. This endemic species, known as "Cimil porini", is a perennial plant with a length of 23-80 cm, with fibrous leaf residues at the base, and with a sparse woolly hairy stem that is usually branched near the base (Güner et al., 2012; Coşkunçelebi et al., 2017). It has been reported in many ethnobotanical studies that various *Tragopogon* species are used for medicinal purposes, especially in wounds and gastrointestinal disorders, among the people and in traditional treatment systems (Guarrera et al., 2005; Farzaei et al., 2013; Farzaei et al., 2014; Tuzlaci & Doğan, 2010; Altundag & Ozturk, 2011; Cakilcioglu et al., 2011).

In our study, it was aimed to examine the time-dependent effects of *T. dshimilensis* of the *Tragopogon* genus, which has been reported to be effective in wound treatment in ethnobotanical studies, on wound healing in diabetes-induced rats.

2. Material and Methods

2.1. Extraction

Dr. Mutlu GULTEPE identified plant samples collected from the Maçka/Trabzon province in Turkey (40°38'02.4"N, 39°23'39.6"E). A voucher specimen has been deposited with the designation ESPH 017 at the Herbarium of the Vocational School of Espiye, Giresun University. The collected samples' aerial sections were separated and dehydrated at room temperature. Using Soxhlet apparatus, the desiccated, powdered aerial parts were extracted for eight hours in 100 mL of methanol. Afterwards, the extracts were filtered with filter paper, and the solvents were removed with a rotary evaporator.

2.2. Animals

The Gazi University Local Ethics Committee for Animal Experiments (G.Ü.ET-15.053) approved all animal experimentation procedures. We utilized 36 male Wistar albino rats that weighed between 200 and 250 grams each. The rats were housed separately, subjected to a light/dark cycle that lasted for 12 hours, kept at room temperature, and given a diet consisting of normal rat food and water.

2.3. Induction of diabetes

For the purpose of inducing diabetes, a single intraperitoneal dosage of streptozotocin (STZ) (60 mg/kg, Sigma-Aldrich, USA) was administered. The STZ was formed in a sodium citrate buffer (0.1 mol/L, pH 4.5). Blood glucose levels were monitored 72 hours after STZ induction using a glucometer, and individuals with levels more than 250 mg/dL were classified as diabetic (Kaltalioglu et al., 2020).

2.4. Excisional wound model

The animals were separated into three primary groups, as shown in Table 1. Diabetes was induced in DM and ETD groups. The animals were administered intramuscular doses of ketamine HCl and xylazine HCl (50 mg/kg and 5 mg/kg, respectively) to induce anesthesia. Shaving and sterilizing

the rat's dorsum. Six full-thickness excisional skin wounds were opened on each rat using an 8-mm punch (Acuderm, USA). In the ETD groups, wounds were treated topically with *T. dshimilensis* extract (50 mg/kg, dissolved in physiological saline). There was no treatment administered to the NDM and DM groups. Six animals from each group were killed on days 3 and 7 by intracardiac blood aspiration while under anesthesia.

Table 1. Experimental design

Groups	Procedure
1. Non-diabetic (NDM)	Non-diabetic, wounded, but no treatment was given; they were sacrificed on the third day after being wounded (n = 6)
	Non-diabetic, wounded, but no treatment was given; they were sacrificed on the seventh day after being wounded (n = 6)
2. Diabetic (DM)	Diabetic, wounded, but no treatment was given; they were sacrificed on the third day after being wounded (n = 6)
	Diabetic, wounded, but no treatment was given; they were sacrificed on the seventh day after being wounded (n = 6)
3. *T. dshimilensis* (ETD)	Diabetic, wounded, ETD was given; they were sacrificed on the third day after being wounded (n = 6)
	Diabetic, wounded, ETD was given; they were sacrificed on the seventh day after being wounded (n = 6)

2.5. Measurament of wound area and wound closure rate

During the wound healing process, the wound areas were photographed and calculated using a computer program (ImageJ, NIH, USA). The wound closure rate was computed as (Kaltalioglu et al., 2020):

Wound closure rate (%) = [(wound area on day 0 – wound area on day 3 or 7)/wound area on day 0] × 100.

2.6. Statistical Analysis

The results were given as the mean ± standard deviation (SD) and compared using the post-hoc Tukey test following a one-way ANOVA. $P<0.001$ was considered statistically significant (SPSS v.16, IBM, USA).

3. Results and Discussion

Wound healing is a complicated process characterized by inflammation, epithelialization, and angiogenesis. Various systemic and local factors, especially diabetes, may delay or prevent the process by affecting wound healing. Considering that there is currently no complete healing in diabetes, it is important to effectively treat the complications caused by diabetes in order to improve the declining quality of life of diabetics. It has been reported in ethnobotanical studies that many plants in the world are traditionally used as wound healing agent. The effect of *T. dhsimilensis* extract on the healing time of diabetic wounds was demonstrated in our study.

Epithelialization is the process of coating an epithelial surface that has been devoid of cells. Successful wound closure requires the initiation, maintenance, and completion of epithelialization (Pastar et al., 2014). Delayed wound closure is an important complication in diabetics. In our study, we found that the wound area of DM groups was greater than that of NDM groups ($P<0.001$) on days 3 and 7 (Table 2 and Figure 1). This result supports the thesis that wound healing is delayed in diabetics.

Table 2. Effects of *T. dshimilensis* extract on wound areas (mm^2). [a] $P < 0,001$ when compared to NDM on day 0, [b] $P < 0,001$ when compared to DM on day 0, [c] $P < 0,001$ when compared to intragroup day 3, [d] $P < 0,001$ when compared to DM on same day, [e] $P < 0,001$ when compared to NDM on same day.

Groups	Day 0	Day 3	Day 7
1. Non-diabetic (NDM)	62,09 ± 3.50	29,84 ± 3,17[a]	7,04 ± 1,15[ac]
2. Diabetic (DM)	62,89 ± 3.73	43,80 ± 2,05[abe]	24,07 ± 1,68[abce]
3. *T. dshimilensis* (ETD)	61,35 ± 1,75	38,56 ± 1,82[ab]	9,04 ± 0,89[abcde]

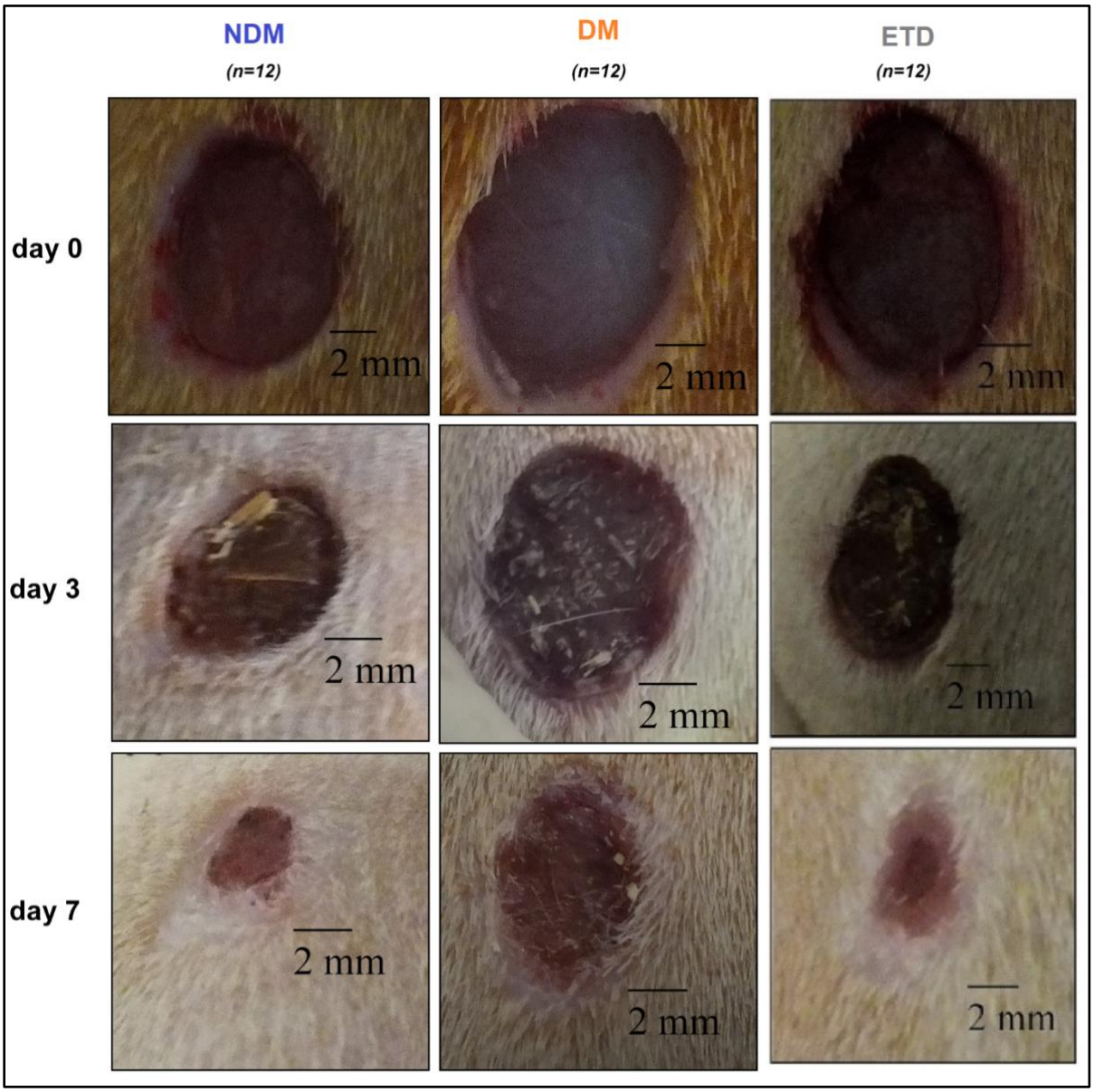

Figure 1. Experimental wound model and time-dependent change of the wound area

On the contrary, the wound area in the ETD group was found to be lower compared to the DM group on day 7 ($P<0.001$) (Table 2 and Figure 2). However, the change on day 3 was not statistically significant compared to the DM group ($P>0.001$) (Table 2 and Figure 2).

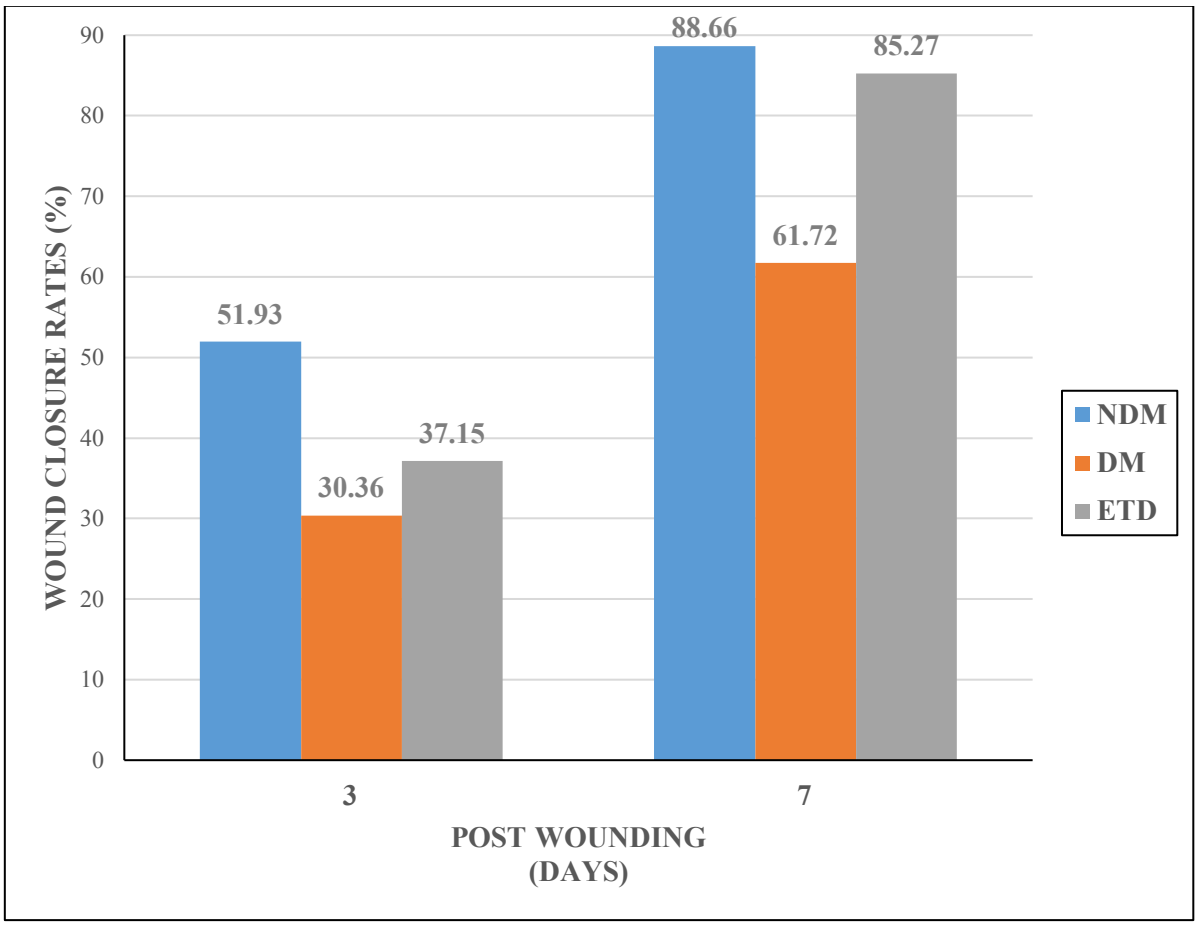

Figure 2. Effects of *T. dshimilensis* extract on wound closure rates (%)

Therefore, it can be said that *T. dshimilensis* extract contributes to the acceleration of diabetic healing by improving epithelialization. Hashemnia et al. (2014) reported in an *in vivo* study that application of *T. porrifolius* (another member of *Tragopogon* genus) extract on wounds accelerated healing. This healing effect of the plant extract may be due to its rich phenolic compounds (flavonoids, phenolic acids, tannins, etc.). In our previous study, we reported that *T. dshimilensis* species has antioxidant activity and contains chlorogenic acid, caffeic acid and ferulic acid as major phenolic compounds (Kaltalioglu & Coskun-Cevher, 2016). Chen et al. (2013) reported that chlorogenic acid increased cell proliferation and epithelialization and accelerated healing by showing antioxidant activity in their study with Wistar rats. Song et al. (2008) reported that caffeic acid had a positive effect on incision wounds in mice. Ghaisas et al. (2014) reported that ferulic acid application increased epithelialization and hydroxyproline levels compared to the control group in their study in diabetic rats. These studies in the literature support our findings.

4. Conclusion

In conclusion, it can be said that administration of *T. dshimilensis* extract accelerates wound closure depending on time in diabetes-induced rats. It can be used in this context to develop new pharmaceuticals for diabetic wound repair.

Acknowledgements

This paper is part of Dr. Kaan Kaltalioglu's doctoral thesis, which he presented in 2017 to the Gazi University Biology Department.

Conflict of Interest

None

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ORAL PRESENTATION - FULL PAPER

ITS SEQUENCE ANALYSIS IN *CARTHAMUS TINCTORIUS* GENOTYPES

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Abstract

Carthamus L. (Asteraceae) is represented by about 20 species in the Eastern Mediterranean and Irano-Turanian regions. The origin center of the genus is considered to be the eastern part of the Mediterranean region. *Carthamus tinctorius* L. (2*n*=2*x*=24), commonly known as safflower, is one of humanity's oldest crops. The species is economically important as it is used by the public as a natural food colourant as well as for its medicinal properties. In this study, the internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA (nrDNA) were analysed in twenty-nine safflower genotypes. The Parsimony and Bayesian analyses were performed using PAUP and MrBayes programs, respectively. As a result, it was determined that there are no differences were found in safflower genotypes in terms of ITS sequences. This is consistent with the occurrence of a population genetic bottleneck during domestication, as reported in the literature. The findings of this study will be beneficial for *Carthamus tinctorius* in breeding programs in Türkiye as well as in other countries of the world.

Key Words: Asteraceae, Safflower, Türkiye.

1. Introduction

The genus *Carthamus* L. (Asteraceae) is represented by about 20 species and the distribution center of the genus is Eastern Mediterranean and Irano-Turanian region (Sehgal et al. 2009; Bowles et al. 2010). Vilatersana et al (2000a, 2005) divided the genus *Carthamus* into two sections, (section *Carthamus* L. and section *Atractylis* Rchb.) using DNA markers. The distinction of these sections was supported by different molecular analyses (Sasanuma et al. 2008; Bowles et al. 2010; Mehrotra et al. 2013). Section *Carthamus* (*C. tinctorius* L., *C. palaestinus* Eig, *C. oxyacanthus* M. Bieb., *C. persicus* Desf. ex Willd., *C. gypsicola* Iljin and *C. curdicus* Hanelt) has a diploid (2*n*=2*x*=24; Hanelt 1963; Bir and Sidhu 1980; Vilatersana et al. 2000b; Garnatje et al. 2006; Anjali and Srivastava 2012; Yazdani 2013; Uysal et al. 2018), while section *Atractlis* has polyploids (Vilatersana et al. 2007; Sehgal et al. 2009; Sardouei-Nasab et al. 2023).

Safflower (*Carthamus tinctorius* L.), the only cultivated species in the genus, is one of humanity's oldest crops (van Zeist and Walterbolk-van 1992; Knowles and Ashri 1995; Sung et al 2010). *C. tinctorius* is economically important due to an oilseed crop which is used as a more affordable substitute for saffron or a source of edible oil and has numerous medicinal properties. In addition, the flowers of the species are used as raw medicine, natural dye and food colouring in folk

medicine (Han 1988; Kim, 1992; Kang et al. 1999; Lee et al. 2002; Chapman and Burke 2007; Dempewolf et al. 2008; Emongor 2010; Asgarpanah and Kazemivash 2013; Kim et al. 2016; Houmanat et al. 2016; Vilatersana et al. 2022).

Many morphological, palynological, karyological and molecular studies have also been carried out for *C. tinctorius* (Chapman and Burke 2007; Sehgal et al. 2009; Chapman et al. 2010; Bowles et al. 2010; Shao et al. 2012; Bülbül 2013; Houmanat et al. 2016; Uysal et al. 2018; Sardouei-Nasab et al. 2023) that is widely cultivated as a source of high quality vegetable and industrial oil and forage for livestock in various agricultural production systems (Knowles 1989; Singh and Nimbkar 2007; Sehgal et al. 2009; Shafiei-Koij et al. 2020). Until now, various molecular studies such as conservative intron-spanning PCR markers, RAPD, SSR, ISSR, AFLP, EST-SSR and SNP have been performed on cultivars, accessions or populations of the *C. tinctorius* (Sehgal and Raina 2005; Chapman and Burke 2007; Sung et al. 2010; Mayerhofer et al. 2011; Tekkanat 2014; Houmanat et al. 2016; Singh et al. 2022; Sardouei-Nasab et al. 2023). ITS analyses intend to analyse the phylogenetic relationships among taxa belonging to the genus *Carthamus* (Sehgal et al. 2009; Vilatersana et al. 2000a, 2022).

In plants, the 18S-5·8S-26S rDNA locus is frequently used for molecular systematic studies. Some researchers have even shown that this region can be used to assess phylogenetic relationships between cultivated species and their wild relatives (Baldwin,1992). The ITS region consists of ITS1, 5.8S and ITS2.The transcribed internal spacers ITS1 and ITS2 are located between the genes encoding 5.8S, 18S and 26S nuclear ribosomal RNA (nrRNA) subunits (Baldwin,1992). The nuclear genomic ITS1-5.8S-ITS2 regions of rDNA are contiguous and can be PCR amplified and studied together as a single unit. Especially ITS1 and ITS2 regions evolve relatively faster, which has made ITS regions one of the most important markers for phylogenetic studies (Arnheim et al. 1980; Francisco-Ortega et al. 2001; Sonnante et al. 2003; Alvarez and Wendel 2003; Renner et al 2007; Pettengill and Neel 2008; Hřibová et al 2011). In some species, it has even proven useful in studies at the species and species population level due to the high variation of the ITS sequence (Yuan and Küpfer 1995; Desfeux and Lejeune 1996; Kollipara et al. 1997; Aïnouche and Bayer 1999; Sonnante et al. 2003). It has also been used to determine the ancestry of hybrid species and to study the origin of polyploid species (Sun et al. 2002; Liu et al. 2006; Wang et al. 2007).

As far as we know, there are ITS analyses of the *C. tinctorius* for phylogeny studies or individuals belonging to different populations (Vilatersana et al. 2000a, 2022; Sehgal et al. 2009; Bowles et al. 2010). In these studies, a complete solution could not be obtained among genotypes (Bowles et al. 2010). For this reason, it was aimed to examine the internal transcribed spacer (ITS) region to contribute to the selection of safflower varieties by using genotypes that were reported to differ among genotypes in previous chromosomal and molecular studies and to develop an additional marker for breeding studies. In this study, the twenty-nine genotypes belonging to *Carthamus tinctorius* were analysed in terms of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA).

