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The Seventh International Mediterranean Symposium on Medicinal and Aromatic Plants

November 18-20, 2021 / İzmir - TURKEY

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ABSTRACTS & PROCEEDINGS BOOK
18– 20 November 2021, Turkey
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The Seventh International Mediterranean Symposium on Medicinal and Aromatic Plants

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ABSTRACTS & FULL PAPERS

November 18th – 20th, 2021

Izmir – Turkey

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Dear Colleagues,

Having respected scientific board and organizing committee members from all over the world, MESMAP Symposium series started in 2013. The first Mediterranean Symposium on Medicinal and Aromatic Plants (MESMAP-2013) was held on April 17-20, 2013 in Gazimagosa (Famagusta), Turkish Republic of Northern Cyprus (TRNC), which was organized by Faculty of Pharmacy, Eastern Mediterranean University (EMU) joint with AMAPMED (Association of Medicinal and Aromatic Plants of the Mediterranean).
MESMAP-2 Symposium was held on April 22-25, 2015 in Antalya – TURKEY, which was organized by academicians from Gazi University (TURKEY), Gaziantep University (TURKEY), Kilis 7 Aralik University (TURKEY), Yüzüncü Yıl University (TURKEY), Association of Pharmaceutical Teachers of India (APTI – INDIA) joint with AMAPMED (Association of Medicinal and Aromatic Plants of the Mediterranean). INDUSTRIAL CROPS AND PRODUCTS JOURNAL with high impact factor from ELSEVIER group published a special issue covering some of the full papers selected after scientific evaluation.

MESMAP-3 Symposium which was held on April 13-16, 2017 in Girne (Kryneia) – Turkish Republic of Northern Cyprus (TRNC), was the third event of MESMAP symposium series on Medicinal and Aromatic Plants. After scientific evaluation selected full papers published in Indian Journal of Pharmaceutical Education and Research (IJPER), indexed with THOMSON REUTERS. MESMAP-4 Symposium, which was held on April 18-22, 2018 in Sherwood Breezes Resort Hotel Antalya – Turkey, was the fourth event of MESMAP symposium series on Medicinal and Aromatic Plants. Then, the fifth one was MESMAP-5 symposium, which was organized as joined meeting with ISPBS-5 at Cappadocia on April 24-28, 2019. After scientific evaluation selected full papers of MESMAP-5 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. MESMAP Symposiums provide a platform for herbal medicines, biology, chemistry, plant biotechnology, botany, ethnobotany, phytopharmacology, pharmacognosy, food, agriculture and forestry, phytochemistry and aromatherapy. Afterwards, MESMAP-6 Symposium was organized on October 15-17, 2021 and this symposium was supported TÜBİTAK 2223-B National Scientific Meetings Grant Program. After scientific evaluation selected full papers of MESMAP-6 Symposium were published in MOLECULES, indexed with THOMSON REUTERS. This symposium was the seventh meeting series of MESMAP, and you can find abstracts of all the scientific works presented in MESMAP-7 in this ABSTRACTS & PROCEEDINGS BOOK. We would like to encourage MESMAP-6 participants to submit the full papers to the contracted journals. After scientific evaluation, selected full papers will be published in 'Molecules', 'Annals of Phytomedicine', 'International Journal of Agriculture, Environment and Food Sciences' and 'Current Perspectives on Medicinal and Aromatic Plants (CUPMAP), after scientific evaluation. We are also proud to announce that MESMAP international symposiums are indexed by Web of Sciences Conference Proceedings Citation Index-Science (CPCI-S) / Scopus Index.

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Sincerely,
Symposium Chair

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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:

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KEYNOTE SPEAKER

LIVERWORTS ARE GOOD SOURCES OF BIO- AND PHARMACOLOGICALLY ACTIVE COMPOUNDS

Professor Yoshinori Asakawa

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Abstract

The Marchantiophyta (liverworts) are rich sources of polyphenols, especially, cyclic and acyclic bis-bibenzyls which are rare natural products in the plant kingdom together with bibenzyls as well as the characteristic terpenoids. At present more than 110 bis-bibenzyls have been found in liverworts. They are biosynthesized from dimerization of lunularic acid *via* dihydrocoumaric acid and prelunularin. The structurally unique cyclic and acyclic bis bibenzyls show various biological activities such as antimicrobial, antifungal, cytotoxic, muscle relaxation, antioxidant, tubulin polymerization inhibitory, and anti-oxidant and antitrypanosomal activities and among others. The present paper deals with the distribution of bis-bibenzyls and bibenzyls, and several characteristic *ent*-sesqui- and diterpenoids in liverworts and their structures. Bio- and total syntheses, and biological activities of bis-bibenzyls are also surveyed [1-5].

Keywords: Liverworts, bis-bibenzyls, *ent*-terpenoids, cytotoxicity, antimicrobial, antiviral

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KEYNOTE SPEAKER

HOW MANY DISCOVERIES HAVE BEEN LOST BY IGNORING THE PROPER APPLICATION OF BIOSTATISTICAL METHODS

Professor Irfan Ali Khan

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Abstract

Biostatistics has been defined as the application of the science of statistics to a wide range of problems in biology and medicine. However, the use of biostatistical methods is constantly increasing in sciences which are touching newer horizons every day. The development of theories is closely associated with statistical methods. The doctrine of heredity rests on statistical basis. Therefore, a good understanding of biostatistics is essential as the methods of biostatistics are indispensable tools for the design and analysis of data and in the interpretation of experimental results for dependable conclusions. A preliminary acquaintance will help not only in applying biostatistical methods but also in a better appreciation of their potential value. This article is primarily written to outline the logical basis of the statistical approach to experimental problems, commonly used in agricultural, biological and medical experimentations.

The main purpose of this article is to provide non-biostatisticians with the ability to understand and utilize basic biostatistical concepts and tools and to facilitate their capacity to seek and utilize biostatistical expertise as may be required when conducting their own research or reviewing that done by others. Many statistical techniques are used to analyses data, but learning just a few of the basic and not widely used approaches will allow you to produce meaningful data analysis. These techniques fall into two general categories; descriptive and inferential. They can serve many purposes: to summarize the data in a simple manner, to organize it so it is easier to understand and to use the data to test theories about a larger population.

Therefore, this topic is divided into the following sections, to understand the applications of different statistical methods and to solve the unsolved problems, arise at different stages while dealing with the subject.

KEYNOTE SPEAKER

EXPERIMENTAL THERAPEUTIC APPROACH TO AUTISM SPECTRUM DISORDER FROM KAMPO MEDICINES IN A NEUROSTEROID DEFICIENCY MODEL

Professor Kinzo Matsumoto

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Abstract

Autism spectrum disorder (ASD) is a developmental disorder with sociability deficits and restrictive, repetitive behaviors, core symptoms of ASD. The prevalence of ASD is about 4-times higher in males than in females. Dysfunction in the GABAergic and dopaminergic systems in the brain is reportedly implicated in the pathophysiology of ASD. However, because the exact cause of ASD remains unclear, no appropriate therapeutic drugs for ASD have been developed yet.

We have investigated the physiological roles of neurosteroid allopregnanolone (ALLO) for many years. ALLO is a positive allosteric modulator of the $GABA_A$ receptor and biosynthesized from progesterone by the type I 5α -reductase and 3α-hydroxysteroid dehydrogenase activities. In our recent studies, SKF105111 (SKF), a type I 5α -reductase inhibitor, reduced brain ALLO levels and thereby induced ASD-like behaviors in male mice, but it did not show such effects in female mice. These findings allowed us to hypothesize that ALLO deficiency may cause ASD and provide an animal model of ASD.

Using this model, we elucidated the effects of Kamishyoyosan (KSS), a Kampo formula that has been long used to treat neuropsychiatric symptoms, particularly in the female. We found that KSS could mitigate ASD-like behaviors by facilitating GABAergic and dopaminergic systems. Moreover, female mice, which were insusceptible to SKF, exhibited ALLO deficiency and ASD-like behaviors after ovariectomy (OVX). OVX-induced ASD-like behaviors were also mitigated via the facilitation of GABAergic and dopaminergic systems by KSS administration. It is unclear which herbs or chemical constituents of KSS account for the effects. However, our findings suggest that KSS is beneficial for the treatment of ASD and that Kampo medicines like KSS have therapeutic potentials for ASD.

Key Words: Allopregnanolone, autism spectrum disorder, experimental therapy, animal model, Kampo medicines, Kamishyoyo-sa

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INVITED SPEAKER

NATURAL COMPOUNDS INDUCE IMMUNOGENIC CELL DEATH IN CANCER

Professor Marc Diederich

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Abstract

Apoptosis and autophagy were traditionally considered as the most prominent cell death or cell death-related mechanisms [1]. By now multiple other cell death modalities were described and most likely involved in response to epigenetic treatments. It can be hypothesized that especially necrosis-related phenotypes triggered by various treatments or evolving from apoptotic or autophagic mechanisms, provide a more efficient therapeutic outcome depending on cancer type and genetic phenotype of the patient. In fact, the recent discovery of multiple regulated forms of necrosis [1, 2] and the initial elucidation of the corresponding cell signaling pathways appear nowadays as important tools to clarify the immunogenic potential of non-canonical forms of cell death induction [3, 4]. This presentation will cover the effect of epigenetically active compounds and highlight their activity leading to non-canonical or immunogenic cell death [5-10].