2. Material and Methods

The research materials were provided by Dr. Rahim ADA (Table 1). Total genomic DNA was extracted by the 2X CTAB method as previously described and modified by Doyle and Doyle (1987), Soltis et al (1991) and Cullings (1992) from leaves belonging to *C. tinctorius* genotypes. For PCR amplifications; amplification of the ITS region was performed using ITS1-ITS4 primers (White et al. 1990; Garcia-Jacas et al (2006). ITS sequences of the outgroup (*Carduncellus cuatrecasasii*) and some taxa belonging to the genus *Carthamus* were downloaded from GeneBank and included in the analysis (Table 1).

Table 1. Samples analysed in the study

	Laboratory codes	Genotypes	ITS Gene Bank numbers
1	TCAR1 (*Carthamus tinctorius*)	Black sun 2	OQ938753 (In this study)
2	TCAR2 (*Carthamus tinctorius*)	J-41	OQ938754 (In this study)
3	TCAR3 (*Carthamus tinctorius*)	CB	OQ938755 (In this study)
4	TCAR4 (*Carthamus tinctorius*)	F4	OQ938756 (In this study)
5	TCAR5 (*Carthamus tinctorius*)	KS03	OQ938757 (In this study)
6	TCAR6 (*Carthamus tinctorius*)	KS07	OQ938758 (In this study)
7	TCAR7 (*Carthamus tinctorius*)	CT-8-3	OQ938759 (In this study)
8	TCAR9 (*Carthamus tinctorius*)	J-19	OQ938760 (In this study)
9	TCAR10 (*Carthamus tinctorius*)	A13	OQ938761 (In this study)
10	TCAR11 (*Carthamus tinctorius*)	A29	OQ938762 (In this study)
11	TCAR12 (*Carthamus tinctorius*)	H3	OQ938763 (In this study)
12	TCAR13 (*Carthamus tinctorius*)	E12	OQ938764 (In this study)
13	TCAR14 (*Carthamus tinctorius*)	C12	OQ938765 (In this study)
14	TCAR15 (*Carthamus tinctorius*)	J51	OQ938766 (In this study)
15	TCAR16 (*Carthamus tinctorius*)	G16	OQ938767 (In this study)
16	TCAR18 (*Carthamus tinctorius*)	F6	OQ938768 (In this study)
17	TCAR19 (*Carthamus tinctorius*)	C11	OQ938769 (In this study)
18	TCAR20 (*Carthamus tinctorius*)	Y 11-8-14-1	OQ938770 (In this study)
19	TCAR21 (*Carthamus tinctorius*)	A30	OQ938771 (In this study)
20	TCAR22 (*Carthamus tinctorius*)	F5	OQ938772 (In this study)
21	TCAR23A (*Carthamus tinctorius*)	J29	OQ938773 (In this study)
22	TCAR24A (*Carthamus tinctorius*)	C 2-8-1	OQ938774 (In this study)
23	TCAR25 (*Carthamus tinctorius*)	E5	OQ938775 (In this study)
24	TCAR26 (*Carthamus tinctorius*)	H7	OQ938776 (In this study)
25	TCAR27A (*Carthamus tinctorius*)	H14	OQ938777 (In this study)
26	TCAR28 (*Carthamus tinctorius*)	Dinçer	OQ938778 (In this study)
27	TCAR29 (*Carthamus tinctorius*)	Remzibey	OQ938779 (In this study)
28	TCAR30 (*Carthamus tinctorius*)	E1	OQ938780 (In this study)
29	TCAR31 (*Carthamus tinctorius*)	KS06	OQ938781 (In this study)
30	*Carthamus tinctorius*		GU969647
31	*Carthamus tinctorius*		GU969648
32	*Carthamus tinctorius*		GU969650
33	*Carthamus oxyacanthus*		GU969639
34	*Carthamus oxyacanthus*		GU969640
35	*Carthamus oxyacanthus*		GU969641
36	*Carthamus palaestinus*		GU969642
37	*Carthamus palaestinus*		GU969643
38	*Carthamus persicus*		GU969644
39	*Carthamus glaucus*		GU969624
40	*Carthamus glaucus*		GU969625
41	*Carthamus glaucus*		GU969626
42	*Carthamus glaucus*		GU969627
43	*Carthamus lanatus*		GU969628
44	*Carthamus lanatus*		GU969629
45	*Carthamus lanatus*		GU969632
46	*Carthamus lanatus*		GU969633
47	*Carthamus leucocaulos*		GU969634
48	*Carthamus leucocaulos*		GU969635
49	*Carduncellus cuatrecasasii*		AH009511

All obtained sequences were aligned with the Bioedit program (version 7.0.5.3; Hall 1999). MrBayes 3.2 program was used for Bayesian analyses. In this analysis, Markov Chain Monte Carlo

(MCMC) algorithm was run, and analysis was started with random trees and 64×10^4 generations where repeated trees were sampled every 10 generations. Probability values calculated for each chain were checked using the Tracer v1.7 (Rambaut et al. 2018) program and 20% of the sampled trees were burned-in. The Bayesian inference tree was visualized with the FigTree v1.4.0 program. Parsimony analyses were performed with the PAUP 4.0 beta version (Swofford, 2002). In this analysis, the heuristic search method (TBR swapping algorithm and 1,000 random replications) was used. For the bootstrap (BS) analyses (Felsenstein 1985), 1000 heuristic search replicates were used and set at the default settings. CI (Consistency index), RI (Consistency index) and HI (homoplasy index) were given for the strict consensus tree, with the exclusion of the uninformative characters.

3. Results and Discussion

This study demonstrates the use of ITS sequence variation to generate additional molecular markers suitable for assessing the difference among Safflower genotypes. In this study, ITS sequences of some taxa in the genus *Carthamus* and genotypes of *C. tinctorius* are aligned, and the total length of the data matrix is 662 bp. In this data matrix, 613 characters are continuous and 26 characters are variable. The number of Parsimony-informative characters is 23. The consistency index (CI), retention index (RI) and homoplasy (HI), which are seen as a measure of confidence in the data to reconstruct phylogenetic relationships, are calculated as 0.963, 0.989, and 0.037, respectively. According to the Parsimony and Bayesian analyses based on ITS data, the topological distribution of taxa on the combined tree (Figure 1) is highly consistent with the previous karyological and molecular analyses (Vilatersana et al. 2000a,b; 2005; 2022; Garnatje et al. 2006; Chapman and Burke 2007; Uysal et al. 2022; Sardouei-Nasab et al. 2023).

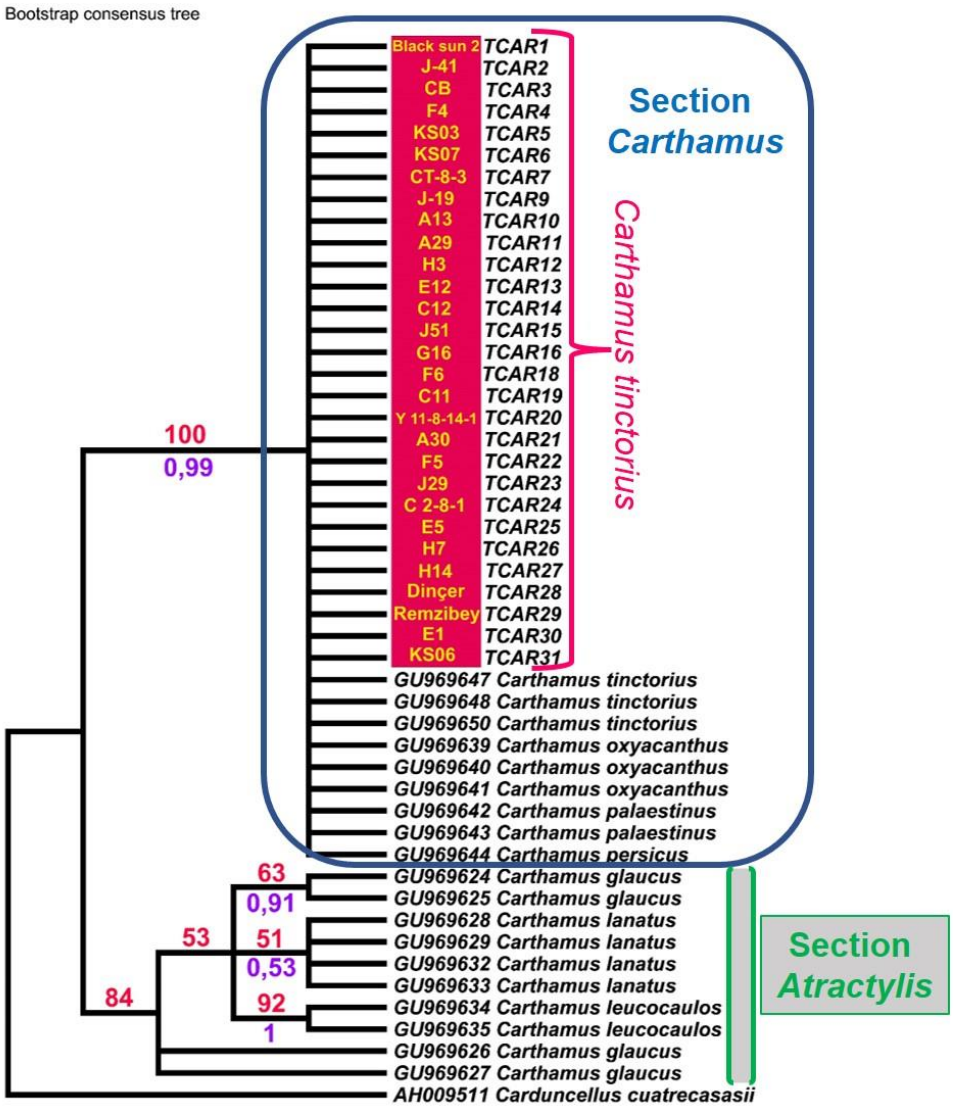

Figure 1. Combined tree obtained from Parsimony and Bayesian analysis of ITS data.

In this study, ITS data of different genotypes belonging to *C. tinctorius* and some taxa belonging to *Carthamus* (diploid) and *Atractylis* (polyploid) sections of the genus *Carthamus* reported by Bowles et al (2010) were aligned to increase resolution and a phylogenetic tree was created (Figure 1). The present study shows a clear sectional distinction according to ploidy levels, in agreement with previous reports (Vilatersana et al 2000a; 2005). The clade showing the *Carthamus* section containing taxa with diploid chromosome number (2n=2x=24; Bir and Sidhu 1980; Garnatje et al. 2006; Uysal et al. 2018) is strongly supported (BS/PP:100/0.99), results which are consistent with previous studies (Vilatersana et al. 2000a; Bowles et al 2010). However, some researchers have reported that the success of phylogenetic analyses in determining interspecies relationships within the genus is limited (Vilatersana et al. 2000a; Vilatersana et al. 2005; Chapman and Burke 2007). In addition, it was emphasized that the phylogenetic relationships among the taxa within the *Carthamus* section, which have morphological differences could not be resolved with the ITS data (Vilatersana et al. 2005; Bowles et al. 2010). Bowles et al. (2010) indicated the relationships within the section *Carthamus*

have not been resolved and the closest wild relative of cultivated safflower remains unclear and they suggested introgression as the reason why the species boundaries could not be determined exactly within the *Carthamus* section. In fact, some researchers suggested that these species may be races of a single species (Ashri and Efron 1964; Imrie and Knowles 1970; Bowles et al. 2010). Due to the inconsistency between the morphological and molecular data, it was emphasized that more work should be done to determine the species within the genus *Carthamus* (Bowles et al 2010).

In this paper, there is no difference between the ITS sequences of the genotypes, in contrast to the variation previously reported by karyomorphological and molecular analyses of the genotypes studied (Tekkanat 2014; Uysal et al. 2018). This may be related to the fact that the population may have gone through a bottleneck during domestication, as previously reported in the literature (Chapman and Burke 2007).

4. Conclusion

Consequently, the findings of this study will be useful in the breeding programs of *Carthamus* tinctorius. It is also important that Safflower is a modern oilseed plant in the Asteraceae family. Because these studies on safflower will make an important contribution to comparative analyses in examining the evolution of other oilseeds in the Asteraceae family (Chapman and Burke 2007).

Acknowledgements

We would like to thank TUBITAK (Project No: 117Z222) and S.U. BAP (Project No: 19401172) for their financial support.

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ORAL PRESENTATION - FULL PAPER

IMPACT OF ORGANIC AND INORGANIC FERTILIZERS APPLICATION ON THE ANTIBACTERIAL ACTIVITIES OF DILL (*ANETHUM GRAVEOLENS* L.)

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Abstract

The aim of this study was the investigation of dill (*Anethum graveolens* L.) antibacterial activities. The plant was grown with different levels of ammonium nitrate (AN: 3, 6, 9, and 12 kg da^{-1}) and different levels of farmyard manure (FM: 750, 1000, 1250, and 1500 kg da^{-1}). The ethanol, *n*-hexane, and dichloromethane extracts of dill (*Anethum graveolens* L.) were evaluated for their potential as antibacterial agents against six bacterial strains, including four gram-positive strains [*S. epidermidis*, *S. aureus*, *E. faecalis, Methicillin-resistant (MRSA)*], three gram-negative strains (*E. coli*, *P. aeruginosa*, *K. pneumoniae*), and one fungus (*C. albicans*). The tested minimum inhibitory concentration (MIC) values for the gram-positive and gram-negative strains ranged from 39 µg ml^{-1} to 1250 µg ml^{-1}. Except *S. epidermidis*, ethanol and *n*-hexane extracts showed significant antibacterial activity against all bacterial strains. The highest antibacterial activity against all tested bacterial species was observed with the 750 kg da^{-1} FM and 6 kg da^{-1} AN, 12 kg da^{-1} AN applications. In conclusion, dill extracts can be used as antibacterial agents for improving food safety.

Key words: Farmyard manure, Ammonium nitrate, *Anethum graveolens*, Antibacterial activity

Acknowledgements

This study is part of a MSc Thesis and it was supported by Scientifc Research Project Fund (2017.10.07.1205), Faculty of Agriculture, Bolu Abant Izzet Baysal University, Turkey.

Introduction

Currently, the demand for healthy food is increasing due to the health and sustainable environmental considerations of these products (Sangkumchaliang and Huang, 2012). This is more important in the case of medicinal plants that are directly related to human health (Khalesro et al., 2012). Medicinal and aromatic plants contain useful secondary metabolites

for humans. The essential oils and aromatic compounds of these plants are used in various uses, such as perfume, food flavoring, spice, food additives, and also making herbal medicines (Hadian et al., 2014).

Dill (*Anethum graveolens* L.), the only species belonging to the genus Anethum (Jana and Shekhawat 2010a), is an annual plant belonging to the family Apiaceae or Umbelliferae. It has 90-120 cm and thinly branched stems, finely divided leaves, and small umbrellas of yellow flowers (Elsayed et al., 2020). Anethum graveolens L. has been used in ayurvedic medicine since ancient times and is an herb used for its essential oil and as a spice generally. Ayurvedic uses of dill seeds are carminative, stomachic, and diuretic. The seeds and aerial parts of plant have various volatile components; Carvone and α-phellandrene, which are the dominant odors of dill seed, limonene, dill ether, and myristicin constitute the most dominant odors of the green part. Other compounds isolated from the seeds are coumarins, flavonoids, phenolic acids, and steroids (Jana and Shekhawat, 2010b).

Studies have shown that although chemical fertilizers are the needs of today's agriculture and are necessary for increasing the yield of plants, the increasing concerns of environmental pollution, product contamination, and their high cost have encouraged the replacement of chemical fertilizers with organic fertilizers to increase yield. (Siddiqui et al., 2011; Savci, 2012). Organic fertilizers, such as animal manure, by having the essential and needed elements of the plant (high-consumption and low-consumption) while eliminating or reducing chemical fertilizers, increase the water retention capacity and the cation exchange capacity of the growth, yield, and quality of the product, especially in the production of medicinal plants. (Kapoor et al., 2004; Wu et al., 2005; Pandey et al., 2016). Organic cultivation of medicinal plants reduces the possibility of negative effects on their medicinal quality. This is why many consumers of medicinal plants prefer organic herbal compounds (Griffe et al., 2003). According to Rostaei et al., (2018) study, the use of Farmyard manure increased the quality of dill.

In various studies, inactivating properties of dill on some microorganisms have been determined. Enterococcus spp, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus cereus*, Listeria spp, *Pseudomonas aeroginosa* bacteria are examples of this situation (Badr et al., 2017, Yaldız et al., 2018, Nada et al., 2018; Çapan, 2020).

The aim of this research was to determine the antimicrobial activity of *Anethum graveolens* affected by different levels of FYM and AN.

Materials & Methods

Growing conditions and treatments

This study aimed to investigate the antibacterial activity of dill (*Anethum graveolens* L.) under the influence of different fertilizer sources. The field experiment was carried out in

the Bolo Abant Izzet Baysal University research farm (40°28′43″N, 31°12′39″E; altitude: 830) for two years (2016-2017) in the form of a randomized complete block design in the form of split plots with three replications. The plant was grown with different levels of ammonium nitrate (AN: 3, 6, 9, and 12 kg da^{-1}) and different levels of animal manure (FMY: 750, 1000, 1250, and 1500 kg da^{-1}). Each experimental plot consisted of five rows with a distance of 30 cm between each row and 20 cm between each plant and the plot size was 5.6 square meters. The soil was rich in phosphorus (14.86 g kg^{-1}), potassium (53.73 g kg^{-1}), and organic matter (13.6 $^{-1}$), classified in the resi-loam category and had a neutral quality (pH = 7.25). According to the climatic data, the average temperature, humidity, and total rainfall from April to August in the two experimental years were 8.18 °C, 61%, and 208.8 mm, respectively. Different total doses of FYM (750, 1000, 1250, and 1500 kg day^{-1}) were added to the respective plots one week before planting. Also, 30 kg of diammonium phosphate (DAP) and half of the dose of ammonium nitrate were used as basic fertilizers at the same time as planting. Ammonium nitrate left after the first harvest was added as a top dressing. The field was regularly irrigated by a drip system. Harvesting was done at the beginning of flowering at noon. The samples were dried in the shade.

Preparation of sample solution

The samples (10 g) were grinded and extracted at 60 °C by Soxhlet extractor for 8 h, using *n*-hexane (*n*-Hg), dichloromethane (CH_2Cl_2), ethanol (EtOH) as solvents. The resulting extract was filtered through Whatman Filter Paper Grade 1. The solvent was take away by a Rotary Evaporator. Then, the extracts were move to the bottles that were tared and kept in the refrigerator (-20 °C) until the activity study was done (Tawaha et al., 2007).

Antimicrobial activity

American Type Culture Collection (ATCC) from dissimilar bacterial stains was used for the appraisal of antibacterial activity. *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), methicillin-resistant MRSA (ATCC 43300) and *Enterococcus faecalis* (ATCC 29212) were used as Gram-positive species, and *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 4352), *Pseudomonas aeruginosa* (ATCC 27853) were used as Gram-negative species, and as a yeast-shaped fungus *Candida albicans* (ATCC 10231) were determined by the microbroth dilutions technique the Clinical Laboratory Standards Institute (CLSI) recommendations (Yaldız et al., 2019). Mueller-Hinton broth for bacteria, RPMI-1640 medium buffered to pH 7.0 with MOPS for yeast strain was used as the test medium.

Two-fold serial dilutions ranging from 5000 µg mL^{-1} to 4.9 µg mL^{-1} were prepared in the medium. The inoculum was prepared using a 4-6 h broth culture of each bacteria and 24 h culture of yeast strains adjusted to a turbidity equivalent to a 0.5 Mc Farland standard, diluted in broth media to give a final concentration of 5 × 105 cfu mL^{-1} for bacteria and 0.5 × 103-2.5

× 103 cfu mL^{-1} for yeast in the test tray. The trays were covered and placed in plastic bags to prevent evaporation. The trays containing Mueller-Hinton broth were incubated at 35 °C for 18-20 h and the trays containing RPMI-1640 medium were incubated at 35 °C for 46-50 h. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the compound giving complete inhibition of visible growth. As control, the antimicrobial effects of the samples were investigated against test microorganisms. According to the values of the controls, the results were evaluated.

Results & Discussion

Evaluation of antimicrobial activity (Figure 1) shows the results obtained in screening the antimicrobial activity of n-hexane, dichloromethane, and ethanol extract prepared from samples.