Keywords: Natural compounds; cell death; immunity

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INVITED SPEAKER

AROMATIC PLANTS AS ANTIBACTERIALS – MORE THAN INHIBITORS OF BACTERIAL GROWTH

F. Bucar[1*], K. Šimunović[2], D. Ramić[2], A. Klančnik[2], F. Alperth[1], O. Kunert[3], S. Ochensberger[1], M. Waditzer[1], S. Smole Možina[2]

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Abstract

Antimicrobial resistance has become a major cause of concern for public health. In several collaborative projects, preparations of aromatic plants were characterized phytochemically and evaluated for antibacterial activity. Aside from studying direct antimicrobial effects including search for synergism, also inhibition of bacterial cell adhesion or resistance modulatory activity have been evaluated. In a recent study, essential oil as well as waste material from the hydrodistillation of *Lavandula angustifolia* had particular anti-biofilm effects. RNAseq data provided new insights into the influence of the essential oil on gene expression in *Campylobacter jejuni* [1]. *Satureja montana* extracts revealed antimicrobial activity by influencing bacterial efflux pumps, similar effects were discovered for *Curcuma zanthorriza* [2,3,4]. Extracts and essential oil of the roots of *Peucedanum ostruthium* disturbed *Campylobacter jejuni* membrane integrity at concentrations far below their respective minimum inhibitory concentrations. Furthermore, anti-QS and anti-adhesion activity could be confirmed for *Juniperus communis* fruit extracts and essential oil [5,6].

Key Words: Antibacterial, anti-adhesion, efflux inhibition, *Satureja*, *Lavandula*, *Juniper*.

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INVITED SPEAKER

ON THE SOME REMARKABLE FEATURES OF HESPERIDIN

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Abstract

Hesperidin (HES) represents a type of flavanone glycoside mainly found in citrus fruits. It has been reported that it has to scavenge free radicals it reveals its antioxidant property. Since Hesperidin is known for its antioxidant, anti-inflammatory, and neuroprotective effects and provides modulation drug-metabolizing enzymes without toxicity this presentation consists of both *in silico* and *in vivo* research results (summaries of a couple of published papers by our studies) on different aspects mentioned above. Due to its protective effects in a wide range of applications, we could suggest that the HES has a promising molecule to overcome many damages including cells to organs in living organisms.

Key Words: Hesperidin, *in silico*, *in vivo*, radiation protection, pre-eclampsia, epilepsy

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INVITED SPEAKER

PHYTOCHEMISTRY OF SOME ALKALOID CONTAINING PLANTS FROM AZERBAIJAN

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Azerbaijan has a rich flora, more than 4,500 plant species have been registered in the country. We have investigated different group of compounds in the *Datura innoxia, D. stramonium, D. stramonium* var. *tatula*, *Peganum harmala* and *Thalictrum minus* samples from Azerbaijan. All samples were investigated for their alkaloidal compounds first. Alkaloids were extracted with 95% ethanol, alkaloid-rich extracts were prepared by using acid-base extraction method. Flash column chromatography and preparative Thin layer chromatography (TLC) were applied for isolation of pure substances. Alkaloids of each species were determined by GC-MS, HPLC-UV, LC-MS, UV-, IR-, NMR-spectroscopy methods. *Datura* species *(Solanaceae)* are known as essential sources of tropane alkaloids. Plants possess anti-asthmatic, analgesic, spasmolytic, mydriatic effects [1]. Atropine, scopolamine, apoatropine, anisodamine, aposcopolamine, norhyoscyamine, norapoatropine were identified as major alkaloids in the *Datura* extracts by performing GC-MS method. Scopolamine was observed as a major compound in the *D. innoxia* seeds, isolated and identified with ^{1}H and ^{13}C NMR spectroscopy. As a result of quantitative analysis with HPLC-UV method, 0.32 mg/g and 4.68 mg/g atropine were observed in the aerial parts of *D. innoxia and D. stramonium* respectively. Isomers of six 306/124 - tropane derivatives were identified by LC-MS method in the alkaloid-rich extract of *D. stramonium* var. *tatula*. In folk medicine of *P. harmala (Nitrariaceae)* have been used as a remedy due to analgesic, anti-inflammatory, antiseptic and diuretic properties. The plant contains of indole and quinazoline group alkaloids [2]. *L*-vasicine, 4-(3-propynyloxy)quinazoline, vasicinone, harmine, harmol were observed as major alkaloids in the GC-MS analysis of plant extracts. The highest concentration of alkaloids is noted in seeds. Three alkaloids were isolated and identified as harmaline, harmine and vasicine based on UV, IR and NMR spectroscopy data.
T. minus L. (*Ranunculaceae*) is widely spread around the Greater Caucasus Mountains. Plant has been used as a folk medicine for treating dysentery, bedsore, fungal infection and lung inflammation. Berberine has been isolated from plant root and confirmed with ^{1}H and ^{13}C NMR spectroscopy.

Key Words: *Datura, peganum, thalictrum,* alkaloids, flora of Azerbaijan

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INVITED SPEAKER

MYRICETIN ALLEVIATES RAW 264.7 MACROPHAGES DAMAGE INDUCED BY HIGH GLUCOSE LEVELS

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Abstract

Myricetin is a natural polyhydroxyflavonoid, which can be extracted from the bark of bayberries (Myrica spec.) and in the leaves and stems of vine tea (*Ampelopsis grossedentata*). Previous research has shown that myricetin and its derivatives possess several pharmacological activities Chronic high blood glucose level represent has a major adverse impact on human health, including immune function. The purpose of this study was to assess how high glucose levels impede immune functions and further whether dietary polyphenols i.e., myricetin can restore immune functions. Our results showed that cell viability of macrophages RAW 264.7, endocytosis and phagocytosis of macrophages were significantly inhibited under high glucose levels. Moreover, the secretion of inflammatory cytokines was also significantly decreased, while the level of cellular reactive oxygen species (ROS) was significantly increased. Treatment with myricetin, significantly improved cell viability, endocytosis and phagocytic abilities and restored the secretion of inflammatory cytokines. In addition, the level of ROS was reduced. The finding of this study reinforces that long-term hyperglycemia could impair the body's immune function, where myricetin could be used as an immunomodulator to enhance the immune functionality and the mechanism may be related to the regulation of the immune-related genes Malt1, Gpr183, Kdm6a and Tcirg1 as confirmed by the fold-change mRNA expression levels of RAW 264.7 macrophages when treated with myricetin.

Key Words: Myricetin; high glucose; macrophage; immunomodulatory; RNA-seq

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INVITED SPEAKER

ROLE OF ENDOPHYTIC RHIZOBACTERIA IN INDUCING SYSTEMIC RESISTANCE AND BIO-CONTROL OF FUNGAL PATHOGENS OF MEDICINAL PLANTS

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Abstract

Increasing the productivity of medicinally important plants under present climate scenario relies on adopting sustainable agriculture practices. Moreover, Global concern over the demerits of agrochemicals has diverted the attention of researchers towards the utilization of multiple potentials of plant growth promoting rhizobacteria (PGPR) for sustainable and chemical free farming of medicinal plants. PGPR are root colonizing bacteria that are involved in plant growth promotion, disease suppression and in imparting disease resistance. The important medicinal plants such as *Chlorophytum borivillianum* and *Withania somnifera* used in more than 100 medicinal preparations for a wide range of applications though are abundant in the Indian forests, particularly Satpuda ranges of in North Maharashtra region, however fungal diseases, low germination rate, and traditional cropping methods have hampered their yield. Moreover, no biotechnological inputs have been tried so far for improving the growth and yield of such medicinally and economically valuable crop therefore; the present work was aimed to exploit the *A. feacalis* as a bioinoculant for enhancing the yield and to control the fungal pathogens of these that severely affect the growth and yield of these medicinal plants. Application of antibiotic-producing Pseudomonads that also secrete defense-inducing enzymes is known to impart resistance to medicinal plants. Here, we report induction of the induced systemic resistance by six strains of rhizosphere fluorescent Pseudomonas possessing 2,4-DAPG antibiotic genes against fungal phytopathogen, isolate EP5 (*P. fluorescence*) showed 76.5% inhibition of fungal pathogens and promoted seed germination (96.6%), mean root length (15.3 cm), shoot length (12.6 cm) and vigor index (2104.9) in *C. borivillianum*. Challenge inoculation with fungal pathogens gave higher activity of peroxidase (PO), polyphenol oxidases (PPO), phenylalanine ammonia lyase (PAL) and transcinnamic and caused induction of induced systemic resistance.

Keywords: Bioocntrol; Induced Systemic Resistance; Defense related enzymes

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INVITED SPEAKER

ANTHOCYANINS AS PROTECTIVE AGENTS AGAINST METABOLIC SYNDROME-RELATED DYSFUNCTIONS: POTENTIAL MECHANISMS UNDERLYING PREVENTIVE AND THERAPEUTIC BENEFITS

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Abstract

Metabolic syndrome (MetS) is the name for a group of risk factors which severely increases the risk of type II diabetes and cardiovascular diseases. Current evidence supported the potential of natural bioactive compounds to alleviate MetS components. Due to their multiple properties, anthocyanins (ACN), a class of polyphenolic compounds widely found in several Mediterranean medicinal plants, provided evidence of protective effects in metabolic-related disorders contributing to vascular homeostasis, reducing hypertension, inflammation and platelet aggregation, and also improving insulin resistance and dyslipidemia. Additionally, ACN can reduce fat accumulation in adipose tissue decreasing oxidative stress and inflammation and mitigating adipocytokine dysregulation. However, it is now considered that the in vivo beneficial effects of these phytochemicals are unlikely to be explained just by their antioxidant capability. Several plant antioxidants exhibit hormetic properties, by acting as "low-dose stressors" that may prepare cells to resist more severe stress. The discovery of specific genes regulated by Antioxidant Responsive Element – ARE (HO-1, NQO1, gGCS) and pathways (Nrf2 mediated adaptive response) affected by antioxidants, led to the hypothesis that ACN may act as modulators of gene regulatory and signal transduction pathways. Furthermore, ACN positively regulate glucose transporters density and function in both skeletal muscle and adipose tissues via the insulin signaling pathway and induce switching of the cells from an anabolic to a catabolic state through the upregulation of AMPK signaling. Here, the ACN-mediated protective effects against obesity, type 2 diabetes and vascular dysfunction will be discussed focusing on the underlying molecular mechanisms.