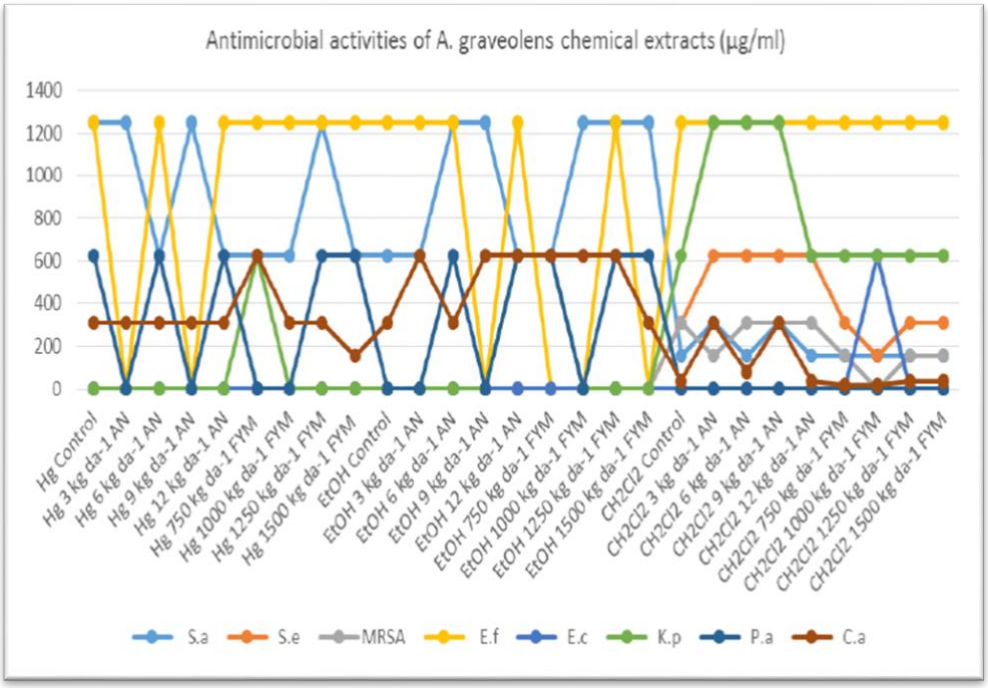

Figure 1. Antimicrobial activities of A. graveolens chemical extracts (µg ml^{-1}).

The results indicated that almost all *n*-hexane extracts were active against *E. faecalis* (1250 µg ml^{-1} mic). Also, *n*-hexane extracts were active against *S. aureus* ATCC 29213 bacteria and *C. albicans* ATCC 10231 fungi. FYM application with various levels enhanced the antibacterial activity. *n*-hexane and ethanol extracts were not active against *S. epidermidis*, MRSA, and *E. coli* bacteria (Table 1).

Table 1. Antimicrobial activities of *A. graveolens* chemical extracts (µg ml^{-1})

	MICROORGANISMS							
Extracts	S.a	S.e	MRSA	E.f	E.c	K.p	P.a	C.a
Hg 1	1250	-	-	1250	-	-	625	313
Hg 2	1250	-	-	-	-	-	-	313
Hg 3	625	-	-	1250	-	-	625	313
Hg 4	1250	-	-	-	-	-	-	313
Hg 5	625	-	-	1250	-	-	625	313
Hg 6	625	-	-	1250	-	625	-	625
Hg 7	625	-	-	1250	-	-	-	313
Hg 8	1250	-	-	1250	-	-	625	313
Hg 9	625	-	-	1250	-	-	625	156
ETOH 1	625	-	-	1250	-	-	-	313
ETOH 2	625	-	-	1250	-	-	-	625
ETOH 3	1250	-	-	1250	-	-	625	313
ETOH 4	1250	-	-	-	-	-	-	625
ETOH 5	625	-	-	1250	-	625	625	625
ETOH 6	625	-	-	-	-	625	625	625
ETOH 7	1250	-	-	-	-	-	-	625
ETOH 8	1250	-	-	1250	-	-	625	625
ETOH 9	1250	-	-	-	-	-	625	313
CH_2CL_2 1	156	313	313	1250	-	625	-	39
CH_2CL_2 2	313	625	156	1250	-	1250	-	313
CH_2CL_2 3	156	625	313	1250	-	1250	-	78
CH_2CL_2 4	313	625	313	1250	-	1250	-	313
CH_2CL_2 5	156	625	313	1250	-	625	-	39
CH_2CL_2 6	156	313	156	1250	-	625	-	20
CH_2CL_2 7	156	156	-	1250	625	625	-	20
CH_2CL_2 8	156	313	156	1250	-	625	-	39
CH_2CL_2 9	156	313	156	1250	-	625	-	39

S.a: *S.aureus* ATCC 29213; S.e: *S. epidermidis* ATCC 12228; MRSA: Methicillin-Resistant; E.f: E. Faecalis ATCC 29212; E.c: *E. coli* ATCC 25922; K.p: *K. pneumoniae* ATCC 4352; P.a: *P. aeruginosa* ATCC 27853; C.a: *C. albicans* ATCC 10231

All extracts indicated varying levels of antimicrobial activity against *S. aures* bacteria and *C. albicans* fungiexcept the ethanol extracts against *S. epidermidis*, MRSA, and *E. coli* bacteria (Table 1). The fertilization methods did not have significant effect on the antibacterial properties of the samples. However, the highest antibacterial activity against almost all tested

microbial species was observed with FYM applications of 1000-1500 kg da^{-1} and 6-9 kg da^{-1} AN treatments.

When all chemical extracts were evaluated together, the dill dichloromethane extracts showed the highest antibacterial activity against all tested microbial species, especially the highest antibacterial activity within all tested microbial species was observed with *E. faecalis* (1250 µg ml^{-1} mic) (Figure 1). However, the antimicrobial activity of *C. albicans* fungi was found the lowest in all dichloromethane extracts both FYM and AN fertilizer. Furthermore, AN fertilizer showed higher antibacterial activity for all tested bacteria than FYM applications.

The dill dichloromethane extracts indicated the highest antibacterial activity against all microbial species. Especially, the highest antibacterial activity within all tested microbial species was observed with *E. faecalis* (1250 µg ml-1 mic) (Figure 1). However, the antimicrobial activity of C. albicans fungi was found the lowest in all dichloromethane extracts both FYM and AN fertilizer. Furthermore, AN fertilizer indicated higher antibacterial activity for all bacteria than FYM applications.

The higher activity of all extracts can be explained based on the chemical structure of their major constituents such as dillapiole and anethole, which have aromatic nuclei containing polar functional groups (Farag et al., 1989). Arora and Kaur (2007) reported that aqueous extracts of dill showed a broad-spectrum antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurim*, *Shigella flexneri* and *Salmonella typhii*. Extracts of dill leaf and seed were studied for antimicrobial activity by agar well diffusion technique against *S. aureus*, *E. coli*, and *C. albicans*. The leaf extracts showed no antibacterial activity, whereas the extract of dill seed exhibited inhibition of the growth of *C. albicans* (19 mm. inhibition zone) (Rasheed et al., 2010). The results of the present study compare favorably with previous studies, showing similar antibacterial activities to those reported in the studies by Sharopov et al. (2013) and Arora and Kaur, (2007), but different antibacterial activities than those reported in the study by Delaquis et al. (2002).

Conclusion

All chemical extracts were evaluated together, the dill dichloromethane extracts showed the highest antibacterial activity against all microbial species, especially the highest antibacterial activity within all tested microbial species was observed with *E. faecalis* (1250 µg ml^{-1} mic). As suggested by the present study, a suitable ratio of FYM in the soil, up to 1250 kg da^{-1} FYM, can increase the antibacterial activities. According to our results, it can be concluded that the application of different doses of FYM is also a way for increasing the antimicrobial activities of dill. Thus, this study confirms the bioactive potential of dill.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION - FULL PAPER

A STUDY ON THE GUM ISOLATION OPTIMIZATION IN DIFFERENT FENUGREEK GENOTYPES

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Abstract

Gum content is an adhesive, emulsifier, thickener, geller, bulker and stabilizer agent used in food material. This agent has been identified as a food hydrocolloid in the food industry. Fenugreek has more quality gum content compared to other plants as guar, tara and locust bean gums. This plant is an annual plant belonging to the Fabaceae family, and it can be used in different industries such as food, cosmetics, medical or etc. So, this study was conducted to determine gum content variability of the different fenugreek genotypes (18 genotypes, 3 cultivars) depending on the different applications under different growing conditions. These applications are ordered as temperatures (30, 60, 90 °C), time (1, 3, 5 h), pH (pH=3, pH=10) and flour:water ratio (1:30 and 1:60 g/mL). The gum content of fenugreek genotypes showed high huge variation among the applications and growing conditions. The gum content of fenugreek ranged from 1,44% to 91,43% under different growing conditions. The highest gum content was found from PI 426971 genotype (91.43%) in 90 °C, 5 h, pH 10 and 1:60 flour: water ratio and followed by PI 251640 genotype (87.67%) in 90 °C, 5 h, pH 10 and 1:30 flour: water ratio under irrigated conditions. The highest gum contents were found from PI 660995 (in 90 °C, 5 h, pH 10 and 1:30 flour: water ratio) and PI 426971 (in 90 °C, 5 h, pH 10 and 1:60 flour:water ratio) genotypes with 84,14% and 83,02% under dryland conditions, respectively. According to result of the study, the high fenugreek gum content was obtained from high temperature (90 °C), time (5 h) and pH=10 (basic media) under both irrigated and dryland conditions.

In a conclusion, the different applications affected the gum content of different fenugreek genotypes under different growing conditions.

Key Words: Fenugreek, gum content, optimization.

1. Introduction

Fenugreek is a significant plant belongs to the Leguminosae family. The fenugreek seeds contain different kinds of protein, amino acids and fatty acid contents (Taylor et al., 2002). This plant can be used to decrease blood glucose and cholesterol levels besides in the pellagra, appetency loss and gastrointestinal disorderliness in traditional medicine (Mathur and Mathur, 2005).

In addition, previous research revealed that this plant also had important favorable influences on anticancer, antidiabetic and antimicrobial activities because of including different bioactive contents (Aasim et al., 2010; Mehrafarin et al., 2011).

Gums are significant industrial raw ingredients, and many researchers conducted comprehensive studies on the gum due to sustainability, biodegradability and biosafety (Bains et al., 2022; Kaur et al., 2021).

Fenugreek seeds include 26,8 soluble fiber with similar characters to guar seed and psyllium husk. This plant gum has equal galactose and mannose (1:1) contents and the galactose ratio showed the highest water solubility compared with guar and locust bean gum. The galactose and mannose ratio has some effects on the physicochemical characters of galactomannans, and it is inversely connected to the gum solubility. (Dhull et al., 2022).

The gum content of fenugreek can be used as adhesive, and a stabilizer and an emulsifying agent to change food materials (Camlica and Yaldiz, 2022). Therefore, fenugreek gum is preferred over other natural hydrocolloids as an excellent ingredient for various food applications (Dhull et al., 2022). Food hydrocolloids show effects as a thickener, gelling, stabilizer bulker and emulsifier agents in food systems (Phillips and Williams, 2009).

This study was carried out to optimize the fenugreek gum, which has an important place in the food industry, with different applications. In this context, the effects of different temperatures, pH, flour:water ratio and time on the gum ratio of different origin 18 fenugreek genotypes were determined grown under irrigated and dryland conditions.

2. Materials & Methods

2.1. Plant materials and growth conditions

The different fenugreek seeds obtained from the United States of America, Department of Agriculture (USDA) and 3 local genotypes were used in this research (Table 1).

Table 1. The knowledge of fenugreek genotypes and cultivar used in the study

No	Accession code	Origin	No	Accession code	Origin
1	Çiftçi	Türkiye-Eskişehir	12	PI 381062	Iran
2	Gürarslan	Türkiye-Ankara	13	PI 426971	Pakistan
3	Berkem	Türkiye-Diyarbakır	14	PI 426973	Pakistan
4	PI 173820	Türkiye	15	PI 469264	Egypt
5	PI 194020	Ethiopia	16	PI 568215	Türkiye
6	PI 215615	India	17	PI 572538	Egypt
7	PI 251640	Ethiopia	18	PI 613633	Australia
8	PI 286532	India	19	PI 617076	Bulgaria
9	PI 296394	Iran	20	PI 639185	Armenia
10	PI 302448	India	21	PI 660995	Armenia
11	PI 302449	India			

The research was conducted at the BAIBU, Faculty of Agriculture research and application area. The climatic data were noted, and the temperature was found between 8,7-21,8 ˚C, the precipitation changed between 0-142,6 kg/m and between 56,1-76,7% humidity (BMGM, 2021).

The soil properties were found clayey, 7,56 pH, rich organic matter (3,71%) and potassium ratio (1083,10 kg/ha) and poor in phosphorus (0,50 kg/ha) (Table 2). The field experiment was conducted according to a split block design with three replications in the 2020 year.

Table 2. Soil properties of experimental area

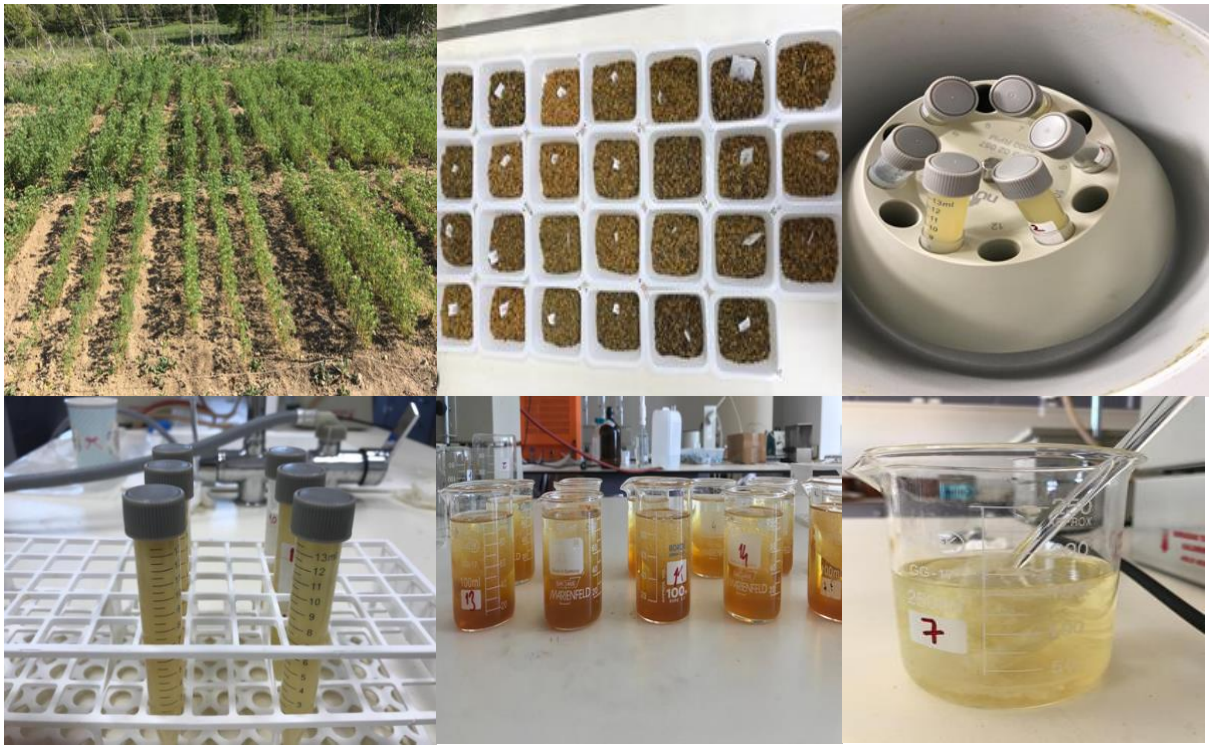
Texture	Organic matter (%)	pH	EC	P_2O_5 (kg/ha)	Potassium (kg/ha)	Lime (%)
Clayey	3,71	7,56	0,04	0,50	1083,10	11,14

The harvests of fenugreek genotypes were done between 27 July-18 August in the experiment. The moisture ratio of fenugreek seeds were measured according to the report by Pandey and Awasthi (2015).

2.2. Isolation and optimization of fenugreek gum content

The gum isolation was conducted by using ground fenugreek seeds with distilled water (1:40). The ground fenugreek seeds mixed with distilled water were heated depending on the different temperatures in a shaking water bath at different hours, pH and flour:water ratio. The mixture was filtered and centrifuged 20 min at 4400 rpm and the supernatant was decanted. Finally, 80% ethanol was added to the final concentration with a 1:3 ratio to precipitate fenugreek gum from the solution. The ethanol solution was repeated three times to obtain pure and a white gum content.

30, 60, 90 ˚C tempretures, 1, 3, 5 h, pH=3, pH=10, and two fenugreek flour:water ratio (1:30 and 1:60 g/mL) were applied to determine the optimization of fenugreek gum contents (Figure 1).



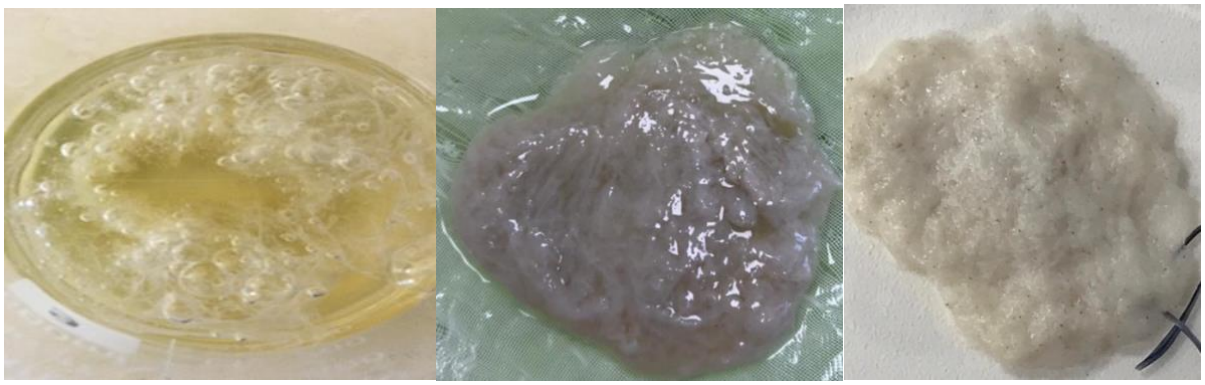


Figure 1. Photographs from gum isolation and optimization stages

3. Results & Discussion

The fenugreek genotypes showed large variability in gum contents with the application of different optimization conditions under two growing conditions.

The gum content ranged from 4,04% to 91,43% under irrigated conditions with an average of 40,22%. The highest gum content was found from the application of 90 °C, 5 h, pH=10 and 1:60 flour:water application in PI 426971 genotype, and followed by PI 251640 genotype with the same temperature, hour, pH and 1:30 flour:water application. The lowest gum content was determined in PI 568215 genotype with the application of 30 °C, 1 hour, pH=3 and 1:30 flour:water application. PI 286532 genotype followed with 7,17% with the application of the same conditions except 3 hours under irrigated conditions.

With the application of 30 °C in different other properties, the gum contents had large variability from 4,04% to 61,31% with an average of 30,93%. Generally, 5 hours and 1:30 flour:water applications were found better application for the higher gum content under 30 °C under irrigated conditions.

The gum contents changed between 8,35-73,94% with an average of 43,57% under irrigated conditions under 60 °C. PI 381062 genotype had the highest gum content with 3 and 5 h, 1:30 and 1:60 flour:water and the same pH=3. The lowest gum content was found from the PI 469264 genotype in 3 h, pH=10 and 1:30 flour:water application and followed by the PI 568215 genotype with the same hour and pH=3, 1:60 flour:water application.

Generally, 3 and 5 h with 1:30 flour:water application revealed high gum content under 60 °C.

The gum content ranged from 1,80% to 91,43% with the large variability and an average of 46,16% at the highest temperature (90 °C).

At 90 °C, the highest gum contents were noted in PI 426971 (5h, pH=10 and 1:60 flour:water applications) and PI 251640 (5h, pH=10 and 1:30 flour:water applications) genotypes. The lowest gum contents were found in PI 639185 and PI 469264 genotypes with 1 h, pH=10 and 1:30 flour:water application.

Under dryland conditions, the gum contents of fenugreek genotypes ranged from 1,45% to 84,14% with the application of different temperature, hours, pH and flour:water applications.

At 30 °C, the gum content changed between 1,45-49,54% with an average of 28,60%. The highest gum contents were found from the PI 639185 (49,54%), PI 286532 (47,43%) genotypes and Berkem cultivar (44,94%) with the 1 h, pH=10 and 1:60 flour:water applications.

The lowest gum content was determined in PI 251640 genotype in 1 h, pH=10 and 1:60 flour:water application and followed by the PI 296394 and PI 572538 genotypes 1 h, pH=3 and 1:30

flour:water application. Generally, 5 h, pH=10 and 1:60 flour:water application were found better for the gum content at 30 °C under dryland conditions.

The gum content ranged from 13,44% to 78,72% with an average of 38,25% at 60 °C in fenugreek genotypes and cultivars. The highest gum contents were found from the PI 426971 genotype and Gürarslan cultivar at 3 h, pH=3 and 1:60 flour:water application. The lowest value was observed from the PI 426973 genotype with 5 h, pH=10 and 1:60 flour:water application.

5 h and 1:60 flour:water applications were found more effective to obtain high gum content in different fenugreek genotypes at 60 °C.

The fenugreek genotypes showed high differences for gum content depending on the gum optimization at 90 °C. The gum content changed between 1,44-84,14% with an average of 40,57%. The highest gum content was obtained from PI 660995 genotype at 5 h, pH=10 and 1:30 flour:water application. The lowest gum content was found in PI 302449 genotype with 1 h, pH=10 and 1:60 flour:water application.

Generally, 5 h and pH=10 were the more effective application to obtain high gum content at the highest temperature (90 °C).

Table 3. General gum content average of fenugreek genotypes at different temperature, time PH and flour:water applications

Temperature (°C)	IC-Gum ratio (%)	DC-Gum ratio (%)
30	30,93	28,60
60	43,57	38,25
90	46,16	40,57

IC: Irrigated conditions, DC: Dryland conditions

The gum content of fenugreek can change depending on the different applications. Because all applications had different effects on the gum content, and this content showed large variability among the fenugreek genotypes. Also, the same genotype showed different gum contents under different applications. So, it is concluded that the isolation process and genotypes revealed different gum ratios depending on growing conditions.

The results of this study showed that the genotypes which have high gum ratio can be used to contribute to biopharmaceutical applications (Kumar Shukla et al., 2017). When the gum ratio values of fenugreek genotypes were compared with literature studies; It was found close to the average gum ratio as 53,53% in the fenugreek endosperm and higher than the average gum ratio of the whole seed as 23,72% (Khatir, 2017). Differences in gum ratios in fenugreek can be attributed to genotype differences, gum isolation method differences and ecological conditions.

4. Conclusion

The fenugreek genotypes revealed different gum contents with different gum optimization applications under irrigated and dryland conditions. Under irrigated conditions, the average of the applications changed between 40,22-46,16% with the increasing temperature. The 90 °C was found the better application for the high gum content of fenugreek under irrigated conditions.

Similar with the irrigation conditions, the highest gum contents were obtained from the high temperature (90 °C) in dryland conditions.

The average gum content of fenugreek genotypes increased with the increasing temperature (from 30 °C to 90 °C) both in irrigated and dryland conditions.

As a result of the study, the highest gum content was found under irrigation conditions.

Acknowledgements

The research was part of the Ph.D. thesis of first author. We thank to the Scientific and Technological Research Council of Turkey (Project code: 219O465) (TUBITAK) for support and the USDA for supplying fenugreek seeds.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION - FULL PAPER

BIOLOGICALLY ACTIVE SECONDARY METABOLITES AND MEDICINAL USEAS OF Stachys schtschegleevii

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Abstract
Stachys schtschegleevii is a plant with medicinal properties that has been used for treating various diseases for centuries. Recent research has revealed that this plant contains a diverse range of biologically active secondary metabolites, including flavonoids, terpenoids, and phenolic acids. These compounds have been found to possess a wide range of pharmacological activities, such as antioxidant, antimicrobial, anti-inflammatory, antitumor, and antidiabetic effects. One of the diseases that *Stachys schtschegleevii* has shown promising results in treating is rheumatoid arthritis (RA). RA is an autoimmune disorder that primarily affects the joints, causing inflammation, pain, and stiffness. Recent studies have demonstrated that *Stachys schtschegleevii* is effective in controlling RA-related pathological and inflammatory outcomes. Moreover, *Stachys schtschegleevii* has also been found to interact with the active site of the coronavirus protease enzyme, inhibiting the amino acids of the active site during the catalytic process. This indicates that *Stachys schtschegleevii* may have potential applications in the treatment of coronavirus. Although *Stachys schtschegleevii* has been traditionally used in Iran for treating colds and other diseases, it is not well-known in Turkey. This paper aims to highlight the various bioactive compounds present in this plant and their potential applications in the treatment of human diseases. The review will also summarize recent studies on the pharmacological properties of *Stachys schtschegleevii*, including their mechanism of action and potential therapeutic benefits. This information may be useful for researchers and healthcare professionals interested in natural product-based drug discovery and development.
Key Words: *Stachys schtschegleevii*, Rheumatoid arthritis, Covid, Pharmacology.

1. Introduction

For centuries, aromatic plants have been used as raw materials for cosmetic and pharmaceutical industries, and in the last decade, their significance has only grown. Essential oils extracted from these plants have made them a prime contender for medicinal plant evaluation. Recently, interest in medicinal plants has increased greatly among researchers, physicians, and the general public. This is due to a growing awareness of the potential health benefits of natural remedies and a desire to seek alternatives to synthetic drugs that may cause harmful side effects. Advancements in technology have made it easier to study the chemical composition of plants, leading to the discovery of new drugs and plant-based therapies for various ailments. Moreover, the demand for organic and natural products has fueled the growth of the herbal supplement industry, making medicinal plants a lucrative market. Among the huge source of these precious plants, the species of *Stachys* genus shows a wide range of pharmaceutic characteristics which can relieve the pains of many chronic diseases.

The *Stachys* L. genus is a member of the Lamiaceae family, which is the sixth largest family of flowering plants, comprising over 245 genera and 7886 species (Zhao et al., 2021). The Lamiaceae family has a long history of use in traditional medicine, dating back to ancient times, and many of its species were cultivated during the Greek and Roman periods (Selvi et al., 2022). Within the Lamiaceae family, the Stachys L. genus is the third largest, consisting of approximately 370 species and 435 taxa worldwide (Satil and Açar, 2020).

The genus Stachys is widely distributed, with a presence in various regions such as the Mediterranean and Southwest Asia, followed by North and South America, and North Africa. However, it is not found in Australia and New Zealand (Tundis et al, 2014).

Iran and Turkey are recognized as the main centers of diversity for the Stachys genus. In Turkey, the Stachys L. genus comprises 91 species and 118 taxa, with 57 of these taxa being endemic, resulting in a high rate of endemism of 48% (Guner et al., 2000; Akçiçek et al., 2016). Iran is also renowned for its diversity of the Stachys genus, with 35 species, 13 of which are endemic, resulting in an endemism rate of 37%.

Stachys species are characterized by growing either as annual or perennial herbs or as small shrubs with simple, stalked, or stalkless leaves. The number of inflorescences ranges from four to many-flowered, typically forming a spike-like inflorescence at the end. The calyx tubes are tubular-campanulate, 5- or 10-veined, and have a regular or weakly bilobed shape with five subordinate teeth. Corolla has a narrow tube with 2 lips; upper lip usually apartment or dome-shaped and hairy, while lower lip 3-lobed and either glabrous or hairy. Nutlets are oblong to ovate and have a rounded apex (Tomou et al 2020).

Stachys species have been extensively studied by international researchers, and their ethnopharmacological use has been substantiated by several pharmacological and phytochemical studies. Of particular interest are the antioxidants, renal protective, anti-inflammatory, analgesic, anxiolytic and antidepressant properties (Bahadori et al., 2019; Elfalleh et al., 2019; Slobodianiuk et al., 2021; Kanjevac et al., 2023). The therapeutic characteristics attributed to these species have been associated with their content of phytochemicals, and thus the genus Stachys has received much attention for screening its bioactive secondary metabolites from various plant parts. Hundreds of natural compounds such as polyphenols (e.g. flavone derivatives, phenylethanoid glycosides, lignans), terpenes (e.g. triterpenes, diterpenes, iridoids), phenolic acids, and essential oils have been isolated from this genus (Nazemiyeh et al., 2006; Şerbetçi et al., 2010; Karioti et al., 2010; Piozzi et al., 2011; Napolitano et al., 2022). However, the bioactive compound composition resulting in various pharmaceutical characteristics is highly variable depending on the species and environmental conditions (Chrysargyris et al., 2020; Ergün, 2021). *Stachys schtschegleevii* as the endemic species to Iran is morphologically similar to Stachys inflate. It has been widely used in Iranian folk medicine to treat rheumatic fever, bacterial infections, and respiratory inflammatory diseases under the name of "Poulk" (Sonboli et al., 2005; Abichandani et al., 2010; Hazrati et al., 2020; Mirtaheri et al., 2022). Given the significant characteristics of this species and the need to develop breeding studies, this study aimed to comprehensively investigate recent research on the biologically active compounds and pharmaceutical aspects of Stachys schtschegleevii.

2. Secondary Metabolites isolated from *Stachys schtschegleevii*

Previous studies proved that *Stachys* L. is a rich source of secondary metabolites which are biologically active and causing the plant to be evaluated as a good source of pharmacologicaly subxstanses. These metabolites include phenolic derevitives, falavonoids, falavons, glycosides and terpens.

2.1. Essential oils isolated from *Stachys schtschegleevii*

Stachys species, known for their aromatic properties, contain a variety of unique volatile compounds that belong to the secondary metabolites. Germacrene, caryophyllene and its isomers, and α-Pinene are some of the most prevalent volatiles in *Stachys* species, as demonstrated by previous studies (Ebrahim and Somae, 2004; Ebrahimabadi et al., 2010; Goren et al., 2011; Tundis et al., 2014; Bahadori et al., 2020). Various studies have investigated the essential oil composition of *Stachys schtschegleevii* and the impact of different factors on its yield and composition. In the aerial parts of *Stachys schtschegleevii*, α-pinene, β-phellandrene, germacrene D, β-pinene, and α-phellandrene were identified as the key volatiles (Norouzi-Arasi et al., 2004). In another study by Sonboli et al. (2005), forty-five volatile compounds were detected in the leaves of *S. schtschegleevii*, among which a-pinene, germacrene-D, limonene, and piperitone were the predominant constituents. Terpenes were found to be the majority of the essential oils. Rezazadeh et al. (2006) reported that sesquiterpenes (54.2%) were the most abundant compounds in the essential oil of the aerial parts of S. shchegleevii, with germacrene D as the main component, followed by limonene, valencene, and a-pinene. Hazrati et al. (2018) also found that α-pinene, germacrene-D, bicyclogermacrene, β-eudesmol, and spathulenol were the main components of the essential oil obtained by the best drying method. However, drying methods such as oven, microwave, and sunlight drying resulted in a significant decrease in certain monoterpene hydrocarbons such as α-pinene, β-pinene, germacrene-D, and myrcene. Nasrollahi et al. (2019) identified the major volatile constituents of *S. shchegleevii* essential oil as spathulenol, valencene, and germacrene-D in stem and germacrene-D, valencene, α-pinene, and spathulenol in leaf. Hazrati et al. (2020) observed significant variations in the essential oil composition of *S.schtschegleevii* during different growth stages. α-Pinene, germacrene-D, spathulenol, and β-pinene were the main components during flowering, while spathulenol, germacrene-D, α-pinene, α-cadinol, and β-eudesmol were the main components during vegetative stages. At the seed set and maturity stages, α-pinene, α-cadinol, germacrene-D, and spathulenol were the major constituents. However, Mohammadi et al. (2022) reported that α-campholenal, germacrene-D, β-pinene oxide, and α-pinene were the major constituents of the essential oil obtained from the aerial parts of *S. shchegleevii* and that water deficit increased the essential oil content. Despite the variations in the composition of *S. schtschegleevii* essential oil due to various factors, many studies have reported that germacrene-D and other sesquiterpene hydrocarbons are the main compounds in its essential oil. Germacrene-D is a sesquiterpene hydrocarbon commonly found in essential oils of various plant species. It has a characteristic earthy, woody aroma and is commonly used in perfumery and flavors. Germacrene-D is also known for its pharmacological properties, such as antimicrobial, antioxidant, and anti-inflammatory activities, which have potential therapeutic applications in medicine. In addition, Germacrene-D has been studied for its role in plant defense against pests and pathogens. Its biosynthesis is complex and involves several enzymatic reactions (Casiglia, et al., 2017).

2.2. Phenolic compounds isolated from *Stachys schtschegleevii*

Flavonoids are a characteristic feature of the genus *Stachys*, with previous studies indicating the presence of several atypical flavonoid types within this genus (Tundis et al., 2014; Marin et al., 2004; Serrilli et al., 2005). Apigenin and luteolin derivatives are mainly found in the petals of the genus *Stachys*, while scuteUarein, isoscutellarein, and baicalein derivatives are found in the herbs and roots of the genus Stachys (Kartsev et al., 1994). Flavonoid variation patterns *in Stachys* species exhibited more diversity. The highest flavonoid compounds in ten *Stachys* species from Iran were flavones (derivatives) and the lowest were dihydroflavonols, flavonols, flavanones, isoflavones and chalcones (Kharazian, N., & Mohammadi, 2014).

Some studies have been showed that *Stachys schtschegleevii* has high total flavonoid content. However, Nasrollahi et al. (2019) reported that the content of total flavonoids in the leaves of *S. schtschegleevii* is sixth times more than stems. Nazemiyeh et al. (2006) studied the phytochemical profile of *S. schtschegleevii* stems and found four flavonoids, including two p-coumaroyl derivatives of apigenin (Apigenin 7-(6″-E-p-coumaroyl)-β-D-glucopyranoside, Apigenin 7-O-β-D-glucoside), Chrysoeriol 7-(6″-E-p-coumaroyl)-β-D-glucopyranoside and 3′-Hydroxy-isoscutellarein-7-O-[6‴-O-acetyl]-β-D-glucopyranoside. Although the most common representative flavones in Stachys species were xanthomicrol, in *S. shchegleevii* circimaritin was detected in addition to xanthomicrol (Nazemiyeh et al., 2006). Maleki-Dizaji et al. (2008) also identified the main flavonoids and caffeic acid derivatives in *S. schtschegleevii* as apigenin 7-*O*-β-[6”- (*p*- coumaroyl)]-glucoside, chrysoeriol 7-*O*- β-[6”-(*p*- coumaroyl)]-glucoside, and acteoside. The discovery and characterization of betonyoside and acteoside, caffeic acid conjugates from S. schtschegleevii, could have important implications for chemotaxonomy. The distribution of flavonoids and their glycosides within the genus *Stachys* is widespread, and many of these compounds have been identified in this particular plant (El-Ansari et al., 1995). Despite numerous studies on the pharmacological effects of *S. shchegleevii* plant extracts, there is still a need for a comprehensive analysis of phenolic compounds and other secondary metabolites, including iridoids, diterpenes, and glycosides.

2.3. Biological activities of *Stachys schtschegleevii*

In Irainia and Turkish folk medicine many species of *Stachys* has been applied for treatment of cough, colds, stomach ache, and cardiac disorders and as disinfectant, anti-spasmodic, antipyretic agents (Tomou et al 2020). The Stachys plant was also used to treat wounds as an external ointment and to relieve cramps, abdominal pain, dizziness, fever, gout, and menstrual cramps when taken orally. *Stachys schtschegleevii* has been traditionally used for Asthma, Rheumatism, Infectious disease, Influenza (Maleki et al., 2001). The bioactivity of *S. shchegleevii* extracts and constituents has been extensively studied, revealing significant antibacterial and antifungal properties, as well as antioxidant, anxiolytic, antihypertensive, anti-inflammatory, antinephritic and hyaluronidase activities. In this part, we focus on the recent findings regarding the bioactivity of *S. shchegleevii* extracts and isolated compounds, with particular attention given to their antimicrobial, antioxidant, anxiolytic, and anti-inflammatory properties. The hydroalcoholic extract of the aerial parts of *S. schtschegleevii* attenuates the inflammatory reaction against carrageenan-induced rat paw oedema and also exhibited anti-inflammatory activity through thin layer chromagoraphy (Maleki-Dizaji et al., 2008). Simillary, Maleki-Dizaji et al., (2008) reported that the hydro-alcoholic extract of *S. schtschegleevii* exhibited anti-inflammatory effects. Methanol, n-hexane, dichloromethane extracts of the non-flowering aerial parts of *S. schtschegleevii* has been showed high antibacterial effect and were active against ampicillin-resistant *Staphylococcus aureus* and *Escherichia coli* (Abichandani et al., 2010). The essential oil obtained from hydrodistillation of *S. shchegleevii* leaves and its two major monoterpenes, a-pinene and limonene, were tested by the disk diffusion method against three Gram-positive bacteria (*Staphylococcus aureus*, *S. epidermidis* and *Enterococcus faecalis*) and three Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*) and showed moderate activity against the tested bacteria (Sonboli et al., 2005). *S. schtschegleevii*'s methanolic extract demonstrated a hypoglycemic effect in diabetic rats and successfully reduced blood glucose levels in type II diabetic patients. As a result, it is now appropriately administered as a protective strategy to mitigate diabetes' adverse effects (Hamidian et al., 2013). Patients with rheumatoid arthritis, a chronic autoimmune inflammatory disease characterized by joint destruction, consumed tea bags made from dried powdered aerial parts of *Stachys shchegleevii*. The results of the study showed that female patients with rheumatoid arthritis experienced significant clinical efficacy, including a decrease in the number of tender and swollen joints, DAS28, and serum levels of MMP-3. This study may provide

further insight for interventional studies aimed at controlling the pathological and inflammatory consequences associated with rheumatoid arthritis (Mirtaheri et al., 2022). A recent study also showed that *S. schtschegleevi* can interact with the active site of the coronavirus protease enzyme, inhibiting the amino acids of the active site during the catalytic process. (Malekmohammad and Rafieian-Kopaei, 2021). Nasrollahi et al. (2019) reported that the leaves and stems of *S. schtschegleevii*'s aqueous and ethyl acetate extracts displayed excellent antimicrobial, antioxidant, and anticancer activities. Hazrati et al. (2020) also concluded the flowering stage of *S. schtschegleevii* is the optimal time for harvesting the plant for the extraction of essential oils for food and pharmaceutical purposes as it exhibited the highest total phenolic accumulation, total flavonoid, and maximum antioxidant activity. The ethanolic extract of *S. schtschegleevii* also showed significant antimicrobial activity against *Listeria innocua*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* as evidenced by the disk diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration. Therefore, *S. schtschegleevii* extract with its high phenolic content and remarkable antimicrobial and antioxidant properties could be used to improve the nutritional value and shelf life of food (Noshad, 2020). In another study, the well diffusion and tube dilution methods to discover the antibacterial activity of the ethanolic extract of S. schtschegleevii against standard bacteria showed that the plant extract has good antibacterial activity. However, the ethanolic extract of the plant Stachys schtschegleevii was more effective against Gram-positive bacteria than against Gram-negative bacteria (Esmaili, 2022).

3. Conclusion

There is a growing interest in exploring the therapeutic properties of endemic plants as a potential source for new drug discovery. Endemic plants are unique to specific geographic locations and have adapted to the local environment over thousands of years, resulting in a variety of compounds with potential therapeutic properties. The development of modern medicine has led to a decline in the use of traditional remedies, resulting in a loss of knowledge and potential therapeutic agents. Therefore, there is a need to rediscover the therapeutic properties of endemic plants using modern scientific approaches such as phytochemical screening, in vitro and in vivo assays, and clinical trials. Potential benefits of screening endemic plants for their therapeutic properties include the discovery of novel compounds, the identification of new drug targets, and the development of cost-effective, locally available therapies for diseases affecting vulnerable communities. Endemism of species of the genus *Stachy* in Iran and Turkey is high. These countries are a rich source of these species. Among the endemic species in Iran, *Stachys shchegleevii* is a plant that contains several biologically active secondary metabolites with potential medicinal uses. These compounds include phenolic acids, flavonoids, iridoids, and terpenoids. The plant has been traditionally used for its anti-inflammatory, antimicrobial, antioxidant, and anticancer properties. Recent studies have also shown its potential in treating neurodegenerative diseases and diabetes. However, further research is needed to fully understand the mechanisms of action and clinical efficacy of these compounds in Stachys schtschegleevii. Nevertheless, the plant is a promising source of natural compounds for the development of new drugs and therapies.

Conflict of Interest

All authors declare no conflict of interest in this study.

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ORAL PRESENTATION - FULL PAPER

RANCIMAT: A RAPID APPROACH FOR DETECTION OF OXIDATIVE DEGRADATION IN FAT-CONTAINING FOOD PRODUCTS

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Abstract
Amount of food consumption has been increasing rapidly due to the world population growth. Therefore, consumption of edible oil, which is one of the basic components of foodstuffs, is increasing fast. Hence, the necessity of delivering the oil to the consumer without deterioration of its current quality has emerged with the increase in the need for oil in the food industry. The deterioration of fat-containing foods is due to oxidation reactions caused by auto-oxidation chain reactions that occur under various conditions (such as heat, light and oxygen). This condition is known as rancidity [1]. Rancidity is one of the major problems affecting the quality of the fat-containing products [2]. So, oxidative stability as one of the most important parameters determining the quality of the foodstuff should be identified [3]. Measuring the oxidative stability of products normally requires months of work. Some instrumental methods such as Rancimat have been developed to measure the oxidation resistance of oil-containing products at increasing temperatures (less than 24 hours and > 100 ºC). On the other hand, determination of rancidity is also performed by conventional methods such as peroxide value measurement. However, it is not possible to identify the shelf-life of the product by these tests only give information about the current status of the product. Rather, Rancimat method accelerates samples to determine if antioxidants are needed to help manufacturers extract the full value from their oils. Rancimat oxidation test is a practical method that does not require extra analysis that consumes a lot of chemical materials and time [4]. In the current study, Rancimat method has been described as an alternative to traditional methods with applications.