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INVITED SPEAKER

NOVEL NATURAL PRODUCTS FROM ALGERIAN MEDICINAL AND AROMATIC PLANTS

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Abstract

Algeria is one of the most original Mediterranean countries by extreme ecological diversity in bioclimatic, morphology, vegetation, and by a Saharan climate in the south. The first African country by area of about 2 million km², Flora of Algeria (spontaneous or native plants) is full of thousands of botanical species belonging to the area of the Flora of North Africa. It consists of a part of the Mediterranean Plant and is a subset of the Flora of Africa.

Algeria is very rich in various natural herbs because of its vast expanses and its many climates: marine, continental, desert and particularly well known for medicinal and aromatic plants; it is necessary to investigate the medicinal value of Algerian medicinal and aromatic plants which might have the tendency to influence human biochemistry since plant species being used as traditional herbal medicine. This review summarizes studies and ethnobotanical information on plants and their constituents used for the treatment of infections caused by different diseases. These results are a very important source of information for the studied area and for the national medicinal flora, which could be a source and database for phytochemical and pharmacological research.

Key Words: Ethnobotany, Medicinal plants, Use value

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INVITED SPEAKER

CHEMICAL CHARACTERIZATION AND BIOPHARMACEUTICAL PROPERTIES OF THREE FRUITS FROM CÔTE D'IVOIRE

Kouadio Ibrahime Sinan[1], Mohamad Fawzi Mahomoodally[2], Nabeelah Bibi Sadeer[2], József Jeko[3], Zoltán Cziáky[3], Annalisa Chiavaroli[4], Lucia Recinella[4], Sheila Leone[4], Simonetta Cristina Di Simone[4], Luigi Brunetti[4], Giustino Orlando[4], Luigi Menghini[4], Claudio Ferrante[4], Gokhan Zengin[1*]

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Abstract

The aim of the present study was to quantify bioactive compounds, determine antioxidant activity and enzyme inhibitory effects of the fruit extracts of *Ficus sur* Forssk., *Gardenia ternifolia* Schumach. & Thonn. and *Uapaca togoensis* Pax prepared from polar solvents (water and methanol). The extracts were tested for total phenolic and flavonoid content, and further screened for phytochemical characterization using HPLC-MS/MS. Antioxidant activities were established using different test systems. α-glucosidase, α-amylase, tyrosinase Acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) were used in the enzyme inhibition assays. Toxicological properties of the extracts were assayed, as well. Additionally, the viability of human prostate cancer PC3 cells was evaluated after treatment with the extracts. Results showed that the aqueous extract yielded the highest TPC (111.19 mg gallic acid equivalent/g) and the methanolic extract the highest TFC (10.87 rutin equivalent/g). HPLC-MS/MS identified 47 compounds in the methanolic extracts of *U. togoensis*, 45 in *F. sur* and 24 in *G. ternifolia*. The aqueous extract of *F. sur* possessed the most potent radical scavenging ability and highest reducing potential. The methanolic *F. sur* extract showed the highest chelating activity (19.58 mg EDTAE/g) and the methanolic extract of *U. togoensis* displayed the highest total antioxidant capacity (3.33 mmol TE/g). *F. sur* was the strongest enzyme inhibitor compared to the other fruits. For instance, the aqueous extract of the fruit significantly depressed AChE activity (2.45 mg GALAE/g) while the methanolic extract was observed to exhibit strong inhibition against BChE (3.12 mg GALAE/g), tyrosinase (72.38 mg KAE/g), α-amylase (0.49 mmol ACAE/g) and α-glucosidase (0.89 mmol ACAE/g). However, *F. sur* were also the most toxic as revealed by the higher grade di heart rate reduction, in the *D.* magna model, and by the increased viability of prostate cancer cells. Further pharmacological screening should be conducted to ascertain the effectiveness of the fruit of *F. sur*.

Key Words: Medicinal plants; bioactive compounds; tyrosinase; α-amylase; antioxidants

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INVITED SPEAKER

INTRODUCING OF 'SPINO' AS NEW DOMESTICATED WILD SPINACH WITH HIGH MINERALS AND MEDICINAL BIOMOLOECULES

Assist. Prof. Dr. Ali Ammarellou

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Abstract

With the increasing of world population, the need for new food and medicine resources is inevitable. The germplasms of domesticated and cultivated plants will not be enough to provide human food and related requirements in future time. Orphan plants, which are potentially nutritious and have the potential to diversify food sources, belong to group of future food sources. After 12 years of field research; Greenhouse and plant breeding experiments based on mass selection program; a type of wild spinach was selected during the breeding process and its nutritional and phytochemical properties were studied. Based on morphological and physiological markers, this selected and developed variety was identified with high mineral content, especially maximum iron, as well as specific medicinal secondary metabolites compared to ordinary and cultivated spinach (*Spinacia oleracea* L.). This new vegetable was registered and introduced for the first time in the field of medicinal plants in Iran under the name of Spino.

Keywords: Medicinal plants, Orphan plants, Future food, Spinach.

INVITED SPEAKER

NEUROPROTECTIVE POTENTIAL AND PHYTOCONSTITUENTS OF THAI TRADITIONAL HERBAL FORMULA "KLEEB BUA DAENG" AGAINST NEUROPSYCHIATRIC DISEASES AND NEURODEGENERATIVE DISEASES

Assist. Prof. Dr. Orawan Monthakantirat

Abstract

Brain disorders such as neuropsychiatric and neurodegenerative diseases, have become a major problem in recent years. Anxiety and depression are neuropsychiatric disorders that mainly result from intense interpersonal relationships. Many reports indicated that the underlying pathogenesis of brain disorders involve various mechanisms such as control of functional disturbance of oxidative stress, neuroinflammation, neurotransmitter dysfunction, and neurotrophic factors also contribute to the pathogenesis of anxiety and depression. Therefore, treatments targeting these mechanisms may achieve effective therapeutic. However, the symptoms of brain disorder often are overlapped among brain disorders. For instance, cognitive decline, slow and involuntary movements, progressive dementia, and changing of personality are the common symptoms of AD. Nevertheless, psychological disorders, anxiety and depression are secondary changes and are associated with neurodegenerative diseases. Since AD is a multifactorial disorder, but none of a single drug acts as multiple functions. Traditional medicine contains multiple chemical constituents, which are more effective than single chemicals in addressing the pathogenesis of multifactorial disorders through their effects on multiple targets. Thai traditional herbal formula, Kleeb Bua Daeng (KBD) which consists of three medicinal plants i.e. *Piper nigrum*, *Centella asiatica* and *Nelumbo nucifera* was evaluated on cognitive impairment, anxiety and depression by using unpredictable chronic mild stress (UCMS) mice model and the underlying molecular mechanism of its action. Anhedonia, memory, anxiety and depressive-like behavioral were investigated. The results showed that UCMS induced the anhedonia, cognitive impairment, anxiety, and depression. Daily treatment with KBD formula restored all neurodegenerative symptoms as similar as imipramine and vitamin E. To clarify mechanisms whether KBD formula impairs the stress-related gene, brain-derived neurotrophic factor (BDNF), cAMP response element binding (CREB), serum- and glucocorticoid regulate kinase 1 (SGK1), glucocorticoid receptor (GR), FK506 binding protein 5 (FKBP5), Interleukin 1-beta (IL-1β) IL-6 and tumor necrosis factor-alpha (TNF-α) were assessed by quantitative real time PCR (QPCR). The results revealed that UCMS mice significantly reduced the fold difference relative BDNF, CREB and GR mRNAs expression while the SGK1, FKBP5, IL-1β, IL-6 and TNF-α mRNAs expression were significantly increased when compared with non-stress mice in both of frontal cortex and hippocampus. As well as imipramine treatment, KBD treatment was significantly improved these changes when compared with vehicle treated UCMS group in both of brain regions. The chemical analysis of the KBD extract by high performance liquid chromatography fingerprint method. The present investigation demonstrated that UCMS procedure induced anhedonia, memory and cognitive deficits, anxiety and depression, which is due to the up-regulation of hypothalamic-pituitary-adrenal axis, reduction of neuronal plasticity and neurogenesis, oxidative brain damage and against MAOs activities. Daily treatment with the KBD formula was found to reverse all symptoms in UCMS mice. Therefore, these results confirm the use of KBD formula as an effective traditional medicine for daily stress life event related to learning and memory impairment, anxiety, and depression.

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INVITED SPEAKER

THE INFLUENCE OF TREATED WASTEWATER IRRIGATION ON GROWTH, YIELD AND ESSENTIAL OIL COMPOUNDS OF SICILIAN OREGANO (*Origanum vulgare* ssp. *Hirtum* (Link) Ietswaart)

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Abstract
The use of treated wastewater represents a sustainable cultivation practice for irrigation of aromatic plants. Despite a number of benefits are well-known, the effects of this source of irrigation water on the essential oil yield and quality need to be more investigated. The aims of this study were to assess the effects of freshwater and treated wastewater on plant growth and yield, essential oil yield and composition of oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) and soil characteristics. Treated wastewater was produced by a pilot-scale horizontal subsurface flow constructed wetland system located in a small city of Sicily (Italy). The system had two separated and parallel units providing a total surface area of 100 m^2. The two units were planted with giant reed and umbrella sedge, respectively. An experimental open field of oregano was set up close to the system. Two years and two different sources of irrigation water were tested in a split-plot design for a two-factor experiment. Treated wastewater had higher values of mineral and organic constituents than freshwater, on average. In both years, the short-term irrigation with freshwater and treated wastewater increased plant growth, dry weight and essential oil yield of oregano plants. However, it did not significantly affect the essential oil content and composition in comparison with the control. Furthermore, the two main factors did not significantly determine any variation of the soil chemical composition. On the basis of results, treated wastewater can be considered an alternative to freshwater for the cultivation of oregano due to the fact that it does not greatly influence the yield quality and quantity of this species in the short-term.
Key Words: constructed wetlands, non-conventional water resource, irrigation, oregano, sustainability, Sicily.