Key Words: Rancidity, lipid oxidation, induction time, accelerated oxidation test, fats and oils.

1. Introduction

Lipid oxidation is one of the main reasons of oxidative spoilage in fat and fat-containing foods. It is an economic problem for the food industry since it makes food less acceptable, resulting in the formation of potentially toxic products in foods containd lipid, as well as a variety of flavors, colors and odors called oxidative rancidity [1]. This situation accelerates with the temperature, light and oxygen depending on the shipping and storage conditions (Figure 1).

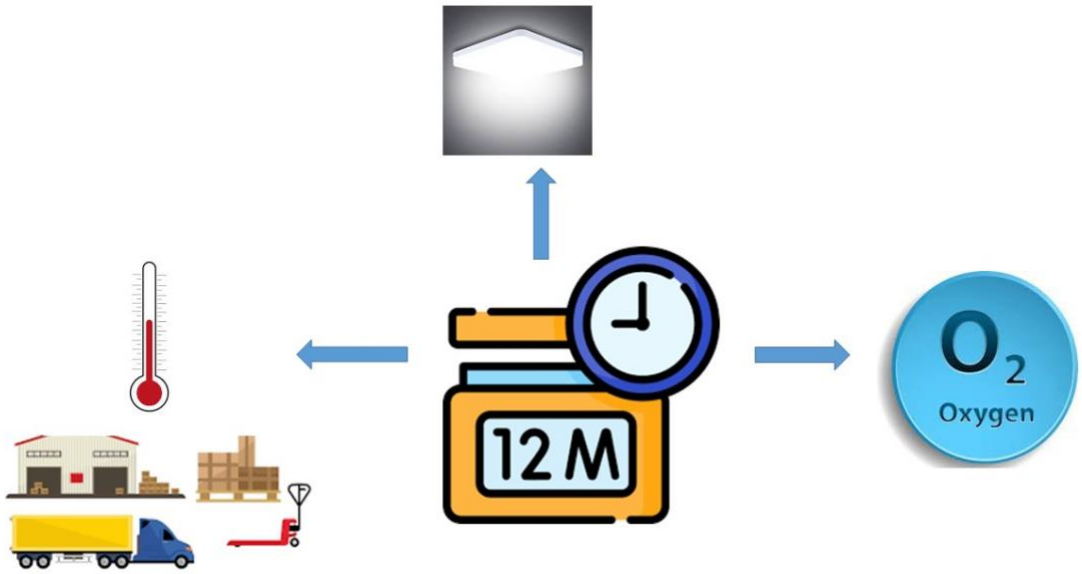

Figure 1. Some factors that cause the lipid oxidation process

The lipid autooxidation reaction is also called as the free radical chain reaction. This process takes place in three stages as seen in Figure 2. Initiation is the stage where free radicals are formed. The formation of a free radical begins with an initiating factor. In the presence of the mentioned factors (Figure 1), the hydrogen molecule is withdrawn from the unsaturated lipid and forms the lipid radical (R•). Propogation stage consists of the formation of hydroperoxides. They are the major products of auto-oxidation and are called primary oxidation products. As a result of their decomposition, secondary oxidation products (aldehydes, ketones, alcohols, acids) are formed. Actually, these secondary products are the cause of unpleasant odor and taste [5]. In the last step (termination), the oxidation ends. Non-radical products are formed [6]. These non-radical products have no flavor.

Lipid Oxidation

Initiation

$$RH \rightarrow R^{\bullet} + H^{\bullet}$$

Propagation

$$R^{\bullet} + {}^{3}O_2 \rightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$

Termination

$$ROO^{\bullet} + R^{\bullet} \rightarrow ROOR$$

$$R^{\bullet} + R^{\bullet} \rightarrow RR$$

Figure 2. Lipid oxidation stages

Natural and synthetic antioxidant additives can be used to prevent these auto-oxidation reactions [2,7]. Recently, phytochemicals (such as phenolics, flavonoids and carotenoids) have become very popular to replace synthetic antioxidants. Generally, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate) and TBHQ (tertiary butyl hydroquinone) are consumed in food prodycts [8]. However, TBHQ, one of these synthetic additives, is banned in Japan, Canada and some European countries due to its carcinogenic effects. BHA is also not applied to food formulations in Japan [9]. Thus, there is a general preference for natural additives to replace synthetic antioxidants. This is the reason why research activities on these subjects have increased in recent years. On the other hand, a rancid food product is irreversible. However, if natural oil is known to degrade in the near future, this process can be slowed, or even prevented by adding antioxidants beforehand. In this case, the most important process seems to be the detection of this oxidation.

4. Determination of Lipid Oxidation

The quality of fat-containing food products is one of the main parameters affecting the acceptability of the product by the consumer and its market value. Therefore, it is very important to perform product quality control in the food industry to produce consumer acceptable product and to maintain the quality. The quality of the products is evaluated using parameters such as oxidative stability, phenolic profile, antioxidant activity, peroxide value, carotene and tocopherol contents [10]. Among these properties, peroxide value is generally used as an indicator of primary lipid oxidation. However, this indicator is insufficient when the related product goes to a secondary oxidation and becomes rancid [11].

Oxidative stability is one of the most important parameters determining the quality of oils. Measuring the oxidative stability of oil-containing products normally requires months of work. Thus, some instrumental methods have been developed to measure the oxidation resistance of oil-containing products under accelerated conditions due to hydroperoxide degradation and at increasing temperatures (less than 24 hours and > 100 ºC) [3–5,12–14]. Differential Scanning Calorimetry (DSC), Active Oxygen Method (AOM), Thermogravimetric Analysis (TGA), Fourier Transform Infrared Spectra (FTIR), Schaal Furnace and Oxygen Bomb tests have been reported as accelerated methods used to measure the oxidation resistance of fats and oils.

DSC is a thermal analysis method. It has been used for more than fifty years to evaluate the lipid oxidation in several products [15,16]. Another method for determination of oxidative stability in lipid-containing products is AOM [17]. TGA can also be used to measure the stability against oxidation by defining that the mass of the relevant sample changes through thermal degradation [18]. In addition, FTIR is used for determination of oxidative stability resulting from degradation in fats [19]. Schaal Furnace and Oxygen Bomb tests are also accelerated methods used to measure the oxidation resistance of oil [20]. Finally, the Rancimat method is a simple accelerated method that does not involve analytical analysis such as titration, and is performed continuously [21].

5. Ransimat Accelerated Oxidation Test

Rancimat is a practical method that does not require a lot of chemical material and time-consuming extra analysis. Rather, it is a simple accelerated method that does not involve analytical analysis such as titration. Hence, it is performed continuously. While this method examines the oxidative stability of oil-containing products, it includes only three operational variables: product amount, air flow rate and temperature [10].

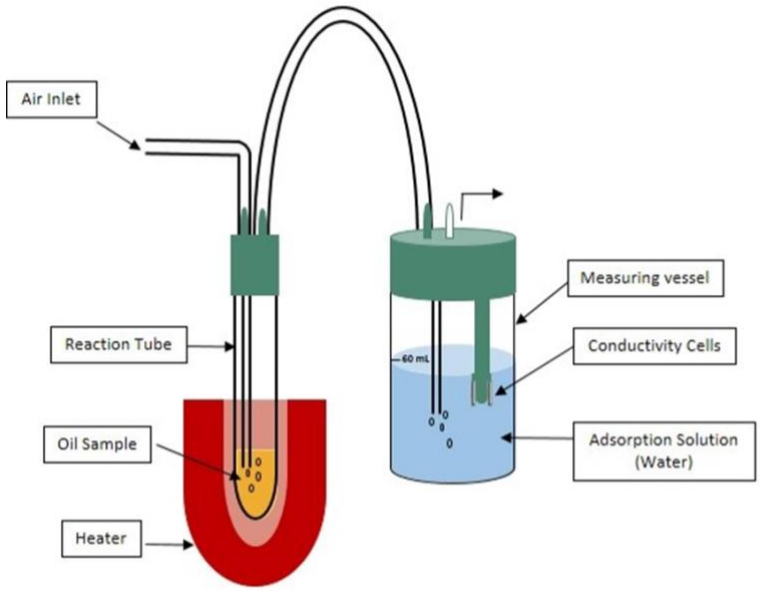

Figure 3. Schematic representation of the Ransimat device

Figure 3 shows the Rancimat apparatus with its elements during the process [22]. Air is passed through the oil sample kept at a certain temperature. Volatile organic acids separated from the oil as a result of heating are transported to containers containing pure water with the help of thin capillary pipes passing through the oil sample. A linear relationship is observed between oxidation in oil and conductivity of water. In the instrument brought to accelerated oxidation conditions (>100°C), the turning point at which the sudden change in the conductivity by time is detected as "induction time". This property is a quality parameter used to measure the oxidation stability of oils.

In Figure 4, the induction time values can be seen from the sample diagrams of a study in which conductivity versus time was measured [23]. It can be observed that the stability of the oil sample treated with antioxidant increased by 46% (4.98 versus 3.41).

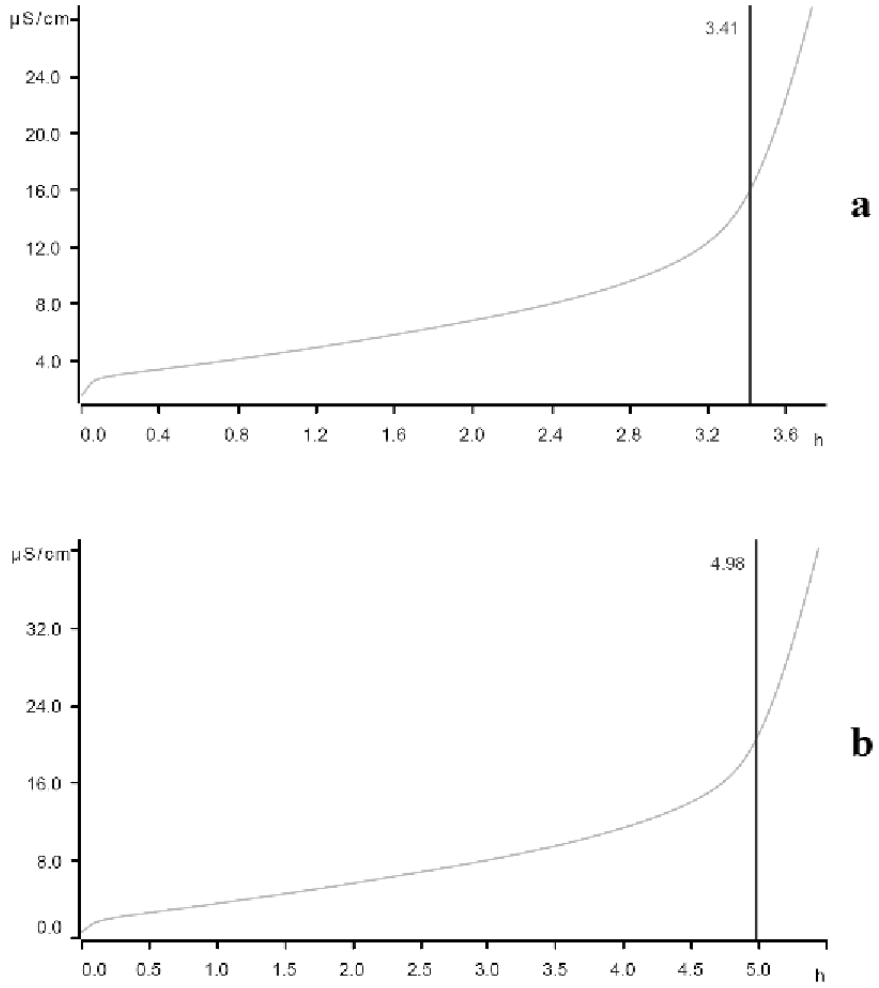

Figure 4. Oxidative stability curves of a) untreated oil sample b) treated oil sample

On the other hand, kinetic and thermodynamic parameters are used to understand the lipid oxidation process nature in a product [24]. So,it is possible to enhance the quality of the sample by preventing degradation toxic materials with the findings of kinetics and themodynamics. Arrhenius model can describe the temperature dependence of oxidation [22]:

$$\ln k = \ln A - \frac{E_a}{R}\frac{1}{T} \quad (1)$$

k= Reaction rate constant (h^{-1})

E_a= Activation energy (kJ mol^{-1})
A= Frequency factor
R= Universal gas constant (J mol^{-1} K^{-1})
T= Temperature (K)

Thermodynamic structure of the process is characterized by means of enthalpy (ΔH°), entropy (ΔS°) and Gibbs free energy (ΔG°) changes. The following equations are used to calculate these parameters [2]:

$$\ln \frac{k}{T} = \left[\left(\frac{k_B}{h}\right) + \left(\frac{\Delta S^\circ}{R}\right)\right] - \left(\frac{\Delta H^\circ}{R}\right)\left(\frac{1}{T}\right)$$

(2)

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

(3)

k_B =Boltzmann constant (1.38065×10^{-23} J K^{-1})

h= Planck's constant (6.62608×10^{34} J s)

6. Conclusions

Lipid oxidation process can be decelerated/avoided by adding the necessary additives, if the oxidation process of any oil-containing product is determined. Therefore, measuring the induction time through Rancimat method would allow manufacturers to achieve as much value as possible from the related product by determining its shelf-life, and deciding before sale whether further action is needed to extend it. Otherwise, the rancidity of oils and fats becomes a factor that can immediately lower the selling price of these products to customers in the food and cosmetic industries. The products that remain stable over longer periods are more valuable as they lead to a higher quality end product.

Conflict of Interest

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

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ORAL PRESENTATION - FULL PAPER

ENZYME INHIBITORY AND PHYTOCHEMICAL STUDIES ON *Pistacia vera* LEAVES COLLECTED from GAZİANTEP

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Abstract
Known as 'Pistachio', *Pistacia vera* L. is a species in the Anacardiaceae family. It is reported in the literature that various *Pistacia* species have numerous ethnobotanical uses. In this study, in addition to pancreatic cholesterol esterase enzyme inhibition, the *in vitro* antidiabetic (*α*-amylase and *α*-glucosidase enzyme inhibition) and antiobesity (pancreatic lipase enzyme inhibition) potentials of *P. vera* leaves collected from Gaziantep province were investigated. The phytochemical content of the 80% ethanol extract prepared from *P. vera* leaves was investigated by RP-HPLC technique. While the presence of gallic acid and methyl gallate in the extract was determined by RP-HPLC analysis, the extract was standardized on pentagalloylglucose (1.11 mg/g plant). The inhibitory effects of *α*-glucosidase, *α*-amylase, pancreatic cholesterol esterase and pancreatic lipase of the 80% ethanol extract were evaluated. The extract had an inhibition value of 100% on the *α*-glucosidase enzyme at a concentration of 2 mg/ml, while at the same concentration this value was 99.70 ± 0.26 for the reference compound acarbose. The *α*-amylase inhibitory activity of the extract at 2 mg/ml concentration was found to be 88.51 ± 3.15%. While the extract reached the inhibition of the highest pancreatic lipase enzyme (57.19 ± 2.86%) at 2 mg/ml; this value was 0.25 mg/ml for inhibition of pancreatic cholesterol esterase enzyme (39.45 ± 1.78%). The findings from the experiments revealed that P. vera leaves have antiobesity potential and a strong antihyperglycemic activity. In the light of these results, it was thought that *P. vera* leaves could be a potential source for isolation studies directed by antihyperglycemic and antiobesity activity.

Key Words: Antidiabetic; Phytochemistry; *Pistacia vera*; RP-HPLC

1. Introduction

Diabetes mellitus (DM) can be defined as a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both insulin secretion and insulin action. (1). The prevalence of type 2 diabetes in the world tends to increase rapidly. (2). Diabetes mellitus and obesity are strongly and complexly related to each other. Obesity is defined as a risk factor for Type 2 DM (3) .Since DM may cause disturbances in lipid metabolism, it is important to discover candidate molecules that affect diabetes complications and to act on enzymes that play a role in lipid metabolism. Lipase is an enzyme involved in the breakdown, digestion and transport of lipids (4). By inhibiting the cholesterol esterase enzyme, the bioavailability of dietary cholesterol is reduced, and this process may play a role in the treatment of atherosclerosis and hypercholesterolemia (5). By inhibiting specific enzymes that play a role in the pathophysiology of hyperlipidemia, diabetes and obesity, these diseases can be treated or it may be possible to alleviate the complications of the diseases. Medicinal plants emerge as important sources for the discovery of new safe and effective natural drug molecules (6).

Known as 'Pistachio', *Pistacia vera* L. is a species in the Anacardiaceae family. Many uses of various *Pistacia* species, including ethnobotany, are reported in the literature (7). The fruits of the P. vera plant are used all over the world. The records that the fruits of the species were consumed as food date back to 7000 BC. Different parts of various species of the genus *Pistacia* have long been used as beneficial medicines for diseases. The use of fruit seeds of the *P. vera* species as a stomach, heart, liver and brain tonic is an example of this. Regarding diabetes, which is one of the focal points of our study; There is information in the literature that various *Pistacia* species such as *P. terebinthus* and *P. atlantica* are used in folk medicine against diabetes in different geographies (8)

In this study, *in vitro* antidiabetic (*α*-amylase, *α*-glucosidase enzyme inhibition) and antiobesity (pancreatic lipase enzyme inhibition) potentials and pancreatic cholesterol esterase enzyme inhibition of the extract prepared from *P. vera* leaves collected from Gaziantep were investigated. In addition, the phytochemical content of 80% ethanol extract prepared from *P. vera* leaves was investigated by RP-HPLC technique. After qualitative analysis by RP-HPLC, the extract was standardized over pentagalloylglucose (PGG).

2. Material and Methods

2.1. Plant Material

The leaves of *P. vera* were collected in August 2019 from Gaziantep, Turkey. The plant material was described by Osman Tugay (Department of Pharmaceutical Botany, Selçuk University, Konya, Turkey). Prepared herbarium materials were stored in Gazi University Faculty of Pharmacy Herbarium. (GUEF 3831).

2.2. Extraction

After the leaves were dried in the shade, they were cut into small pieces with the help of a grinder. Dried leaves (10 g) were extracted with 200 ml of 80% ethanol for 24 hours at room temperature, and this process was repeated three times. The resulting filtrates were combined, then concentrated at 45°C (80% ethanol extract: 21.33% w/w dry plant) using a rotary evaporator.

2.3. Enzyme Inhibitory Activities

2.3.1. Alpha-Glucosidase Inhibitory Activity

α-Glucosidase (Sigma Co., St. Louis, USA) Type IV enzyme was incubated with the samples for 15 minutes, then p-nitrophenyl-*α*-D-glucopyranoside solution was added to the mixtures as a substrate. After 35 minutes, the absorbance of the formed p-nitrophenol was read. Acarbose was used as the reference compound in the experiment (9).

2.3.2. Alpha-Amylase Inhibitory Activity

α-Amylase type I-A (EC 3.2.1.1, Sigma) was dissolved in buffer solution. Potato starch was used as substrate solution in the experiment. After the enzyme solution was added, the substrate solution was added to the mixtures incubated at room temperature. After an incubation period of 3,5-dinitrosalicylic acid (DNS) color reagent solution was added, the mixture in the microplate was placed in an oven at 80°C. After 40 minutes, distilled water was added rapidly.

Absorbance values were read at 540 nm in an ELISA microplate reader. In this experiment, acarbose was used as reference material. After the standard maltose calibration chart was prepared, the amount of maltose produced was calculated using the standard maltose calibration chart and the net absorbance obtained (9).

2.3.3. Pancreatic Lipase Inhibitory Activity

Pancreatic lipase (Sigma Co., St. Louis, USA) Type II enzyme prepared in buffer solution was extracted and incubated with a Tris buffer for 15 minutes. p-Nitrophenyl butyrate used as substrate

was added to the mixture. After 30 minutes, the absorbance of the formed p-nitrophenol was read. Orlistat was used as the reference compound (10).

2.3.3. Pancreatic Cholesterol Esterase Inhibitory Activity

Cholesterol esterase (Sigma-Aldrich, Zwijndrecht, The Netherlands) from pig pancreas was dissolved in phosphate buffer. Then, taurocholic acid and p-nitrophenyl butyrate solution used as substrate were added. After the incubation process of the mixture, an enzyme was added and absorbance measurement was performed using kinetic measurement. Simvastatin was used as the reference compound in the experiment (11).

2.4. Analysis of the extract by RP-HPLC

The extract was prepared using a 25% (v/v) acetonitrile solution at a concentration of 5 mg/ml, and then filtered through membrane filters. HP Agilent 1260 series LC System and ACE 5 C18 (5 µm, 150 mm x 4.6 mm) column were used in this analysis. The mobile phase system used in the analysis was as follows; Solvent A (acetonitrile: H_2O: formic acid [50:50:0.5]) and Solvent B (H_2O: formic acid [100:0.5]). The gradient system was used to separate the peaks in the analysis. A mixture of standard substances containing gallic acid, methyl gallate and PGG was used for qualitative analysis.PGG, used in the standardization process of the extract, was isolated from *Rhus coriaria* by Gök et al. 2020 (12). The calibration equation and correlation coefficient determined for the PGG molecule were obtained as follows, respectively ($y= 43.941x + 4.4608$, $r^2 = 0.9998$).

Statistical Analysis

The results obtained from the analyzes performed in three series were averaged. All values were expressed as mean ± standard deviation (S.D.), Microsoft Excel and GraphPad Instat software were used for linear regression analyzes and calculations. The difference in $p< 0.05$ values in the analysis was considered statistically significant ($^{*}p< 0.05$, $^{**}p< 0.01$, $^{***}p< 0.001$). The correlation coefficient was calculated using Microsoft Excel 2016.

3. Results and Discussion

In this study, the extract prepared with 80% ethanol based on *P. vera* leaf; α-glucosidase, α-amylase, cholesterol esterase and pancreatic lipase inhibitory activities were evaluated. When the inhibition of the extract on enzymes is evaluated; Concentration-dependent increased inhibition values were observed in α-glucosidase, α-amylase, pancreatic lipase enzymes. The highest inhibition values in all three enzymes were reached at 2 mg/ml concentration. These inhibition values were calculated as 88.51 ± 3.15, 100.10 ± 1.15 and 57.19 ± 2.86 for these enzymes, respectively. The inhibition value reached in the α-glucosidase enzyme was calculated as 99.70 ± 0.26 higher than the reference substance acarbose at the same concentration. The highest inhibition values of α-amylase and lipase enzymes at 2 mg/ml concentration of the extract were calculated to be lower than the reference substances acarbose (98.91 ± 0.39) and orlistat (79.97 ± 2.35), respectively. In the pancreatic cholesterol esterase enzyme, unlike other enzymes, the highest enzyme inhibition value was reached with a value of 39.45 ± 1.78 at 0.25 mg/ml concentration (Table 1).

As a result of the qualitative analysis performed by RP-HPLC method, the presence of gallic acid methyl gallate and PGG compounds in the extract was determined. The retention times of the compounds were determined as 5.9, 14.2 and 32.3 respectively. In addition, the extract was

standardized on pentagalloylglucose (1.11 mg/g plant). The chromatogram of the extract for the analysis is shown in Figure 1.

Table 1. Inhibitory effects of *P. vera* crude leaf extract on *α*-glucosidase, *α*-amylase, pancreatic lipase and pancreatic cholesterol esterase enzyme

Samples	Concentration (mg/ml)	Inhibition % ± SD			
		α-Glucosidase	*α*-Amylase	Pancreatic lipase	Pancreatic Cholesterol Esterase
Extract	0.25	70.88 ± 4.75***	43.26 ± 0.33***	19.67 ± 4.52***	39.45 ± 1.78***
	0.5	96.16 ± 0.29***	81.68 ± 4.53***	28.89 ± 2.61***	30.04 ± 0.56***
	1	98.00 ± 0.27***	83.08 ± 1.91***	41.57 ± 2.22***	35.06 ± 0.00***
	2	100.10 ± 1.15***	88.51 ± 3.15***	57.19 ± 2.86***	39.38 ± 2.98***
ACA/OR/SIM 0.25	0.25	98.49 ± 0.15***	83.92 ± 2.25***	75.94 ± 3.19***	42.82 ± 1.54***
ACA/OR/SIM 0.5	0.5	99.25 ± 0.10***	89.25 ± 0.11***	77.05 ± 2.06***	60.09 ± 2.46***
ACA/OR/SIM 1	1	99.60 ± 0.15***	98.18 ± 0.88***	78.69 ± 0.59***	70.83 ± 1.82***
ACA/OR/SIM 2	2	99.70 ± 0.26***	98.91 ± 0.39***	79.97 ± 2.35***	87.11±3.75***

-: No activity, SD: Standard Deviation, ns: Not statistically significant *p<0.05, **p<0.01, ***p<0.001, [a]ACA: Acarbose, [b]OR: Orlistat, [c]SIM: Simvastatin

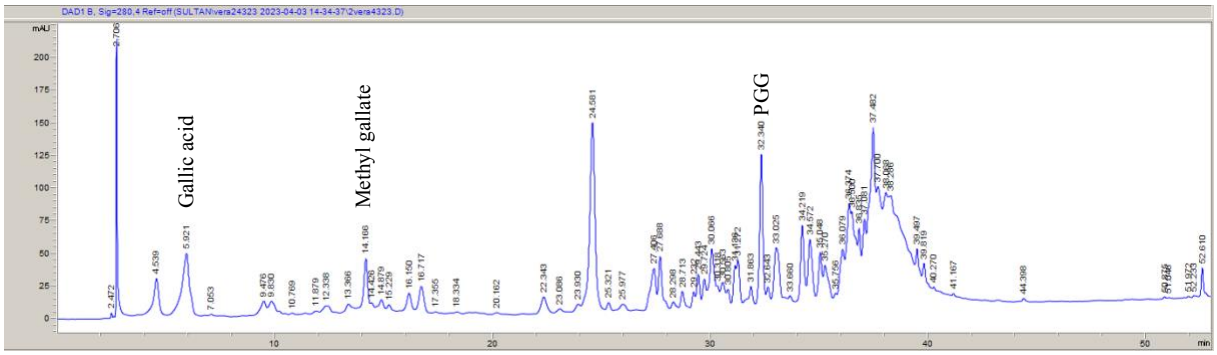

Figure 1. RP-HPLC chromatogram of the extract (280 nm)

In this study, *α*-glucosidase, *α*-amylase, pancreatic cholesterol esterase and pancreatic lipase enzyme inhibitory activities of *P. vera* leaves collected from Gaziantep province were investigated. The results of the study were compared with the study by Gök et al. (2022) on *P. vera* leaves collected from Siirt. Gök et al. reported that 80% ethanol extract prepared from the leaves of the plant showed outstanding *α*-amylase (7.74 ± 0.72 µg/ml IC_{50}) and *α*-glucosidase (11.08 ± 3.96 µg/ml IC_{50}) activities (6). In our study, the IC_{50} value for *α*-amylase was calculated as 26.36 ±1.16 µg/ml and the IC_{50} value for *α*-glucosidase was calculated as 20.75 ± 1.64 µg/ml. When the experimental results were compared with

P. vera leaves collected from Siirt province, it was concluded that both α-glucosidase and α-amylase activities were higher in *P. vera* leaves collected from Siirt province. Gök et al. reported that the extract had a weak inhibition on pancreatic lipase enzyme and calculated the IC_{50} value as 168.43 ± 26.10 µg/ml. In addition, researchers stated that the extract had no effect on the pabcreatic cholesterol esterase enzyme. In our study, the IC_{50} value of *P. vera* leaves on the pancreatic lipase enzyme was calculated as 145.00 ± 5.20 µg/ml. In addition, an inhibition value of 39.45 ± 1.78% on cholesterol esterase enzyme was reached at 0.25 mg/ml concentration in our study. It was concluded that *P. vera* leaves collected from Gaziantep province had a higher activity on pancreatic lipase enzyme and pancreatic cholesterol esterase enzyme than the leaves collected from Siirt province.

Gök et al. calculated the amount of PGG in the extract as 1.295 ± 0.001 g/100 g. The amount of PGG in our extract was calculated as 0.521 ± 0.001 g/100 g. There is information in the literature that PGG has potent α-glucosidase and α-amylase inhibitory activities (13). Considering this literature information, it explains the lower α-glucosidase and α-amylase activity of PGG, which is found in lesser amounts in *P. vera* leaves collected from Gaziantep province, compared to leaves collected from Siirt province.

4. Conclusion

The *in vitro* antihypercholesterolemic, antiobesity and antidiabetic effects of extracts prepared with 80% ethanol from *P. vera* leaves collected from Gaziantep province have not been evaluated before. In this study, the mentioned potentials of the extract were emphasized. The extract of *P. vera* leaf prepared with 80% ethanol showed potent α-glucosidase and α-amylase enzyme inhibitory activities. In addition, it has moderate potential on pancreatic cholesterol esterase and pancreatic lipase enzyme. There is information in the literature that the PGG molecule, whose presence was detected in the plant by RP-HPLC studies, has a significant inhibition on α- glucosidase and α-amylase enzymes. Therefore, the extract was standardized on PGG. As a result of the study; It is thought that *P. vera* leaves collected from Gaziantep can be used as a standardized medicinal herbal product in the prevention and treatment of diabetes. It was also concluded that these leaves could be a source for the isolation of bioactive compounds.

Acknowledgements

This work was supported by Gazi University Scientific Research Projects (02/2019–32). Sultan Pekacar was granted by TÜBİTAK 2211-A National PhD Scholarship Program.

Conflict of Interest

The authors declare that there is no conflict of interest for this article.

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MESMAP – 9
ABSTRACTS & PROCEEDINGS BOOK
03-05 May 2023, Ankara-Türkiye
www.mesmap.com

ORAL PRESENTATION - FULL PAPER

NEUROINFLAMMATION IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE AND PHYTOTHERAPEUTICS

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Abstract

Neuroinflammation is an important mechanism that plays an active role in the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). In the central nervous system (CNS), the process involved in the inflammatory response is called as neuroinflammation and is characterized by the activation of the immune reaction through increasing the microglia and astrocyte population, as well as increasing different concentrations of cytokines and chemokines. Sustaining of neurogenesis as a mechanism of brain repair, mobilization of neural precursors for repair, remyelination, and even axonal regeneration are involved in neuroprotection, but neuroinflammation may be damaging if it causes neuronal damage. The best-known pathophysiological features of AD are the aggregation of neurotoxic forms of amyloid-β proteins in senile plaques and the accumulation of hyperphosphorylated tau proteins in neurofibrillary tangles. Therefore, drug treatments targeting amyloid-β and tau are used in clinical practice. However, FDA-approved anti-AD drugs have only symptomatic efficiency, and the search for alternative and more effective therapeutic targets for the treatment of the disease continues.

Phytochemicals and their derivatives are natural compounds that provide neuroprotection by helping to prevent the initiation and progression of neurodegeneration by altering pathogenic factors. Epidemiological studies show that phytochemicals affect the main pathogenetic mechanisms of AD by targeting oxidative stress, mitochondrial dysfunction, neurotrophic factor deficiency, apoptosis, and abnormal protein accumulation. Curcumin, ferulic acid, hypericum, and resveratrol, important phytochemicals, have been shown to exert preventive and therapeutic effects on depressive disorders by inducing brain-derived neurotrophic factor (BDNF) expression in the hippocampus. These results suggest that phytochemicals contribute to the activation of signaling pathways for neuroprotection by inducing the biosynthesis of neurotrophic factors (NTFs), the Bcl-2 protein family, and antioxidant molecules. Further elucidation of the molecular mechanisms underlying the anti-amyloidogenic effects of blood-brain barrier (BBB)-permeable phytochemicals besides their neuroprotective activities is crucial for synthesizing novel and multifunctional phototherapeutic compounds with high neuroprotective potential for the treatment of AD. In this context, phytochemicals, also known as anti-AD therapeutics, are suggested as a potential therapeutic strategy for the development of antidepressants and anxiolytic agents for the prevention of neurodegeneration. Accordingly, this review discusses the role of inflammation in the developmental period of AD and phytochemical compound-based treatment strategies for AD. In addition, it is highlighted the importance of research on the development of multi-targeted neuroprotective agents based on the structure of BBB-permeable phytochemicals to improve brain dysfunction and prevent neurodegeneration.

Keywords: Alzheimer’s disease, neuroprotection, natural products, herbal medicine, phytochemicals

1. Introduction

Neuroinflammation has been suggested as a key contributor to the progression of Alzheimer's disease (AD). AD is a neurodegenerative disorder characterized by progressive cognitive decline. The cause of the disease is unknown, but neuronal loss, formation of amyloid plaques, and neuroinflammation have been shown to be involved in the pathogenesis. Neuroinflammation is a complex process, involving the activation of glial cells, release of proinflammatory cytokines, and the recruitment of immune cells to the brain. This inflammatory response has been suggested to lead to further neuronal damage, contributing to the progression of the disease (Akiyama et al., 2000; Walker et al., 2019).

Neuroinflammation is a complex process that occurs when the brain is exposed to a variety of different stimuli, such as infection, trauma, or toxins. This inflammatory response can involve a variety of different cell types, including microglia, astrocytes, and even neurons. In response to these stimuli, the cells of the brain release a variety of different cytokines which act as chemical messengers between cells and also have a direct effect on the cells themselves (Lee et al., 2010; Zotova et al., 2010).

Phytotherapeutics are a rapidly advancing area of research that has the potential to provide novel treatments for AD. Plants and their extracts have long been used for traditional medicines, and some of these compounds are known to have anti-inflammatory properties. Phytotherapeutics such as curcumin, resveratrol, and flavonoids have been studied for their potential to reduce neuroinflammation in AD. These compounds have been shown to reduce the production of proinflammatory cytokines, as well as modulate the activation of glial cells. Additionally, they have been shown to improve cognitive functions and reduce the formation of amyloid plaques. Thus, phytotherapeutics offer a promising approach to treating AD by targeting the underlying neuroinflammatory processes (Gezici and Sekeroglu, 2022; Kumar et al., 2023).

Accordingly, this review discusses the role of inflammation in the development of AD and examines phytochemical compound-based treatment strategies. Additionally, the importance of developing multi-targeted neuroprotective agents based on the structure of BBB-permeable phytochemicals as a way to improve brain dysfunction and prevent neurodegeneration is highlighted.

2. Neuroinflammation

Neuroinflammation is a type of inflammation of the nervous system, which includes the brain, spinal cord, and all peripheral nerves. This occurs when the body’s immune system is activated in response to injury or disease, and causes an increase in pro-inflammatory cytokines, immune cells, and other inflammatory molecules in the central nervous system.

Neuroinflammation has been linked to a number of neurological disorders, including Alzheimer's, Parkinson's, and multiple sclerosis. It is also associated with autoimmune diseases, stroke, infections, and traumatic brain injury. Treatment for neuroinflammation typically involves reducing inflammation, managing symptoms, and preventing further damage (Onyango et al., 2021).

Neuroinflammation is a process that involves the activation of immune cells and the release of pro-inflammatory mediators in response to injury or infection of the brain or spinal cord. This process is necessary for tissue repair and resolution of inflammation (Zotova et al., 2010; Walker et al., 2019).

The process of neuroinflammation begins with recognition of an injury or infection by the immune system. Immune cells, such as macrophages, microglia, and astrocytes, are the first to respond. These cells produce and release pro-inflammatory molecules, such as cytokines and chemokines, which activate other immune cells and initiate the inflammatory response (Rogers et al., 1996; Heneka et al., 2015).

Subsequent steps involve the recruitment of additional immune cells, such as T-cells, B-cells, and natural killer cells, to the site of inflammation. These cells produce additional pro-inflammatory cytokines, as well as anti-inflammatory molecules, such as interleukin-10 and transforming growth factor-β (Zhang and Jiang, 2015; Walker et al., 2019)

This inflammatory response also triggers the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These molecules can damage cells and tissue, and are therefore a key component of the inflammatory response (Heneka et al., 2010; Onyango et al., 2021).

3. Molecular Mechanisms Underlying the Neuroinflammation

The process of neuroinflammation is complex, and involves multiple cell types and pathways. The resolution of inflammation is the final step of this process, in which the inflammatory response is reduced and tissue repair begins. This is a critical step in the resolution of the inflammatory response and the return to homeostasis (Ho et al., 2005).

At the cellular level, neuroinflammation is characterized by an increase in the number of microglia, which are the brain's resident immune cells. In response to a stimulus such as infection or injury, microglia become activated and release cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6). These cytokines stimulate an inflammatory response by increasing the production of reactive oxygen and nitrogen species, which in turn can damage cells and lead to further inflammation (Reddy and Beal, 2005; Rajo et al., 2008).

In addition to microglia, astrocytes are also important players in neuroinflammation. Astrocytes are the most abundant glial cell in the brain and can release a variety of cytokines and chemokines, which can both directly and indirectly activate microglia. Astrocytes also play a role in the clearance of debris and toxins from the brain following an insult (Marttinen et al., 2018; Andronie-Cioara et al., 2023).

Finally, neurons can also be involved in neuroinflammation. For example, neurons can release a variety of inflammatory molecules, such as chemokines, which can further activate microglia and astrocytes. Neurons can also be damaged by the reactive oxygen and nitrogen species produced by microglia and astrocytes, leading to further inflammation and possible neuronal death (Sastre et al., 2006; Rojo et al., 2008).

In summary, neuroinflammation is a complex process that involves a variety of different cell types. Microglia, astrocytes, and neurons all play a role in the inflammatory response, releasing cytokines and chemokines that can both directly and indirectly activate other cells. This process can lead to damage to the cells of the brain, which can ultimately lead to further inflammation (Ho et al., 2005; Andronie-Cioara et al., 2023).

4. Pathogenesis and Pathophysiology of Alzheimer's Disease

The pathogenesis of Alzheimer's disease (AD) is a complex process involving genetic, epigenetic, and environmental factors. In the last few decades, research has significantly advanced our understanding of AD pathogenesis (Fan et al., 2020).The exact cause of Alzheimer's disease is not known. However, it is believed to be due to a combination of genetic, environmental, and lifestyle factors. The most common theory suggests that a buildup of certain proteins in the brain, known as amyloid-beta and tau proteins, cause the death of nerve cells. This leads to a decline in cognitive abilities, resulting in the symptoms of Alzheimer's disease. In addition to the buildup of these proteins, other risk factors for Alzheimer's include age, family history, and lifestyle factors such as smoking, high cholesterol, and high blood pressure (Swerdlow, 2007; Li et al., 2022).

Alzheimer's is a progressive disease, meaning it gets worse over time. As the disease progresses, more nerve cells are damaged and cognitive abilities decline further. This causes the symptoms of Alzheimer's to become more severe, including memory loss, confusion, and difficulty with language and thinking. Eventually, a person with Alzheimer's may become unable to care for themselves and require assistance with daily activities (Sanabria-Castro et al., 2017). Alzheimer's disease is a progressive and ultimately fatal neurodegenerative disorder. Its onset and degree of cognitive decline vary from person to person, but its cause is the same: a gradual and irreversible decline of brain cells (Chen, 2018).

In Alzheimer's disease, two abnormal protein structures accumulate in the brain: amyloid plaques and tau tangles. These protein structures damage and eventually kill neurons, which leads to changes in the brain. First, the nerve cells that produce acetylcholine, an important chemical messenger in memory, decline and become unable to communicate with other cells. This disruption impairs memory formation, leading to early changes in the person's ability to remember, reason, perceive and make decisions. As the disease progresses, the hippocampus, a region integral to learning and memory, also begins to shrink. In addition, the cells responsible for forming new memories also decline (Fan et al., 2020; Li et al., 2022).

Other parts of the brain are also affected, leading to problems with speaking, movement, coordination and behavior. The brain continues to shrink and die over time, causing the person to lose the ability to perform basic daily activities. As the brain continues to shrink, the cognitive decline worsens until eventually the person can no longer recognize family members or interact socially. Ultimately, Alzheimer's Disease is a fatal disorder that ends in death (Glass and Arnold, 2012; Jorfi et al., 2023).

5. Neuroinflammation and Molecular Diagnosis of Alzheimer's Disease

Neuroinflammation is an increasingly recognized area of research with implications for the pathogenesis and molecular diagnosis of Alzheimer's disease (AD). In recent years, accumulating evidence has suggested that inflammation is an important factor in the development of AD, and that it can play a role in both the initiation and progression of disease. Neuroinflammation is characterized by changes in the expression of pro-inflammatory and anti-inflammatory cytokines, reactive gliosis, and peripheral inflammatory mediators that enter the central nervous system (CNS). The presence of these mediators has been associated with increased levels of amyloid-β and tau pathology, as well as cognitive decline and neurodegeneration. In addition, neuroinflammatory processes have been linked to synaptic dysfunction and neuronal death, as well as to the activation of microglial cells (Chouliaras et al., 2010; Fan et al., 2020).

The diagnosis of Alzheimer's disease is based on a combination of clinical and laboratory tests. In addition to these tests, molecular genetic testing can be used to detect mutations in genes associated with AD, such as the amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2). These tests can be used to detect genetic mutations that are believed to be associated with an increased risk of developing AD. In addition, biomarkers of inflammation, such as C-reactive protein (CRP) and proinflammatory cytokines, can be measured to diagnose and monitor the progression of AD (Nizzari et al., 2012; Delport and Hewer, 2022).

The understanding of neuroinflammatory processes in AD is still in its early stages, and the precise mechanisms by which these processes contribute to the development of AD remain to

be elucidated. However, the identification of neuroinflammatory markers in the peripheral blood has been proposed as a potential strategy for the early diagnosis of AD. In addition, the potential of anti-inflammatory therapies, such as non-steroidal anti-inflammatory drugs, to modify the course of AD is currently being explored. Neuroinflammation is thus an important area of research that could have significant implications for the pathogenesis and molecular diagnosis of AD (Grant et al., 2002; John et al., 2022).