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INVITED SPEAKER

SOME UNCOMMON BIOACTIVE METABOLITES FROM *SALVIA* SP. (LAMIACEAE) AND *SCILLA* SP. (LILIACEAE)

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Abstract

The ethnomedical uses that can be identifiable nowadays derive from the experience of local people who have developed remedies against a wide range of diseases handing down the knowledge from generation to generation over the millennia. Folkloric medicine represents an eminent source of inspiration in the process of drug development and therapeutic strategies [1, 2]. In order to trap drug lead compounds, the search for new secondary metabolites from plant resources, in general, has long been considered by scientific reports. The genus *Salvia* L. (Lamiaceae) and *Scilla* L. (Liliaceae) are distinguished medicinal herbs in traditional medicine and worldwide ethnomedicine, where the plants are showed prominently various biological activities due to the presence of myriad interesting specialized metabolites including terpenoids, phenolic compounds, and even alkaloids [3, 4]. We herein aim to have a glimpse of the research progress on some uncommon terpenoids especially dammarane-type triterpenes in *Salvia* plants along with some uncommon flavonoids particularly homoisoflavonoids, which are well accepted for potential pharmacological properties such as, such as anti-inflammatory, antifungal, antibacterial, antiviral, antiparasitic, cytotoxic, and enzyme-related Alzheimer inhibitory activities. Thereafter some of our recent researches on Iranian plants, *Salvia russellii* Benth. [5], *Scilla bisotunensis* Speta., and *S. persica* HUSSKEN [6, 7] will be discussed.

Acknowledgments: The author thank University of Kurdistan for logistical supports.

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ORAL PRESENTATION

EFFECT OF OSMOTIC STRESS ON GERMINATION, SEEDLING GROWTH AND PHYSIOLOGICAL PARAMETERS OF *BRASSICA OLERACEA* L.

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Abstract

This study objective was to evaluate the tolerance of *Brassica oleracea* var. *gongylodes* L. to osmotic stress. For this purpose, the osmotic stresses between 0 and -18.05 MPa osmotic potential were obtained using polyethylene glycol-6000 (PEG) solutions. The osmotic potential effect on germination and seedling growth of B. oleracea were determined. Results showed that enhanced osmotic stress led to substantial reduction in seed germination and germination index (%) as well as shoot length, root length and fresh and dry biomass. Seed germination was completely inhibited at -18.55 MPa osmotic potential. This plant supported only moderate osmotic stress up to -1.55 MP. Effects of stress levels (0 to 400 mg/ml of PEG 6000) were also tested in a greenhouse experiment. Increasing PEG concentration decreased strongly the stem diameter and the number, length and area of leaves. At 400 mg/ml of PEG, the shoot dry weight and the root dry weight were significantly reduced compared to control. For all the data, the impact of severe drought was more pronounced than that of moderate drought.

Keywords: *Brassica oleracea* var. *gongylodes* L., osmotic stress, PEG-6000, morphological and physiological parameters.

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ORAL PRESENTATION

ANTIGLYCATION AND ANTIOXIDANT PROPERTIES OF *TRIBULUS TERRESTRIS*

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Abstract

Glycation refers to the interaction between sugars and proteins leading to production of harmful advanced glycation end-products (AGEs). Many types of diseases such as neurodegenerative, cardiovascular, secondary complications in diabetic patients have been shown to an involvement of these products. This study inclines to investigate the antiglycation and antioxidant potential of aqueous and methanolic extracts of *Tribulus terrestris* (TT). The in-vitro glycation system (BSA and glucose) was incubated along with aqueous and methanolic extracts of TT for 28 days at 37 °C. Standard methods such as browning, NBT assay, DNPH assay and assessment of fluorescent AGEs were carried out spectroscopically and flourimetrically to check the amount of glycation products. Antioxidant activity was also assessed for the aqueous and methanolic extracts. The presence of both the extracts of TT showed the similar decrement in the formation of glycation products. There was a significant inhibition in the formation of glycation induced aggregation of BSA. The extracts of the fruits of TT exhibits potential antiglycation and antioxidant activity in-vitro. It may be used as a therapeutic agent for management of diabetes and its complications.

Key Words: Aggregation, Antioxidant, BSA, Glycation, NBT assays, *Tribulus terrestris*

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We acknowledge the financial support by Research Society for Studies of Diabetes in India (RSSDI).

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ORAL PRESENTATION

IN VITRO AND FIELD EXPERIMENTS ON PLANT CULTIVATION AND PROPAGATION OF GALBANUM (*FERULA GUMMOSA* L.)

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Abstract

Despite advances and scientific and technical developments in the field of chemical drug synthesis, there is still a great need for biological resources to produce a number of original plant secondary metabolites. Being the most common treatment methods in modern medicine and the various side effects of using chemical treatments, people naturally prefer to use herbal remedies. Plants as one of the important sources of medicine have played a key role in the health of the people of the world and many valuable medicinal compounds from plant secondary metabolites are extracted. Iran, with more than 8000 plant species, has a very large variety of plant species that the contribution of rangelands in the formation of this rich genetic bank is very vital and key. Use of medicinal plants as *Ferula gummosa* Boiss by-products of the country's rangelands have a long history. Prolonged propagation and long seed cycle in this plant, effects of continuous scratching and Galbanum irregularity on the crown of the plant (for gum extraction) and the geographical conditions of natural habitats, has caused this plant in the list of endangered plants in world. Therefore, introducing new methods of propagation of this plant and also providing alternative methods of producing medicinal compounds under control conditions is one of the essential needs for maintaining the germplasm of this valuable medicinal and industrial plant. In this paper some *In vitro* and the field experiments on plant cultivation and production of Galbanum (*Ferula gammosa* L.) medicinal gum and its micropropagation will be presented.

Keywords: *Ferula* sp., galbanum, cultivation, propagation.

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ORAL PRESENTATION

ESTABLISH OF A NATIONAL LIVE COLLECTION OF *VIOLA* SP. (LCV) IN IRAN AND ORGANIZING PROGRAM FOR ITS INTERNATIONALITY COVERAGE

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Abstract

The Violaceae comprises 806-1000 predominately tropical and temperate species in 25 currently recognized genera. Viola section is one of the largest groups of the Violaceae family. Infra generic classification has varied, but recent phylogenetic analysis indicates that the genus can be subdivided into two subgenera and 16 sections worldwide. Except the Endemic Iranian species other violet species of Iran distributed in other countries as: Turkey, Syria, Lebanon, Cyprus, Palestine, Iraq, Talysh, Caucasus, W. Siberia, Europe, Mediterranean region, Balkan Peninsula, S. E. European Russia, Palestine, Caucasus, Crimea, Turkmenistan, Uzbekistan, Asia Minor and Central Asia, Arabia Peninsula, Afghanistan, Pakistan, S.E. France, Bulgaria, Hungary, Romania, N. Africa, According to the routine process and standard of plant flora studies, all reports and sources have been compiled and references of published plant flora are based on collected and stored dried and fixed samples in national and international herbariums. This valuable work has its limitations as well as its numerous advantages. Samples collected from different climates and geographical areas, despite the apparent differences in their morphology, can be of the same species and the same variety and genotypes. Due to the fact that most of the identifications and reports of herbarium are based on morphological studies and their physical characteristics and are less identified and classified based on molecular information, so the probability of error in diagnosis will be high. On the other hand, dry specimens of leaves, stems and flowers can not be regenerated, growth and reproduction, and their seeds lose their ability to grow and germinate after a limited time. For these reasons, the creation of Live Plant Collections (LPC) has the more particular importance. In these collections, the collected plant specimens, whether as whole live plants or as seeds, are continuously grown and kept alive in greenhouse and field conditions. Under these conditions, climatic and environmental differences will be minimized and the detection of samples will be very accurate. Simultaneous and comparative studies between different masses will also be possible. In these collections, researchers can access samples and fresh plant tissues in all seasons and any research can be done on them in terms of techniques and statistical requirements and experimental design. The five hot point of Viola sp. habitats were considered in Iran. These 5 important wild habitats included: Gilan, Mazandaran, Golestan, Arasbaran and Northern Alborz chain Mountain. With transferring of 20 ecotypes ofIranian Viola to National Live Collection of Viola (NLCV), the first live collection of Iranian Viola was established since 2020 in University of Zanjan (Research Institute of Modern Biological Techniques). In the next program, we consider live ecotypes of Viola in the other regions, including Turkey, the countries of the North Caspian Sea; Iraq, Russia, China, India, Pakistan and Afghanistan to this collection and internationalize the level and scope of activities. All stages of Viola collection establishing will displayed in this paper.

Keywords: Medicinal and aromatic plants, secondary metabolites, *Viola,* live collection.

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ORAL PRESENTATION

CHANGES OF ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS IN SAFFLOWER ACCORDING TO DEVELOPMENT PERIODS OF SORBITOL FOLIAR APPLICATIONS

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Abstract

Secondary metabolites, which are natural sources of antioxidants, vary according to growth and development periods. Cultural practices have an important role in this change. Sorbitol plays an important role in increasing both phenylalanine ammonia-lyase (PAL) activity in cells and enzymes that are precursors in the biosynthesis of secondary metabolites. The aim of this study was to determine the effects of foliar sorbitol applications (control (water), 5, 25, 50 and 100 g/L) on the total phenolic (TPC), flavonoid (TFC) contents and antioxidant activity of the safflower plant at different growth stages (pre, full and post flowering). The TPCs and TFCs were determined by Folin-ciocalteu and aluminum chloride colorimetric assays, respectively. Antioxidant capacities were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. Among sorbitol applications, treatment with 5 g/L (142.65 mg GAE/g dry weight) pre-flowering and 100 g/L (125.12 mg GAE/g dry weight) full flowering stage significantly increased TPC and antioxidant activity (105.2 µg/mL). Sorbitol treatments did not show a significant change in the total amount of flavonoid. However, when evaluated periodically TFC was the highest (26.044 mg RE/g of dry extract) in the full flowering stage. The results show that sorbitol treatment applied by foliar spray can increase antioxidant capacity and total phenolic content.

Keywords: Bioactive compounds*; Carthamus tinctorius L.;* medicinal plants; osmotic stress.

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ORAL PRESENTATION

A PINITOL-RICH *GLYCYRRHIZA GLABRA* L. LEAF EXTRACT AS FUNCTIONAL SUPPLEMENT WITH POTENTIAL IN THE PREVENTION OF METABOLICALLY INDUCED INFLAMMATION AND INSULIN-RESISTANCE

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Abstract

Glycyrrhiza glabra L. (Licorice) is a small perennial herb that has been traditionally used to treat many diseases. While liquorice roots have been well studied for their pharmacological activities, less research has been conducted on aerial parts. Leaves represent a good source of the inositol D-pinitol, useful in the treatment of insulin resistance-related pathologies. In this study, a methanolic extract from *G. glabra* leaves (GGLME) was prepared and profiled for its polyphenols content. The amount of D-pinitol was also measured by HPLC-ELSD and NMR. The extract was then tested, using two different models, for its in vitro protective effects against insulin resistance induced by palmitic acid (PA), the most prevalent saturated free fatty acid in circulation, comparing its activity with D-pinitol. In the first model the activity against endothelial dysfunction in endothelial cells (HUVECs) was evaluated. GGLME restored tyrosine phosphorylation of IRS-1 and downstream PI3K/Akt/eNOS signaling pathway altered by PA. Next, GGLME activity against lipotoxicity-related hypertrophy, inflammation, and insulin resistance in 3T3-L1 adipocytes exposed to PA was evaluated. GGLME pretreatment decreased PA-induced lipid deposition, PPAR-γ and NF-κB pathway, similarly to D-pinitol, and improved insulin sensitivity increasing PI3K, pAkt, and GLUT1 levels. Interestingly, we demonstrated, in both models, that the extract, as well as D-pinitol, possess insulin sensitizing effects since they induced insulin-PI3K/Akt pathway also under basal conditions. This study confirms that liquorice leaves, considered a waste of resource, gives a phytocomplex endowed with potential therapeutic properties and thus could potentially be reused, and support further in vivo studies on animal and human models. In conclusion, liquorice leaves extract represents a potential candidate for prevention of metabolically induced inflammation, frequently leading to metabolic disorders. Moreover, the use of the phytocomplex, rather than a pure compound, allows avoiding a series of isolation/purification procedures and can be easily scaled up for industrial applications.

Keywords: *Glycyrrhiza glabra* L., insulin resistance, D-pinitol, palmitic acid, adipose inflammation, metabolic disease.

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ORAL PRESENTATION

EFFECT OF A CHRONIC INTAKE OF AN IVORIAN MEDICINAL PLANT EXTRACT ON SERUM ELECTROLYTES AND RENAL BIOMARKERS IN WISTAR RATS

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Abstract

Anthocleista djalonensis A Chev is a plant belonging to the family of loganiaceae and is found in tropical Africa in Madagascar and in the Comoros. All part of this plant is used to treat a variety of ailments on account of their medicinal properties. The effect of hydroethanolic extract of *Anthocleista djalonensis A Chev* on blood electrolytes, and renal biomarkers in Wistar rats. Fifty healthy Wistar rats weighing between 120 to 160 g were assigned to 5 groups of (10) ten rats (5 males and 5 females). Group 1 (control group, received distilled water), group 2 (was administered 400 mg/kg bwt of extract), group 3(received 800 mg/kg bwt of extract), group 4 (was given 1200 mg/kg bwt of extract) and group 5 (satellite, was administered 1200 mg/kg bwt of extract). All treatments were given by oral route for 180 consecutive days. Then, serum electrolytes and renal markers were assayed using standard methods. The results concerning serum electrolytes such as sodium (Na^+); chloride (Cl^-), Calcium (Ca^{2+}); Potassium (K^+) and Magnesium (Mg^{2+}) did not show any significant difference compared to the control groups, notwithstanding the dose administered. Likewise, no significant difference was noticed for renal biomarkers except for uric acid with a significant ($p<0.05$) decrease in male rats. To sum up, the hydroethanolic leaf extract of *Anthocleista djalonensis* A Chev did not entail serum electrolytes imbalance and renal markers disturbance in Wistar rats at experimental doses.

Keywords: Serum electrolytes, *Anthocleista djalonensis*, renal markers.

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ORAL PRESENTATION

DIFFERENCES IN ADJUVANT ACTIVITY OF AN OILY *HIPPOPHAE RHAMNOIDES* EXTRACT IN *AVES* AND *MAMMALIA*

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Abstract

Cell-mediated immune responses represent an important part of adaptive immune defence targeted by vaccination. Furthermore, adjuvants enhance the systemic immunity, improving the antigen presentation. Due to their complex content in biologically active compounds, plants could be an appropriate source for immune adjuvants. This research was performed to evaluate the differences in the local immune enhancing potential of *Hippohae rhamnoides* extract on cell-mediated immunity in two phylogenetically distant species, chickens and rabbits. The intradermal graft rejection test was used in immunologically mature broiler chickens (wattle test) and adult male rabbits (lateral skin), using a xenogeneic lymphocyte suspension (sheep, 10^6cells/ml) in RPMI 1640, injected separately or in combination with an oily sea-buckthorn fruit extract, in separate spots. The increased local responses were used as a means of estimating the adjuvant qualities of the fruit extract, which were statistically interpreted. There was a significant increase ($p<0.001$) after 24, 48 and 72 h in skin responses in rabbits, but not in chickens. The results indicated a different pattern of local response dynamics in the two classes, with lower values and a constant decrease from 24 to 72 h in chickens (156.78 to 121.19%) and higher values and a constant increase in rabbits (160 to 248%) when compared to the initial wattle/skin thickness. The diversity of T-cell subpopulations in the two classes, *Aves* and *Mammalia*, supported the differences in the dynamics in xenogeneic lymphocyte rejection response in the studied species. The oily *Hippophae* extract had a lesser effect on the graft-rejection response in longer term than in short term in birds but not in rabbits, proving to be a stronger immune enhancer for the latter species.

Keywords: Skin test, *Hippophae*, adjuvant, *Aves*, *Mammalia*, immune enhancer.

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ORAL PRESENTATION

JOJOBA CULTIVATION AND INDUSTRIAL USE CASE

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Abstract

Jojoba is an industrial plant in the form of a greyish-green bush, perennial and evergreen. The seeds of jojoba (*Simmondsia chinensis* Link), a member of the Simmondsiaceae family known for its superior quality oil seeds, contain oil ranging from 45% to 60%. The feature that distinguishes it chemically from other vegetable oils is that it has a liquid structure called wax although oils produced from jojoba seeds are triglycerides. Jojoba, which is not cultivated economically in Turkey, has an average of 300 ha cultivation area and 150-ton production in recent years in the world. Although jojoba is a plant grown both by seed and vegetatively, it has been stated that vegetative cultivation is suitable for superior yield. Jojoba can easily grow in barren, arid, saline and sloping lands where other plants do not grow, and thanks to this feature, it is a promising plant. Since jojoba has a very strong and deep root system, it also minimizes the loss of landslides exposed to erosion. Some researchers in Turkey have been determined that the commercial cultivation of jojoba can be carried out in the entire Mediterranean coastline, Southeastern Anatolia and the microclimate areas of the Aegean. Jojoba oil is mostly used in the machinery industry, leather production, cosmetics and pharmaceutical industries. Besides, it is used in wax, disinfectants, detergents, floor polishes, shoes, cars and for the preparation of a large number of different substances. Jojoba, one of the main plants used as a raw material in biodiesel studies because it is a sustainable and renewable energy source. The versatile use of jojoba oil, the need for bioactive substances in the pharmaceutical industry and the fact that the raw materials used in the production of biodiesel are plants that are used as food add another dimension to the subject. Considering that today's rapid population growth, nutrition and health problems come to the fore, it would be appropriate to state that oil production should be prioritized, the bioactive material requirements should be given importance and jojoba cultivation should be supported.

Key Words: Jojoba, *Simmondsia chinensis* Link, industrial plant

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ORAL PRESENTATION

DEVELOPMENT OF NEW MOLECULAR TOOLS TO ASSESS ARGAN OIL AUTHENTICITY: DETECTION OF OLIVE OIL AS A POTENTIAL ADULTERANT

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Abstract

Argan oil is a traditional product obtained from the argan tree (*Argania spinosa* L.), which is endemic only to Morocco. Both cosmetic and food grade argan oil are commercialized worldwide, attaining very high prices in the international market. For that reason, argan oil is very prone to be adulterated, in particular with cheaper vegetable oils. Therefore, it is important to develop methodologies that can be used in control and inspection programs in order to guarantee argan oil authenticity and quality. In particular, there is the need for methodologies that allow the accurate identification of vegetable oils illegally added to argan oil. The present work aims at developing novel approaches based on DNA markers to detect the presence of adulterants, using olive oil as case study. A novel olive-specific PCR assay was developed, enabling the clear detection of 1% (w/w) of olive oil in argan oil. Subsequently, a real-time PCR assay with EvaGreen dye was developed for quantitative analysis in a dynamic range of 1-50%, with acceptable correlation coefficient and PCR efficiency considering the type of food matrix. Both, qualitative and quantitative PCR assays can provide simple, fast and high-throughput tools to detect the presence of adulterant oils in argan oil.