In addition to these findings, recent studies have also highlighted the potential for therapeutics to target the inflammatory response in AD. A variety of anti-inflammatory drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, have been proposed for the treatment of AD. Additionally, studies in animal models have suggested that targeting specific pathways involved in neuroinflammation, such as the cyclooxygenase 2 (COX-2) pathway, may be beneficial in reducing the severity of AD symptoms (Liu et al., 2022; Astana et al., 2023).

Overall, understanding the molecular signaling pathways and therapeutics that are involved in neuroinflammation in AD is an important step in developing new treatments for this devastating disease. Further research into the molecular and cellular mechanisms of neuroinflammation in AD is needed to identify potential therapeutic targets and develop effective treatments.

6. The Role of Medicinal Plants as Phytotherapeutics in Alzheimer's Disease

Alzheimer's disease (AD) is a serious medical condition that causes progressive and irreversible changes in mental functioning. Despite extensive research, there is no effective cure for AD. Although some drugs can slow down its progression, there is no known treatment for the disease itself. In recent years, plant-based medicines have been gaining increasing attention as a potential source of treatments for AD. Plants contain a wide range of active compounds, many of which have been shown to have promising pharmacological properties that may be beneficial in treating AD (Gezici and Sekeroglu, 2019). In addition, the use of traditional medicines, such as those derived from medicinal plants, may be attractive to patients because they are often less expensive, have fewer side effects, and have a long track record of safety. In this review, we discuss the current understanding of the potential role of medicinal plants in the management of AD. We also discuss potential future approaches to the development of novel plant-based treatments for AD (Gezici et al., 2020; Sharma et al., 2023).

The most important medicinal plants in the management of Alzheimer's disease are Ashwagandha (*Withania somnifera*), *Gingko biloba*, Rosemary (*Rosmarinus officinalis*), Sage (*Salvia officinalis*), Curcumin (*Curcuma longa*), *Passiflora incarnata,* Green tea (*Camellia sinensis*), *Bacopa monnieri, Centella asiatica,* Lemon balm (*Melissa officinalis*), *Huperzia*

serrata, *Hypericum perforatum, Moringa olifera, Glycyrrhiza glabra, Phyllanthus acidus*, and Ashitaba (*Angelica keiskei*) (Gezici and Sekeroglu, 2022; Abhishek et al., 2023; Sharma et al., 2023; Zafar et al., 2023). Some of them are given in details;

Salvia hispanica and officinalis (Sage): Sage is a Mediterranean herb that has been used for centuries to treat numerous mental and physical ailments, including Alzheimer's disease. Some of its key therapeutic properties include anti-inflammatory, anticonvulsant, antioxidant, and anti-amnesic effects. It is thought to enhance neuronal signaling and memory formation, as well as reduce inflammation and free radical damage in the brain associated with Alzheimer's.

*Curcuma longa (Turmeric, curcumin):*Turmeric is a widely used medicinal herb with numerous health benefits. It is thought to protect against the development and progression of Alzheimer's by reducing inflammation and oxidative damage in the brain. Curcumin, a major component of turmeric, has been found to inhibit the accumulation of damaging proteins associated with the disease.

Ginkgo biloba: Ginkgo biloba is a Chinese herb used for centuries to boost memory and mental functioning. It contains a number of compounds that have demonstrated neuroprotective properties, including antioxidant, anti-inflammatory, and antiamyloidogenic effects. These properties may help slow the progression of Alzheimer's disease.

Bacopa monnieri: Bacopa monnieri is an Ayurvedic herb used to increase mental alertness and memory formation. Studies have shown that its bioactive components, called bacosides, can reduce the amount of damaging proteins associated with the disease, as well as improve memory, learning, and overall cognitive function.

Huperzia serrata: Huperzia serrata is a Chinese herb known for its powerful effects on memory and cognitive function. It is thought to help reduce the symptoms of Alzheimer's by decreasing inflammation and oxidative damage, as well as enhancing neuronal signaling in the brain.

Phytotherapy is a promising approach for the treatment of Alzheimer's disease (AD). Phytotherapy is the use of plant-based medicines to treat and prevent diseases. Plant-based medicines have been used for centuries to treat various medical conditions, and research has shown that they may have potential benefits in the treatment of AD. Phytotherapy has been shown to reduce inflammation, improve cognitive function, and reduce oxidative stress, all of which are associated with AD (Kim et al., 2023). Phytotherapeutics, or plant-based medicines, is an emerging field of research that has the potential to provide effective, safe, and affordable treatments for Alzheimer's Disease. While there are currently no cures for Alzheimer's disease, phytotherapeutics has shown promise in reducing the severity of symptoms

associated with the condition. Plant-based compounds may function as antioxidants, anti-inflammatory agents, and neuroprotective agents, which could help to protect and restore neuron function. A number of different plant-based compounds have been studied, including ginkgo biloba, curcumin, resveratrol, and vinpocetine, among others (John et al., 2022; Kumar et al., 2023).

In addition to the potential for direct therapeutic effects, plant-based medicines may also be beneficial for Alzheimer's patients in terms of providing nutrition. For example, plant-based compounds may provide vitamins, minerals, and other important nutritional components that are often lacking in the diets of Alzheimer's patients. This can help to support overall health and wellbeing, which could help to reduce the severity of symptoms (Acero et al., 2023).

Overall, while more research is needed to fully understand the potential of phytotherapeutics in Alzheimer's Disease, the early evidence is promising. Plant-based compounds may provide direct therapeutic benefits, as well as nutritional support, which could help to reduce the severity of symptoms and improve the quality of life for Alzheimer's patients (Santos-Neto et al., 2006; Kim et al., 2023).

7. Conclusion

This review examines treatment methods based on phytochemical compounds and highlight about how inflammation contributes to the onset of AD. In order to improve brain dysfunction and stop neurodegeneration, it is crucial to develop multi-targeted neuroprotective agents based on the structure of BBB-permeable phytochemicals. In conclusion, neuroinflammation in the pathogenesis of Alzheimer’s disease is now thought to be an important underlying cause, making it a promising target for therapeutic interventions. Further research into the molecular and cellular pathways of phytochemicals should be conducted to understand how phytochemicals could be used to target specific pathways and combat Alzheimer’s Disease. Given the potential protective effects of phytochemicals, dietary and lifestyle changes should be promoted to ensure increased consumption of these beneficial compounds. However, more research is needed to determine the exact processes involved before the promising potential of phytotherapeutics can be realized. While phytotherapeutics offer a safe and suitable option for targeting inflammation in Alzheimer’s, further research is necessary before implementation of these treatments can be considered.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Ethics approval and consent to participate: Not Applicable.
Declaration of Competing Interest: The authors declare that they have no competing interests

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03-05 May 2023, Ankara-Türkiye
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POSTER PRESENTATION - FULL PAPER

IN VITRO ASSESSMENT OF NEUROBIOLOGICAL EFFECTS OF COMMERCIAL COCOA POWDER SAMPLES FROM TÜRKİYE*

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Abstract

Cocoa powder is obtained from *Theobroma cacao* L. (Malvaceae), which is often used for flavoring in food industry. It is also well-known to be rich in polyphenols, which are beneficial for human health including neuroprotection. As cholinergic hypothesis proposed for pathology of Alzheimer's disease (AD), cholinergic deficit has been reported due to hydrolysis of acetylcholine by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). In this study, inhibitory effect of ethanol extracts of the cocoa powders with different brands purchased from several supermarkets in Ankara (Türkiye) against AChE and BChE closely related to AD was evaluated. Moreover, the impact of the same extracts on the elimination of free radicals, which affect the formation and progression of AD, was studied. The antioxidant effects of the samples were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric reducing antioxidant power (FRAP), and metal-chelating activity assays. Total phenolic and total flavonoid contents in the extracts were also determined by Folin-Ciocalteu and aluminium chloride colorimetric methods, respectively. Among our samples, the sample coded as RY12 had a high AChE inhibition activity by 56.46 ± 2.12% (inhibition% ± standard deviation, S.D.) at a final concentration of 200 µg/mL. On the other hand, the sample coded as RY7 had a strong BChE inhibition activity by 41.29 ± 0.09% with a final concentration of 200 µg/mL. DPPH radical scavenging activity, FRAP, and metal-chelating activity experiments revealed that the ethanol extracts had modest antioxidant activity. The total flavonoid content of the samples could not be determined since the concentration of quercetin in all samples except of RY5 was less than 0.016 mg. According to the results of total phenol quantification calculated on the basis of gallic acid in the extracts, where each sample was determined to contain moderate phenol content. The results of the study showed that the ethanol extracts of cocoa powders purchased from the markets and used by the public in daily life have a moderate level of cholinestarase inhibition and have antioxidant activity at some extent that can be evaluated. Further studies are in progress in our laboratory to determine which substances are responsible for antioxidant and antialzheimer activities.

Key Words: Cocoa powder, antioxidant, cholinesterase inhibition, Alzheimer's disease.

*This study was performed as graduation thesis of Rahmancan Yurduseven, who was then 5th year student at Faculty of Pharmacy, Gazi University under supervision of Prof. Dr. Ilkay Erdogan Orhan.

1. Introduction

Alzheimer's disease (AD) is a progressive neurological disease that damages a person's memory and cognitive abilities, typically affecting the elderly. The particular cause of the disease is unknown, and no specific therapy for AD is now available. According to the cholinergic hypothesis, the quantity of neurotransmitters such as acetylcholine and butyrylcholine, which are broken down by the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), respectively, is reduced in the brains of Alzheimer's disease patients (1). As a result, cholinesterase inhibitors are frequently used to treat Alzheimer's disease symptoms. To treat Alzheimer's disease, FDA-approved cholinesterase inhibitors (such as rivastigmine, galanthamine, tacrine, and donepezil) as well as *N*-methyl-D-aspartate (NMDA) receptor antagonists (such as memantine) and aducanumab, a monoclonal antibody that reduces beta-amyloid plaques approved in 2021, are currently in clinical trials (2,3). As ADadvances, the creation of reactive oxygen species rises, leading to neurodegeneration *via* oxidative damage in the brain. As a result, antioxidants can also aid in the treatment of AD (4). The antioxidant capacity and AChE and BChE enzyme inhibitory activity of polyphenol-rich cocoa powder samples were assessed. As a result, the inhibitory impact of ethanol extracts of several brands of cocoa powders purchased from different supermarkets in Ankara (Türkiye) on AChE and BChE, which are closely associated to AD, was investigated. Furthermore, the effect of the same extracts on the removal of free radicals influencing the onset and development of AD was studied.

2. Material and Methods

Commercial cocoa powder of 12 different brands was purchased from supermarkets in Ankara, Türkiye. Each cocoa powder sample was then macerated in 96% ethanol (EtOH, 50 mL) for 3 days at room temperature with occasionally shaking by hand. After each sample was filtered through pleated filter paper, the EtOH phases were evaporated under reduced pressure by a rotary evaporator (Büchi, Switzerland) to obtain crude extracts. The obtained crude extracts were stored in the refrigerator at +4 °C until the experiments were carried out.

2.1. Microplate assays for acetylcholinesterase and butyrylcholinesterase inhibition

The samples' inhibitory activities against acetylcholinesterase (AchE) and butyrylcholinesterase (BChE) enzymes were investigated using a slightly modified version of Ellman's method (5). The enzyme sources were electric eel acetylcholinesterase (Type-VI-S, EC 3.1.1.7, Sigma) and equine serum butyrylcholinesterase (EC 3.1.1.8, Sigma), whilst the reaction substrates were acetylthiocholine iodide and butrylthiocholine chloride (Sigma, St. Louis, MO, USA). The cholinesterase activity was determined using 5,5'-Dithio-bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA). Using a multichannel automated pipette (Eppendorf Research, Germany), 140 µL of 0.1 mM sodium phosphate buffer (pH 8.0) was added to the 96-well microplate, followed by 20 µL of the samples/EtOH (negative control) at dilutions ranging from 25-200 µg/mL. Then, using a multichannel automated pipette (Gilson Pipetman, France) 20 µL of 0.2 M AChE/BChE solution was added. After that, it was incubated at room temperature for 10 minutes. To initiate the reaction, 10 µL of 0.2 M acetylthiocholine iodide/butyrylthiocholine chloride were added to the 96-well microplate as substrates. Thiol esters used as substrates are hydrolyzed by AChE or BChE to release thiocholine. As a result of the reaction of thiocholine with DTNB, 2-nitro-5-thiobenzoate (TNB) is formed as the yellow-colored product. The formation rate and color intensity of the product, which formed as a result of the reaction, were assessed using ELISA microplate reader (Molecular Devices, Spectramax i3x microplate reader, USA) at wavelength of 412 nm. In both studies, galantamine hydrobromide (Sigma, USA) was employed as

a reference. Every experiment was carried out in triplicate. Based on a comparison of rates of enzyme reaction between samples and the blank sample (ethanol in phosphate buffer, pH = 8) using the formula (1-S/E)*100, where E is enzyme activity without test sample and S is enzyme activity with test sample, we determined percentage of inhibition of AChE and BChE. IC_{50} values of samples were calculated using GraphPad Prism 6.01.

2.2. Microplate assays for determination of total phenol and total flavonoid contents

Total phenolic content of ethanolic extracts of cocoa samples and was measured using the Folin-Ciocalteau colorimetric approach (6). Gallic acid solutions at various concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL) were produced in order to create the calibration curve. EtOH (96% concentration) was also used to dissolve the extracts. Samples and gallic acid concentrations were combined with 30 µL of Folin-Ciocalteau's reagent (diluted 2, Sigma, USA) and 150 µL of 3.5% sodium carbonate. After 30 minutes of incubation at 40°C, its absorbance at 760 nm was measured with an ELISA microplate reader (Molecular Devices, Spectramax i3x microplate reader, USA).

2.3. Microplate assays for antioxidant activity

2.3.1. DPPH radical scavenging activity

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma, USA) radical scavenging activity was determined by the method of (7) with minor modifications (8). 10 µl extracts and reference (quercetin, Sigma, USA) dissolved in %96 EtOH were mixed with DPPH solution (0.138 mg/ml). The remaining DPPH amount was measured at 515 nm using an ELISA microplate reader (Molecular devices spectramax i3x microplate reader, USA). Analyses were performed in triplicate.

2.3.2. Ferrous ion-chelating activity assay

Carter's modified technique (9,10) was used to assess the metal-chelating influence of the samples and reference (ethylenediaminetetraacetic acid, EDTA, Sigma, USA). In summary, 20 µL of each sample and reference were incubated at room temperature for 10 minutes with 96% EtOH, 2 mM FeCl2 (Sigma, USA), and 5 mM ferrozine (Sigma, USA) solutions. Using an ELISA microplate reader (Molecular Devices, Spectramax i3x microplate reader, USA), the absorbance of the produced ferrozine-Fe^{2+} complex was measured at 562 nm.

3. Results and Discussion

Among our samples, the sample coded as RY12 had a high AChE inhibition activity by 56.46 ± 2.12% (inhibition% ± standard deviation, S.D.) at a final concentration of 200 µg/mL. On the other hand, the sample coded as RY7 had a strong BChE inhibition activity by 41.29 ± 0.09% with a final concentration of 200 µg/mL. DPPH radical scavenging activity, FRAP, and metal-chelating activity experiments revealed that the ethanol extracts had modest antioxidant activity. The total flavonoid content of the samples could not be determined since the concentration of quercetin in all samples except of RY5 was less than 0.016 mg. According to the results of total phenol quantification calculated on the basis of gallic acid in the extracts, where each sample was determined to contain moderate phenol content. The results of the study showed that the ethanol extracts of cocoa powders purchased from the markets and used by the public in daily life have a moderate level of cholinesterase inhibition and have antioxidant activity at some extent that can be

evaluated. Further studies are in progress in our laboratory to determine which substances are responsible for antioxidant and anti-Alzheimer activities.

Table 1. Extract yields and antioxidant assay results

Samples	Yield (% w/w)	DPPH Radical Scavenging Activity (%Scavenging Activity ± S.D.[a]) 2000 µg/mL[b]	DPPH Radical Scavenging Activity (%Scavenging Activity ± S.D.[a]) 1000 µg/mL[b]	Metal-Chelating Activity (%Activity ± S.D.[a]) 2000 µg/mL[b]
RY 1	2.91	34.22 ± 2.78	-[c]	46.26 ± 3.28
RY 2	5.59	15.6 ± 3.44	-	61.89 ± 2.82
RY 3	4.28	11.76 ± 1.72	-	57.82 ± 1.47
RY 4	6.07	-[c]	-	49.55 ± 4.61
RY 5	5.2	67.06 ± 1.85	23.52 ± 1.13	25.19 ± 0.46
RY 6	3.7	31.5 ± 3.44	-	45.35 ± 4.03
RY 7	2.69	27.85 ± 5.43	-	51.71 ± 3.81
RY 8	3.79	38.71 ± 3.57	23.36 ± 0.93	33.38 ± 3.27
RY 9	1.5	-	-	70.21 ± 0.70
RY 10	3.88	-	-	71.07 ± 2.75
RY 11	2.51	34.4 ± 3.57	-	44.20 ± 2.31
RY 12	5.68	8.3 ± 0.19	-	47.86 ± 2.06
References		86.21 ± 1.35[d]		94.25 ± 0.98[e]

[a] Standard deviation (n: 3), [b] Stock concentration, [c] No effect observed, [d] Quercetin- 1000 µg/mL, [e]EDTA- 1000 µg/mL

Table 2. AChE and BChE inhibition assay results for the cocoa extracts

Samples	Acetylcholinesterase Inhibition (Inhibiton % ± S.D.[a]) 200 µg/mL[b]	Butyrylcholinesterase Inhibition (Inhibiton % ± S.D.[a]) 200 µg/mL[b]
RY1	NA*	7.11 ± 0.4
RY2	NA*	17.82 ± 3.39
RY3	2.68 ± 1.56	15.58 ± 2.89
RY4	2.85 ± 1.03	1.48 ± 0.77
RY5	13.57 ± 2.16	10.04 ± 2.25
RY6	16.4 ± 0.41	13.37 ± 1.64
RY7	NA*	41.29 ± 0.09
RY8	11.85 ± 1.11	1.46 ± 1.04
RY9	40.25 ± 0.81	20.54 ± 0.74
RY10	27.75 ± 2.58	12.76 ± 0.87
RY11	21.88 ± 1.15	16.29 ± 3.33
RY12	56.46 ± 2.12 (IC_{50}: 28.62 ± 0.02 µg/mL)	19.31 ± 3.63
Reference[c]	97.11 ± 1.26 (IC_{50}: 0.68 ± 0.05 µg/mL)	72.88 ± 2.61 (IC_{50}: 45.45 ± 5.97 µg/mL)

[a] Standard deviation (n: 3), [b] Final concentration, [c] Galanthamine hydrobromide (100 µg/mL), *NA: No activity

Table 3. Total phenol and total flavonoid contents of the cocoa extracts

Samples	Total Phenol Content[a] ± S.D.[b]	Total Flavonoid Content[c] ± S.D.
RY 1	53.26 ± 2.2	*
RY 2	27.33 ± 4.77	*
RY 3	21.89 ± 1.47	*
RY 4	31.09 ± 3.12	*
RY 5	121.07 ± 4.95	0.9 ± 0.44
RY 6	64.41 ± 1.47	*
RY 7	48.08 ± 2.57	*
RY 8	67.14 ± 3.12	*
RY 9	20.07 ± 2.5	*
RY 10	15.49 ± 1.2	*
RY 11	58.71 ± 1.83	*
RY 12	40.73 ± 2.84	*
	y=1,9282x+0,1226 r^2=0,9993	y=3,2169x+0,0872 r^2=0,9998

[a]Data expressed in mg equivalent of gallic acid to 1 g of extract, [b]Standard deviation (n: 3), [c]Data expressed in mg equivalent of quercetin to 1 g of extract, * Less than 0.016 mg, not calculated.

Conflict of Interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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03-05 May 2023, Ankara-Türkiye
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ORAL PRESENTATION - FULL PAPER

PROPIONIC ACID: PHARMACOLOGICAL PROPERTIES, MOLECULAR MECHANISMS AND HEALTH BENEFITS

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Abstract

Propionic acid, also known as propanoic acid, is a naturally occurring short-chain fatty acid. It can be produced through different methods, including chemical synthesis and fermentation. In fermentation processes, certain bacteria produce propionic acid as a metabolic byproduct. Chemical properties of propionic acid contribute to its versatility in various applications, including food preservation, pharmaceuticals, agricultural products, and industrial processes. On the other hand, propionic acid possesses various pharmacological properties and exerts its effects through multiple molecular mechanisms, which contribute to its potential health benefits. In this review, there is an overview of pharmacological properties, molecular mechanisms, and associated health benefits of propionic acid. The exact molecular mechanisms underlying propionic acid's pharmacological properties are diverse and context-dependent. They involve interactions with various signaling pathways, receptors, enzymes, and transcription factors, including but not limited to those mentioned earlier such as inhibition of cyclooxygenase (COX), activation of peroxisome proliferator-activated receptor gamma (PPARγ), and modulation of G-protein coupled receptors (GPCRs). While propionic acid shows promising potential and has been investigated in various contexts, it is essential to note that further research is needed to fully understand its molecular mechanisms, optimize its therapeutic applications, and evaluate its safety and efficacy in clinical settings. Taken together, consulting scientific literature and healthcare professionals can provide the most up-to-date and accurate information regarding potential health benefits of propionic acid.