Keywords: *Argania spinosa* L, argan oil, authenticity, adulteration, DNA markers, PCR assay

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ORAL PRESENTATION

ANALYSIS OF THE ANTIOXIDANT PROPRETIES OF *SALIX ALBA* (FAM. SALICACEAE), WROWING IN HIGLY POLLUTED AREA

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Objective/Purpose: Plants in generally and particulary some of their parts are rich sources of a variety of biologically active compounds, including phenolics, which have been found to possess various biological properties, as well antioxidant potentials. Antioxidants are substances that neutralize free radicals and their actions. The main objective of this study was to determine the content of phenolic compounds and antioxidant properties of willow bark of *Salix alba* L. (Fam. *Salicaceae*), which grows along the river stream of Sitnica, around the highly polluted area of Kosovo Thermal Power Plants. Antioxidant properties of the extracts were investigated with total phenolics, flavonoids, chlorophylls and carotenoids. **Material and Methods:** Phenolic compounds were extracted from powdered and dried plant material (0.1 g) with 80% (v/v) CH_3OH in ultrasonic bath for 30 min at 4ºC. Thereafter, methanolic extracts were centrifuged at 12000 rpm for 15 min and the supernatants were used for determination of phenolic compound and chlorophyll contents, as well antioxidant activities. Spectrophotometric analyses were performed on SpectraMax 190 Microplate Reader, supported with SoftMax Pro (v. 5.4.1) software. **Results:** Total phenolics were in range 12.63 – 78.53 mg GAE/g DW, flavonoids 9.25 – 67.47 mg CE/g DW, CHLa 13.73 – 86.28 mg/g FW, CHLb 26.06 – 71.50 mg/g FW, CAR 42.26 – 152.58 mg/g FW. **Conclusion/Discussion:** The results in this study indicate that the examined willow bark samples of *Salix alba* L. (Fam. *Salicaceae*), contain certain amounts of polyphenols and flavonoids, chlorophylls and carotenoids with positive correlation and proving to be perfect sources of antioxidants.

Key Words: willow bark, antioxidants, phenolic compounds, polluted area.

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ORAL PRESENTATION

CORRELATION BETWEEN CYTOTOXIC ACTIVITIES AND METABOLOMIC PROFILES OF SELECTED LAMIACEAE SPECIES

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Abstract

Cytotoxicity evaluation of methanolic extracts from the aerial parts of specified Lamiacea species was performed to compare with their metabolite profiles. 11 species were selected from four genera, *Phlomis* L. (*P. pungens*, *P. sieheana*), *Stachys* L. (*S. annua*, *S. cretica*), *Salvia* L. (*S. cryptantha*, *S. tchihatcheffii*, *S. officinalis*, *S. virgata*) and *Teucrium* L. (*T. chamaedrys*, *T. multicaule*, *T. polium*). Antiproliferative effects of the extracts against A549 cells (human lung adenocarcinoma) were determined by MTT assay [1]. Within the metabolomic profiling study, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography quadrupole time of flight mass spectrometry (LC-qTOF-MS) were used for the identification of their primary and secondary metabolites as well as the most abundant ones responsible for any possible activity. As a result of the activity study, *T. multicaule* and *T. polium* were found to have meaningful cytotoxicity against the tested cell line in comparison with *T. chamaedrys* and other species. 320 secondary metabolites were identified by LC-qTOF-MS analysis and 319 primary metabolites were identified by GC-MS utilizations in all *Teucrium* species. The correlation analysis was undertaken to determine the compounds that were positively or negatively responsible for the activity. 25 secondary and 25 primary metabolites were found to have a highly positive correlation ($r \geq 0.9$) with antiproliferative effect, while 58 secondary and 49 primary metabolites showed a highly negative correlation ($r \leq -0.9$). After this preliminary screening, it is aimed to carry out further studies to determine the main compounds responsible for the activity and to experimentally verify the activity.

Keywords: Lamiaceae, cytotoxicity, MTT, metabolomics, LC-QTOF-MS, GC-MS

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ORAL PRESENTATION

HIPPOPHAE RHAMNOIDES ALCOHOLIC EXTRACT'S POTENTIAL USE AS A NON-SPECIFIC PHAGOCYTOSIS ENHANCER IN COWS WITH CLINICAL MASTITIS

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Abstract

Innate cell-mediated defense mechanisms rely on neutrophils as important actors of phagocytosis and bacteria destruction. Their presence is crucial in controlling the *in situ* infection, especially in high yielding cows. The research was carried out to estimate the potential use in therapy of an alcoholic extract of *Hippophae rhamnoides*, by quantifying the changes of non-specific cell-mediated responses to this extract in cows with clinical mastitis, maintained on two farms with different (intensive and extensive) raising technologies. Randomly selected dairy cows showing clinical signs of mastitis were sampled for blood, further subjected to carbon particle inclusion test using an alcoholic seabuckthorn extract, as a measure of phagocytosis. Phagocytic activity index was then calculated as the difference between the natural logarithms of the optical densities of the phagocytosis divided by time (45 and 15 min respectively). Mastitic agents were identified by Sensititre OptiRead after inoculation of milk samples from the same animals on Mueller Hinton agar. The statistical significance of the differences between the groups was interpreted by Student's t test. *Streptococus uberis* represented the dominant bacteria in the mastitic milk sampled on the extensive farm, while *Staphylococcus aureus*/*E. coli* were present in the milk sampled on the other, involving intenisve raising technology. The spontaneous phagocytosis in non-stimulated samples was similar in animals from both farms, but the response to the *Hippophae* extract, significantly (+132.86%, $p<0.0004$) increased on one of the farms, and not on the other (1.06%, NS). Most likely, the effects of the plant extract on the non-specific cell-mediated responses were conditioned not only by plant composition, but also by a complex of external factors, represented by the raising technology.

Keywords: Subclinical mastitis, phagocytosis, therapeutic use, microbiome, *Hippophae rhamnoides*, dairy cows

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ORAL PRESENTATION

THREE VALUABLE BY PRODUCTS: GRAPE, FIG AND POMEGRANATE KERNELS AS SUSTAINABLE RAW MATERIALS FOR COSMETIC INDUSTRY

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Abstract

Demand for efficient use of resources by minimization of waste products in cosmetic industry are increasing, because they are valuable raw materials for sustainability. For the production of safe and environmentally friendly cosmetic products, the sustainability effects are a consideration at all stages of the life cycle of cosmetic products, and therefore the choice of raw materials deserves more attention. In the cosmetics industry, there has been increasing interest in formulating cosmetic products using alternative ingredients that are considered more sustainable. Thus, this review was purposed to explain the relationship between sustainability and the cosmetics industry, emphasizing the importance of using grape (*Vitis vinifera* L.), fig (*Ficus carica* L.) and pomegranate (*Punica granatum* L.) seeds as sustainable raw materials in the cosmetics industry. *'Sustainability can be beautiful and beauty can be sustainable.'*

Keywords: Sustainability, Raw Material, Kernel, Environment Friendly, Green Cosmetics, Innovation

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ORAL PRESENTATION

PHYTOCHEMICAL AND PHARMACOLOGICAL EFFECTS OF TWO INDUSTRIAL HEMP VARIETIES FROM ITALIAN CULTIVATION

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Abstract

In the present study, the phytochemical and pharmacological properties of the essential oils (EOs) from the inflorescences of two industrial hemp varieties, namely Kompolti and Tisza, were studied. EO composition was determined for measuring the levels of terpenes and terpenophenols, and their effects on hypothalamic HypoE22 cell viability were investigated. In hypothalamic HypoE22 cells, neurotransmitter levels were also measured, while the EO mycostatic properties were explored towards different dermatophytes species. The prominent terpenes were iso-caryophyllene, α-humulene and β-caryophyllene oxide in both Kompolti and Tisza EOs, whereas cannabidiol and cannabigerolic acid were the main terpenophenols, respectively. EOs showed antimycotic effects, for which β-caryophyllene oxide could be partly responsible, while cannabidiol could contribute to the stimulation of hypothalamic serotonin release by Kompolti EO. Overall, the present findings highlight pharmacological properties of Kompolti and Tisza EOs, which deserve further investigations and strengthen the interest in industrial hemp inflorescences as valuable source of bioactive extracts and biomolecules.

Key Words: Industrial hemp; phytochemical profile; neuromodulatory effects; antimycotic effects

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ORAL PRESENTATION

IMPROVING THE BIOLOGICAL PROPERTIES OF PLANT EXTRACTS BY THE SYNTHESIS OF METAL NANOPARTICLES

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Abstract

Lithrum salicaria is an herbaceous plant from Lythraceae Family used in folk medicine as aqueous extract for antiseptic, anti-diahorea and astringent effects. The aim of our study was to obtain metal nanoparticles with optimized biological properties. The aerial part of the *Litrium salicaria* was used to obtain the alcoholic and aqueous extract. For the two extracts the polyphenol content, expressed in mg gallic acid/mL extract, was determined by a spectrophotometric method. The aqueous extract contains 2.0393 mg polyphenols/mL. The aqueous extract was used to obtain zinc nanoparticles and silver nanoparticles by green synthesis. This method was chosen because eco-friendly solvents are used. The nanoparticles were physico-chemically characterized by UV-Vis spectrophotometric analysis, FTIR, DLS and TEM method. The antioxidant properties of the extracts and nanoparticles were evaluated by the ferrous ion chelating test, lipoxygenase inhibition test and by the hydroxyl scavenger test. The ethanolic extract showed a higher chelating capacity of the ferrous ion compared to the aqueous one, so the EC_{50} value was 70.95±0.08 µg/mL and 79.58±0.41 µg/mL, respectively. Zinc nanoparticles are twice as efficient in the ferrous ion chelating test as silver nanoparticles. In the lipoxygenase inhibition test, the ethanolic extract was more efficient compared to the aqueous one, the EC50 value being 14.83±0.82 µg/mL. Lipoxygenase inhibition is four times stronger when silver nanoparticles are used compared to zinc nanoparticles. The study showed that metal nanoparticles obtained from plant extracts have more intense biological properties compared to extracts and can be used for new applications in the pharmaceutical field.