Keywords: Propionic acid, health benefits, biological effects, metabolism, dietary intake

1. Introduction

Propionic acid ($C_3H_6O_2$) is a naturally occurring short-chain fatty acid that is produced by bacteria during the fermentation of dietary fiber in the colon. Propionic acid is a carboxylic acid, that is, it contains a carboxyl functional group (-COOH), which gives it its acidic properties. It has a melting point of -20.8°C and a boiling point of 141.1°C. Propionic acid is soluble in water, ethanol, and ether. It is also found in certain foods, such as dairy products and grains. It is also used as a food preservative, and as a result, its salts (propionates) are commonly added to bread, baked goods, and dairy products. In addition, propionic acid and its derivatives have various industrial applications, including plasticizers and solvents (Boyaval and Corre, 1995; Sa'ad et al., 2010).

Propionic acid occurs naturally in a variety of foods, including cheese, butter, milk, and bread. It is also produced industrially by the fermentation of various organic materials such as starch and sugar. Propionic acid and its derivatives are used in a variety of applications, including the production of cellulose acetate and propionate plastics, food preservatives and pharmaceuticals. In addition, propionic acid can be produced industrially through bacterial fermentation of various raw materials such as corn, wheat and sugar. Propionic acid production is used in the food industry as a preservative and in the production of various chemicals and plastics. Overall, propionic acid is a versatile compound that occurs naturally and can be produced industrially for various applications (Kaziro and Ochoa, 1964; Es et al., 2017).

Propionic acid is metabolized in the liver and plays an important role in diverse biological processes, including energy metabolism, lipid metabolism, immune and gastrointestinal functions. After consumption, propionic acid is rapidly absorbed by the body, and metabolized either in the liver or in peripheral tissues. Recent studies have also suggested potential applications of propionic acid in the treatment of metabolic disorders, such as obesity and type 2 diabetes, as well as in the prevention of certain cancers, improving insulin sensitivity, reducing inflammation, and modulating lipid metabolism (Pullammanappallil et al., 2001.

The underlying mechanisms of action and biological effects of propionic acid are not fully understood, but it has been suggested that it may act through the activation of certain signaling pathways, receptors, enzymes, and transcription factors that are expressed in various tissues. Propionic acid has also been demonstrated to induce changes in gut microbiota, which may contribute to its beneficial effects on metabolic health. Consequently, the pharmaceutical properties of propionic acid suggest that it may have potential therapeutic applications in food industry, pharmaceutical industry, and medicine applications (Sa'ad et al., 2010; Duscha et

al., 2020). Accordingly, the pharmacological properties, molecular mechanisms, and health benefits associated with propionic acid were highlighted in this review.

2. Metabolism of Propionic Acid

A naturally occurring carboxylic acid called propionic acid is created by the gut bacteria as dietary fiber and other carbohydrates are broken down. Depending on the organism and the surrounding environment, multiple pathways are involved in the metabolism of propionic acid (James et al., 1956; Sa'ad et al., 2010). The propionate cycle is a metabolic mechanism that predominantly breaks down propionic acid in mammals' livers. Propionate is transformed into succinyl-CoA in this cycle, which then enters the citric acid cycle and is finally oxidized to produce ATP and carbon dioxide. Additionally, the propionate cycle generates intermediates that can be utilized in additional metabolic processes, such as the production of fatty acids and glucose (Flavin et al., 1955; Kaziro and Ochoa, 1964).

Propionic acid has been found to have a number of effects on glucose and lipid metabolism. According to studies, propionic acid can lower the liver's synthesis of glucose and improve the insulin sensitivity of muscle and fat cells. Propionic acid has also been demonstrated to lower triglyceride formation in the liver and serum cholesterol levels, possibly due to its effects on the gut flora (Lemosquet et al., 2009; Heiman et al., 2015).

Propionic acid metabolism: Immune system and energy metabolism are two biological processes that benefit from proper propionic acid metabolism. The propionate route in the liver is used to metabolize propionic acid. Propionic acid is transformed in this pathway into propionyl-CoA, which is then transformed into succinyl-CoA, a crucial intermediary in the citric acid cycle. The citric acid cycle is where succinyl-CoA can be used to produce ATP, which is a kind of energy. In addition to producing intermediates that can be employed in other metabolic pathways, this process also creates energy in the form of ATP (Wongkittichote et al., 2017).

Propionic acid occurrence and production: Natural sources of propionic acid include dairy products, wheat, and some kinds of fermented foods. As a result of bacterial fermentation of dietary fiber, it is also created by the gut microbiota (Es et al., 2017). Propane oxidation and ethylene carboxylation are two examples of chemical processes that can be used to commercially manufacture propionic acid. Although less popular than fermentation, this technique occasionally proves to be more economical. The so-called "Beaupre process," which entails the fermentation of sugars or starches with *Propionibacterium freudenreichii*, a kind of bacteria frequently found in dairy products, is the most regularly used industrial technique for creating propionic acid. Propionic acid is produced by *P. freudenreichii* as a metabolic byproduct during fermentation. The process is typically carried out in large

stainless-steel tanks and takes several days to complete (Wang and Yang, 2013; Yun et al., 2019; He et al., 2021).

Propionic acid and glucose metabolism: In recent years, researchers have been interested in the possible effects of propionic acid on glucose metabolism because it has been demonstrated to have an impact on glucose metabolism (Adler et al., 2021; He et al., 2021). Propionic acid has been shown in studies to promote insulin secretion, which can assist in controlling blood glucose levels. Propionic acid may enhance insulin sensitivity and glucose uptake in a variety of organs, including the liver, muscles, and adipose tissue, according to a number of studies. Propionic acid's effects on glucose metabolism have been linked to its ability to activate the AMP-activated protein kinase (AMPK) pathway. As a crucial control point for cellular energy metabolism, AMPK is activated in response to energy stressors like exercise or low blood sugar. Propionic acid has been demonstrated in studies to activate AMPK and boost glucose absorption in skeletal muscle cells, which may help explain its potential therapeutic effects in diseases including obesity and diabetes. The modulation of the gut flora is another suggested method. Propionic acid supplementation can enhance the abundance of these bacteria and improve glucose tolerance, according to studies that demonstrate that the bacteria that produce propionic acid are diminished in the gut microbiota of persons with type 2 diabetes (Lemosquet et al., 2009; Zhang et al., 2022).

Propionic acid and cholesterol/fatty acid metabolism: The metabolism of fatty acids and cholesterol are significantly impacted by propionic acid. According to studies, propionic acid may be able to prevent the liver from making new cholesterol, hence assisting in the reduction of blood cholesterol levels (Sa'ad et al., 2010). According to one study, propionic acid can alter lipid metabolism by lowering the liver's ability to synthesize fatty acids (Wah et al., 2019). By lowering the expression of genes involved in cholesterol synthesis, propionic acid administration was shown to lower serum cholesterol levels in another animal study (Pourmozaffar et al., 2019). Additionally, propionic acid has been demonstrated to promote fatty acid oxidation, which may aid in preventing the buildup of fat in the liver and other tissues. These effects may have implications for the prevention and treatment of metabolic disorders, such as obesity and type 2 diabetes (Heiman et al., 2015; Xue et al., 2022).

3. Pharmacological Activities and Biological Roles of Propionic Acid

Propionic acid, sometimes referred to as propanoic acid, is an organic molecule that occurs in nature and has a variety of biological functions in both humans and other living things. Its role in the metabolism of lipids and carbohydrates is one of its important biological functions. Propionic acid can be used as a substrate for ATP synthesis through the citric acid cycle in terms of energy metabolism. Additionally, it has been shown that propionic acid stimulates the release of insulin, which can aid in controlling blood glucose levels. Propionic acid

furthermore possesses anti-inflammatory properties that may be helpful in the treatment of inflammatory bowel disease and other inflammatory disorders (Tvrzicka et al., 2011). Furthermore, propionic acid has been shown to inhibit the synthesis of cholesterol in the liver, which can help to lower blood cholesterol levels. In addition, it has been demonstrated that propionic acid prevents the liver's production of cholesterol, which can aid in lowering blood cholesterol levels. Propionic acid also has antibacterial qualities and is frequently used as a food preservative because it can stop the growth of bacteria, yeast, and molds. These outcomes imply that propionic acid may have potential therapeutic uses in the management of metabolic diseases like type 2 diabetes and obesity, as well as in the prevention of some cancers and immune response regulation (Sa'ad et al., 2010; Dhall et al., 2016). Several pharmaceutical properties of propionic acid are presented in the Figure 1.

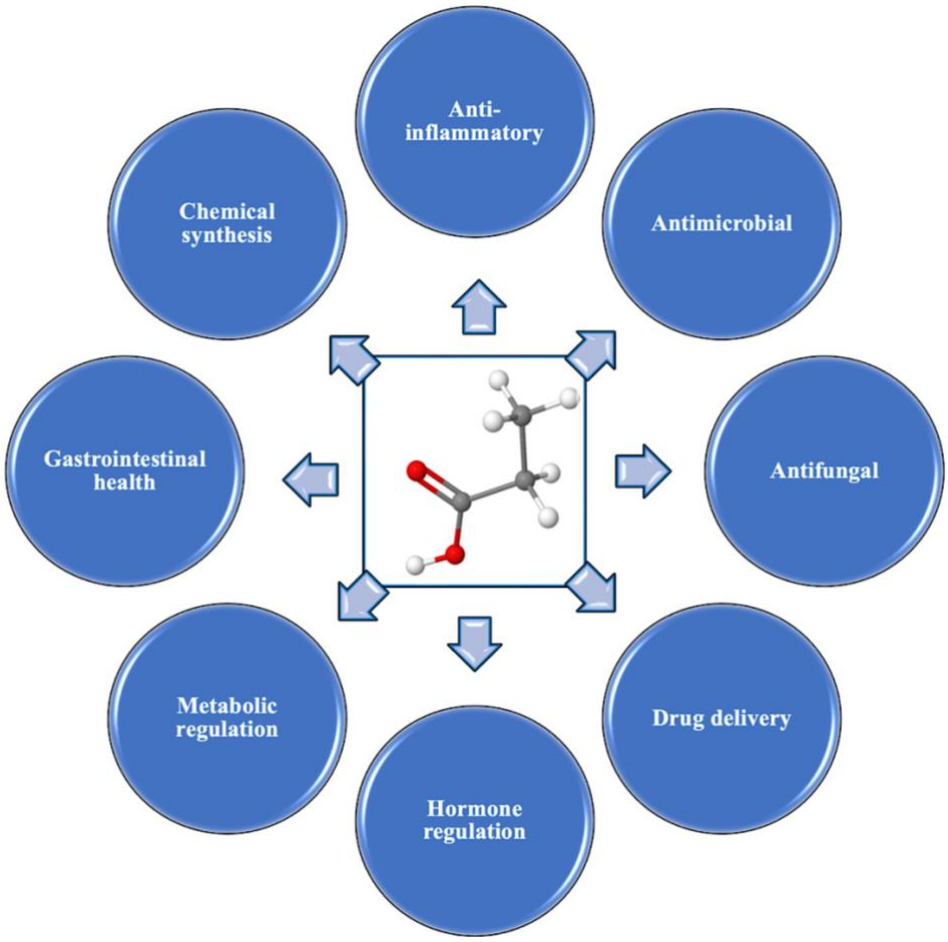

Figure 1. Several pharmacological activities and biological roles of propionic acid

Anti-inflammatory activity: The ability of propionic acid to reduce inflammation has been demonstrated by previous studies. Inhibiting inflammatory mediators and cytokines may assist to reduce inflammation. According to Ahmed et al. (2012) and Zhang et al. (2023), this

property makes it a good candidate for the development of drugs to treat inflammatory diseases such dermatitis, inflammatory bowel disease, and arthritis (Ahmed et al., 2012; Zhang et al., 2023).

Antimicrobial activity: In particular, propionic acid is antibacterial and effective against mold, yeast, and bacteria. It can prevent a variety of microorganisms, including those that cause food deterioration and some infectious diseases, from growing and proliferating. Propionic acid is used as a food preservative and a potential medicinal agent against microbial infections as a result of its antibacterial action (Wang et al., 2014; Antone et al., 2022).

Antifungal activity: Certain fungi have been discovered to be resistant to the antifungal effects of propionic acid. It can inhibit the growth and development of fungi, including those associated with fungal infections in humans. This characteristic underlines its therapeutic promise for fungal diseases (Ravazi-Rohani et al., 1999; Sa'ad et al., 2010).

Drug delivery system: It has been investigated to use propionic acid as a component of drug delivery systems. It can be used in formulations as a solvent or co-solvent to improve the solubility and stability of drugs. Additionally, prodrugs—inactive substances that can be transformed into active medications in the body—have been created using propionic acid derivatives, which improve drug delivery and target (Chen et al., 2018).

Chemical synthesis: Propionic acid is used as an essential component in the manufacture of many different medicinal substances. medications including nonsteroidal anti-inflammatory medications (NSAIDs), antibiotics, and antifungal agents can all be made using it as a precursor. Numerous medicinal compounds are developed and produced thanks to its versatility in chemical synthesis (Li et al., 2011).

Hormone regulation: Propionic acid has been linked to improving insulin sensitivity and glucose uptake, suggesting that it may have benefits in regulating hormones and preventing metabolic disorders, such as diabetes and obesity (Al-Lahham and Rezaee, 2019).

Metabolic regulation: Propionic acid plays a role in glucose and lipid metabolism. It has been demonstrated to improve insulin sensitivity, enhance glucose uptake, and modulate lipid metabolism. These effects can contribute to the management of metabolic disorders, e.g., diabetes and obesity (Sa'ad et al., 2010; Chaput et al., 2011).

Gastrointestinal health: Propionic acid has been investigated for its potential to modify the gut microbiota and alleviate certain inflammatory conditions in the digestive system, such as irritable bowel syndrome (Konopelski and Magilnicka, 2022).

Consequently, propionic acid has crucial physiological functions in the body and may be used therapeutically for a number of diseases. It is a versatile and important molecule that has uses in the food, feed, and pharmaceutical industries.

4. Molecular Mechanisms of Action and Propionic Acid

It has been discovered that propionic acid exerts its biological effects through a variety of cellular mechanisms, including the inhibition of cyclooxygenase (COX), the activation of G-protein coupled receptors (GPCRs), the activation of peroxisome proliferator-activated receptor gamma (PPAR), and the modulation of nuclear factor kappa B (NF-B) signaling. Molecular mechanisms of propionic acid involve several important pathways and targets within the body, which are summarized below (Sa'ad et al., 2010; Xing et al., 2023);

Cyclooxygenase inhibition: COX are enzymes involved in the synthesis of prostaglandins, which play a role in inflammation and pain. Propionic acid, specifically its derivative known as ibuprofen, acts as a non-steroidal anti-inflammatory drug (NSAID) that inhibits COX enzymes, which is involved in inflammation and pain processes. This inhibition ultimately has the anti-inflammatory and analgesic effects of reducing the generation of inflammatory prostaglandins and thromboxanes (Yu et al., 2010).

G-protein coupled receptors (GPCRs): A class of cell surface receptors known as GPCRs mediates a range of cellular reactions. Specific GPCRs may interact with propionic acid to provide a variety of physiological effects. Propionic acid has been seen in a number of investigations to activate GPCRs. GPCRs are essential for a variety of physiological functions, including inflammation, hormone control, and sensory perception. GPR41 and GPR43, which are expressed in adipose tissue, immune cells, and the digestive system, have been discovered to be activated by propionic acid. Propionic acid activation of these receptors has been associated with anti-inflammatory outcomes, enhanced glucose homeostasis, and decreased body weight gain. However, the specific GPCRs affected by propionic acid and their resulting downstream signaling pathways are still under investigation (Sa'ad et al., 2010; Adams et al., 2017; Al-Lahham and Rezaee, 2019).

Peroxisome proliferator-activated receptor gamma (PPARγ): PPARγ is a nuclear receptor involved in the regulation of glucose and lipid metabolism, as well as inflammation. Activation of PPARγ is another mechanism of action for propionic acid. PPARγ is a transcription factor involved in glucose and lipid metabolism, and is also known to have anti-inflammatory effects. Propionic acid has been found to activate PPARγ, leading to improved insulin sensitivity and lipid metabolism (Cai et al., 2018).

Nuclear factor kappa B (NF-κB): A transcription factor called NF-B is essential for controlling the expression of genes related to immune responses and inflammation (Zhuang et al., 2023). Another mechanism by which propionic acid works is through modification of NF-B signaling. The activation of NF-B has been discovered to be inhibited by propionic acid, which results in decreased production of inflammatory mediators and increased insulin sensitivity. Propionic acid may decrease the symptoms of disorders connected to inflammation by regulating NF-B activation. (Tayyeb et al., 2020).

Accordingly, propionic acid exerts its biological activities through multiple cellular mechanisms, including inhibition of COX, activation of GPCRs and PPARγ, and modulation of NF-κB signaling. These mechanisms ultimately result in anti-inflammatory effects, improved glucose and lipid metabolism, and reduced body weight gain.

5. Potential Applications of Propionic Acid

Propionic acid plays a number of physiological roles and has potential uses in a wide range of industries, including the food, plastics, and pharmaceutical industries. It is employed as a flavour and preservative in the food sector. Propionic acid is also used to make a variety of chemicals and polymers. Propionic acid has been researched in the medical field for its possible application in the treatment of metabolic diseases like type 2 diabetes and obesity. Sa'ad et al. (2010); Kumar and Babu (2008). Given that it has been demonstrated to cause cancer cells to undergo apoptosis, it has also been investigated for its possible anticancer qualities. Propionic acid has been researched for its possible medicinal uses in addition to its metabolic roles. Research suggests that it may have anti-inflammatory properties, making it a potential candidate for the treatment of inflammatory diseases. Additionally, propionic acid has been explored for its antimicrobial activity. As a result of its inhibitory properties against different bacteria, yeast, and molds, it is used as a food preservative. Some of notable functions and potential uses of propionic acid are given below (Liu et al., 2012; Vidra and Németh, 2018; Iragavarapu et al., 2023);

1. Energy production: Dietary fiber in the colon ferments to form propionic acid, which is subsequently taken into the bloodstream and delivered to the liver for utilization as an energy source.
2. Lipid metabolism: It has been demonstrated that propionic acid modifies lipid metabolism by inhibiting the liver's ability to synthesize fatty acids. Potential therapeutic uses for this include the management of metabolic diseases like type 2 diabetes and obesity.
3. Anti-inflammatory properties: It has been discovered that propionic acid reduces oxidative stress and inflammation, both of which can result in the emergence of chronic disorders. This suggests that it may have potential as a natural anti-inflammatory agent.

4. Gut health: It has been demonstrated that propionic acid alters the gut microbiota, encouraging the growth of helpful bacteria while inhibiting the growth of harmful bacteria. This could potentially improve gut health and prevent certain gastrointestinal disorders.
5. Food and feed industry: Propionic acid is utilized as a food preservative because it prevents the growth of mold and increases the shelf life of food items in the food and feed industries. Additionally, it can be used as a natural feed supplement for livestock, enhancing feed effectiveness, preventing mold formation in feed, and enhancing the health and performance of the animals. It has also been researched for its potential to act as a biopesticide and serve as an alternative to pesticides made of synthetic chemicals.
6. Pharmaceutical industry: Propionic acid is used in the production of pharmaceuticals, such as antibiotics and anti-inflammatory drugs.

It can be concluded that propionic acid is a biologically active compound with possible health benefits. Physiological roles of propionic acid in energy metabolism, along with its potential therapeutic, agricultural, and industrial applications, highlight its significance in a varierty fields and encourage further research and investigation into its various properties and potential applications (Adams, 1992; Sa'ad et al., 2010; Al-Lahham and Rezaee, 2019; Iragavarapu et al., 2023; Xing et al., 2023).

6. Conclusion

Pharmaceuticals, food preservatives, the manufacture of cellulose acetate and propionate polymers, and other industries and applications all make use of propionic acid. Additionally, it is utilized as a feed supplement in animal nutrition to enhance feed effectiveness and stop the growth of mold. It is a promising substance with prospective uses in the management of many metabolic disorders and diseases. These effects have intricated underlying mechanisms that involve interactions with several signaling pathways and metabolic activities. However, further research is needed to fully understand the mechanisms of action and biological effects of propionic acid in humans and to explore its potential as a therapeutic agent.

Conflict of interest: The authors declare that they have no conflict of interest.

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