Key Words: *Lithrum salicaria*, nanoparticles, antioxidant

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ORAL PRESENTATION

MORPHOLOGICAL AND QUALITATIVE EVALUATION OF CLARY SAGE POPULATIONS FROM SICILY (ITALY)

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Abstract

Clary sage (*Salvia sclarea* L.), known for its aromatic and medicinal properties, belongs to the *Lamiaceae* family. Although the species grows wild throughout Sicily, knowledge of its production and qualitative properties is limited. *Salvia sclarea* L. is an important industrial crop, valued for its herbal-aromatic properties and high-quality essential oils, that is used in food, pharmaceuticals, cosmetics. The aim of this study was to evaluate the agronomic behavior of the species over two years of testing and to characterize the chemical properties of its wild counterparts to identify the most promising accessions for cropping or for use in breeding programs. In three of the evaluated accessions were found the highest yields of flower spikes and the highest values of the number of stems, number of branches and length of the inflorescences. For these tree populations, the composition of essential oils extracted from primary and secondary inflorescences using steam distillation was assessed. The three populations were linalyl acetate/linalool chemotype. Highly significant variations were found for the effective local population and inflorescence type in the composition of the essential oil principal components. In particular, the primary inflorescences were found to be accumulation sites favoured by monoterpenes, and secondary inflorescences were favoured by sesquiterpenes and sclareol. Populations “S. Stefano Quisquina” and “Alcara Li Fusi” performed best on a morphological and production level, whereas populations “Prizzi”and “Alcara Li Fusi” performed best in terms of quality. Population “S. Stefano Quisquina” produced high levels of sclareol. Biotype selection from within the populations should be based on both morphological, production and quality analyses.

Key Words: *Salvia sclarea*, populations, essential oils, quality, yield.

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ORAL PRESENTATION

ASSESSMENT OF THE IN VITRO CYTOTOXIC EFFECTS OF *TANACETUM HARADJANII* (Rech.fil.) Grierson

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Abstract

Recently, cancer is the second cause of death after cardiovascular diseases. Investigation of new cytotoxic compounds in cancer treatment is very popular. Studies show that plant-derived compounds and/or extracts are preferred because they are both effective and have fewer side effects in the treatment. *Tanacetum* L. species are traditionally used for many purposes as well as in folk medicine for their antifungal, antitumor and antimicrobial activities. *Tanacetum haradjanii* is an endemic species belonging to this genus and studies on this species are limited. The aim of this study is to reveal the cytotoxic effects of *Tanacetum haradjanii* extracts prepared with different solvents (methanol and hexane). MTT test was used to determine the cytotoxic effect, and the effects of the extracts were evaluated as % viability. As a result, it was determined that the extracts showed dose and time-dependent cytotoxic effects on the applied cell lines. This study is a pioneer for future studies on the species, and it is necessary to elucidate cell death mechanisms with molecular studies.

Key Words: Deli pireotu, endemic, MTT assay, Turkey.

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ORAL PRESENTATION

A STUDY OF MEDICINAL HALOPHYTES NATIVE TO THE GULF OF SAROS

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Abstract

Halophytes grow in a wide variety of saline habitats, from coastal regions and salt marshes to inland deserts and steppes. They can tolerate harsh environmental conditions, including high salinity, high temperatures, and humidity. These plants thrive in conditions that would be lethal to most glycophytes which makes them attractive to researchers. Halophytes living in these extreme environments have to deal with extreme changes in salinity level, that can be done by developing adaptive responses including the synthesis of several bioactive molecules. Some of them are phenolic compounds known for their biological activities. Due to their high content in bioactive compounds, they display potent antioxidant, antimicrobial, anti-inflammatory, and anti-tumoral activities, and therefore represent key-compounds in preventing various diseases and ageing processes. In many regions of Turkey, plants are often subjected to severe environmental conditions that influence the production of some secondary metabolites involved in stress defence mechanism. The Saros Gulf region is also characterized by higher than average seawater salinities and calcareous saline-sodic soil compared to other regions of Turkey. Various species of halophytes grow naturally in the Saros Gulf region. Some of these native species include *Salicornia europae* L., *Salsola kali* L., *Plantago lagopus*, *Limoniastrum monopetalum* (L.) Boiss., *Suaeda maritima* (L.) Dumort., *Xanthium strumarium* L., *Eryngium maritimum* L., *Limonium narbonense* Mill., *Anchusa calcarea* Boiss. This study highlights the importance of these medicinal halophytes as promising sources of natural antioxidants, which can be used for multiple medicinal and industrial applications. Moreover, these saline lands with these medicinal resources can provide industrial raw material of bioactive natural products, which can be used to replace harmful synthetic derivatives from food, pharmaceutical and cosmetic industries.

Key Words: Halophyte, medicinal, saros gulf, saline, bioactive compounds.

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ORAL PRESENTATION

PHYTOCHEMICAL ANALYSIS OF AERIAL AND UNDERGROUND PARTS OF *URTICA DIOICA*

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Background: *Urtica dioïca* is a perennial herbaceous plant of the Urticaceae family is a spontaneous herb. The leaves of *urtica dioica* are rich in flavonoids, phenolic compounds, organic acids, vitamins and minerals. The root contains the lectin called Urtica Dioica Agglutinin (UDA), polysaccharides, sterols, terpene derivatives, fatty acids and lignans. This species is renowned for its therapeutic properties.
Methods: Phytochemical tests were carried out on aqueous and hydroalcolic extracts (water/ethanol and water/diethylic ether) obtained after maceration of *Urtica dioica* leaves, roots and luminaries. The identification of the presence of polyphenols, flavonoids, tannins, coumarins, etc. was carried out according to specific protocols.
Results: Phytochemical tests showed the presence of flavonoids and coumarins in the leaves and stems. As well as the presence of alkaloids, terpenoids and amino acids at the roots.
Conclusion: The results suggest that our samples of *Urtica dioca* are rich in flavonoids, coumarines, terpenoids which are renowned for their therapeutic effects.

Keywords: *Urtica dioica*, phytochemical tets, flavonoids, coumarins, terpenoids

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ORAL PRESENTATION

ANTIBACTERIAL ACTIVITY TESTING OF NATURAL EXTRACTS; ESSENTIAL OIL, PHENOLS AND ALKALOIDS OF SOME MEDICINAL PLANTS AND FUNGUS AGAINST MULTIRESISTANT DRUG BACTERIA

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Abstract

In order to search for new antibacterial molecules, different studies are targeting herbal medicine, hence the biodiversity of medicinal plants and the large number of secondary metabolites extracted. This research was done to find out which compounds had antibacterial activity, as well as to determine the activity using minimum inhibitory concentrations of biologically active metabolites. Essential oils were extracted using a Clevenger apparatus, phenols were obtained by ethanolic and methanolic maceration followed by evaporation with a Rotavap and alkaloids were obtained utilizing a very exact process. The alkaloid extracted from *Ruta graveolens* and *Rhanterium adpressum* essential oil were tested on three bacteria isolated from Laghouat hospital, *Staphylococcus aureus*, *Aeromonas hydrophilia, Klebsiella pneumoniae*. Antibacterial activity was determined using the disk diffusion method, which involved performing a dilution series (neat, 1/2, 1/10) to test sensitivity and the liquid microdilution procedure to determine minimum inhibitory concentrations. By opening the discs for the pure concentration without dilution of the extract, the alkaloid of *Ruta graveolens* inhibits the two bacteria *Klebsiella* and *Aeromonas*; nevertheless, the bacterium *Staphylococcus aureus* is resistant to the alkaloid. The microdilution method revealed the confirmation, with the Minimum inhibitory concentrations being the initial concentration for the two bacteria, with a value of 54.2 mg / ml. In fact, *Rhanterium adpressum* essential oil has a 10 mm inhibition zone and a minimum inhibitory concentration of 397.1 µg / ml against *Staphylococcus. Ruta graveolens* alkaloid and *Rhanterium adpressum* essential oil may be suitable for treating multidrug-resistant Enterobacteriaceae and Methicillin-resistant *Staphylococcus aureus.*

Keywords: *Ruta graveolens* alkaloid, multidrug resistant bacteria, antibacterial activity, inhibition, *Rhanterium adpressum* essential oil.

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ORAL PRESENTATION

EFFECT OF PLANT AGE ON ESSENTIAL OIL CONTENT AND COMPOSITION OF *SALVIA FRUTICOSA* (MILL.)

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Abstract

Salvia fruticosa species known as Anatolian sage is widely used in many sectors like food, cosmetic, medicine etc. The essential oil content and composition are main guiding properties for determining usage area for this plant. The essential oil content and composition can vary according to many factors like plant cultivar and age. This study was carried out to determine the effect of plant age on essential oil ratio and component ratios for the sage oil. Clonally developed UYSAL and TURGUT candidate cultivars were used in the study. The essential oil content of the cultivars for 5 years was determined by using clevenger apparatus. Essential oil composition was analyzed by GC-MS/FID device using a capillary column. There were significant differences in essential oil composition with respect to age of plant for each cultivar. While, 1,8-cineol varied between 51.63-64.74% over the years for Uysal cultivar, it changed between 47.16-65.55% for Turgut cultivar. β-pinene reached the maximum level in last year for Uysal (14.16%) and Turgut (14.95%) variety. Camphor, one of the main components of sage, was determined as the highest for 2 years old plants for both cultivars, and the lowest value was obtained in 5 years old plants. As a result, the age of the plant for the sage was quite effective on essential oil content and composition.

Keywords: Sage, *Salvia fruticosa*, cultivar, plant age, essential oil.

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ORAL PRESENTATION

ANTIOBESITY EFFECTS OF AN EDDIBLE HALOPHYTE *NITRARIA RETUSA* FORSSK IN 3T3-L1 PREADPOCYTE DIFFERENTIATION AND IN C57B6J/L MICE FED A HIGH FAT DIET-INDUCED OBESITY

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Abstract

Nitraria retusa is a North African halophyte of the Nitrariaceae family well adapted to the arid climate thanks to its fleshy leaves. It is an edible plant, used in Tunisia for several traditional medicine purposes. The present study investigated the antiobesity effects of *Nitraria retusa* ethanol extract (NRE) in 3T3-L1 cells using different doses and in high-fat diet-induced obesity in mice. Male C57B6J/L mice were separately fed a normal diet (ND) or a high-fat diet (HFD) and daily administrated with NRE (50, 100 mg/kg) or one for 2 days with Naringenin (10 mg/kg). NRE administration significantly decreased body weight gain, fat pad weight, serum glucose, and lipid levels in HFD-induced obese mice. To elucidate the mechanism of action of NRE, the expression of genes involved in lipid and carbohydrate metabolism were measured in liver. Results showed that mice treated with NRE demonstrated a significant decrease in cumulative body weight and fat pad weight, a significant lowering in glucose and triglycerides serum levels, and an increase in the HDL-cholesterol serum level. Moreover, mRNA expression results showed an enhancement of the expression of genes related to liver metabolism. Our findings suggest that NRE treatment had a protective or controlling effect against a high fat diet-induced obesity in C57B6J/L mice through the regulation of expression of genes involved in lipolysis and lipogenesis and thus the enhancement of the lipid metabolism in liver. This effect may be due to the improvement of the antioxidant status within hepatic cells by the strong antioxidant activities of many phenolic components present in NRE especially flavonoids such as isorhamnetin aglycones and glycosides.

Key Words: *Nitraria retusa*, antiobesity effect, 3T3-L1 cells, High fat diet-induced obesity, lipid metabolism

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ORAL PRESENTATION

PLANT ESSENTIAL OILS AS AN ALTERATIVE THERAPY OF INFECTIOUS PODODERMATITIS IN SHEEP

Gheorghiță Duca[1,*], Mariana Rusu[1], Emöke Páll [1,2], Diana Ioana Olah[2], Constantin Cerbu[2], Adrian Valentin Potârniche[2], Sergiu Dan Zăblău[2], Aurel Vasiu[2], Carmen Dana Șandru[1,2], Marina Spînu[1,2], Florina Marian [1]

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Abstract

Infectious pododermatitis is a disease of the hoof and interdigital tissue, more frequently diagnosed in sheep. Poor hygiene conditions and/or a humid environment promote the activity of *Fusobacterium necrophorum* and *Dichelobacter nodosus*, whereas the intervention of commensal or saprophytic microorganisms further complicates the disease. The questionable efficacy of the classic antimicrobial treatment leads to enhancement of the overwhelming antibiotic resistance phenomenon. The research aimed at showing that plant extracts could successfully alleviate symptoms of necrobacillary pododermatitis by fighting the local antibiotic resistant microbiome, thus limiting the use of classical antimicrobial agents. The study was performed on samples collected from sheep (n=15) showing lesions of the interdigital skin and sole of the hoof. All strains isolated by standard microbiological techniques for aerobic bacteria were identified with rapid biochemical tests: GN 24 (Tody Laboratories, România) for Gram-negative, and GP 24 (Tody Laboratories, România) for Gram-positive bacteria. The evaluation of antibiotic susceptibility was performed by Kirby-Bauer disc diffusion method using amoxicillin/clavulanic acid, penicillin, imipenem, gentamycin, streptomycin, florfenicol, cefquinome, erythromycin, tylosin, tulathromycin, oxytetracycline, and doxycycline. The activity of essential oils against multiresistant strains was tested by disc diffusion method. The assessment included 9 essential oils derived from plants belonging to genera *Cinnamomum*, *Melaleuca*, *Pelargonium*, *Mentha*, *Thymus*, *Lavandul*a and *Ocimum* at 4 different concentrations: 8%, 4%, 2%, and 1%. Twenty-seven bacterial strains belonging to 13 different genera and 15 species were identified, among which *Escherichia coli*, *Pseudomonas putrefaciens*¸ *Pseudomonas fluorescens,* and *Staphylococcus sciuri*. Tylosin proved to be the least efficient, followed by penicillin and opposed to cefquinome and imipenem, with highest efficacy. Two extracts of cinnamon bark (*Cinnamomum zeylanicum*) showed significant antimicrobial activity, comparable or even superior to that of the most efficient antibiotics, regardless of the concentration. These results promote the further *in vivo* testing of cinnamon essential oil as an efficient alternative therapeutic agent in infectious pododermatitis.

Key Words: Infectious pododermatitis, sheep, antibiotic therapy, *E. coli*, essential oils, cinnamon

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ORAL PRESENTATION

ANTICHOLINESTERASE COMPOUNDS FROM ENDEMIC *PRANGOS UECHTRITZII* BOISS & HAUSSKN

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Abstract

In this study, the anti-cholinesterase and cytotoxic effects of the extracts and isolated compounds from the roots of *Prangos uechtritzii* Boiss & Hausskn (Apiaceae) are reported. two known polyacetylenes; panaxynol and falcarindiol were isolated from the chloroform extract of *P. uechtritzii* roots and identified utilizing spectroscopic methods. Pu-HE and Pu-CE showed potent *in vitro* butyrylcholinesterase (BChE) inhibitory activities with IC_{50} values of 21.19±0.45 and 18.14±0.59 µg/mL, respectively. Falcarindiol (IC_{50}= 27.88±0.91 µM) was the most active component. These findings suggested that **falcarindiol** could be considered as holding remarkable potential to develop novel BChE inhibitors with further research against Alzheimer's Disease (AD).

Key Words: *Prangos*, polyacetylene, AChE, BChE

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ORAL PRESENTATION

METALS ACCUMULATION AND TRANSFER IN *Portulaca oleracea L.* SAMPLES AS EDIBLE WILD PLANTS IN AEGEAN REGION OF MEDITERRANEAN AREA

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Purslane, with the botanic name *Portulaca oleracea L.*, is known to be a common wild edible plant with highly rich nutritional and medicanal characteristics. It is the vegetable richest in Omega-3 fatty acids. Fresh purslane has high nutritional values, in terms of vitamins (A, B1 (thiamin), B2, B6, C, E, niacin, nicotinic acid, beta-carotene, riboflavin, folate etc.) and minerals (especially K, Ca, Fe, Mg, Na, P, Cu and Mn) which are beneficial for human health [1]. In the Middle East, the plant is used in asthma, ulcer, diarrhea, dysentery and hemorrhoids, while it is used for antipyretic, muscle relaxant, antiseptic, antispasmodic, and diuretic purposes. Some studies have shown that purslane consumption helps reduce the occurrence of cancer and heart disease [2,3]. *Portulaca oleracea L.* is widely consumed in Mediterranean countries. The current study aims to determine the properties and heavy metal contents of the purslane plant samples and the surrounding soils taken from 18 points in the Büyük Menderes and Küçük Menderes basins located in Agean Region of the Mediterranean Area. For most of the soil samples, As, Cd, Ni, Zn and Pb elements are above the crustal averages. Strong correlations between Al, Cd, Co, Cu, Li, Mn and Zn elements were determined, which means that the correlated elements are considered to be related to similar sources. The translocation factors (TF) determined between root-leaf indicate that B, Cd, Co, Pb, Li, Ni, Sr, and Zn elements are transferred from the root part to the leaf region. The TF values also point that purslane plant has the ability to accumulate most of the elements examined according to the stem part of its leaves, however the plants are under stress due to the levels of Al, As, Ba, Cr, Cu, Fe, Mn elements.

Key words: Purslane, heavy metals, soil, Mediterrenean
Acknowledgements: The study is supported by PRIMA foundation under the project Valuefarm (Prima2019-11).

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ORAL PRESENTATION

MEDICINAL AND AROMATIC PLANTS EXTRACTS USE IN TEXTILES

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Abstract

Although the effect of fashion on textile products is quite high, in recent years, consumers want not only aesthetic features but also functional features as well as aesthetic features. Therefore, the demand for textile products containing aromatic plant extracts has been increasing in recent years [1]. Aromatic plant extracts are used in textile products not only for their aroma but also for their antibacterial and insect repellent properties. The use of medicinal plant extracts, as well as the use of aromatic plant extracts, is increasing in the textile industry. *Hypercium perforatum L.* extract can be used alone as well as with different essential and non-volatile oils. Jojoba and jasmine oil [2], *santalum album* oil [3], *citrus reticulate* oil [4] were used with *Hypercium perforatum L.* extract. The aim of this paper is to give a review on medical and aromatic plant extracts use in textiles.

Key Words: Medicinal Plants Extracts, Aromatic Plants Extracts, Textiles

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ORAL PRESENTATION

CORRELATION AND PCA ANALYSIS RESULTS IN 6 MORPHOLOGICAL PROPERTIES OF DIFFERENT FENUGREEK GENOTYPES

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Abstract

Fenugreek is one of the important plants among the fabaceae family as chickpea, common bean and soybean. It has anticarcinogenic, antiviral, and antioxidant effects as pharmacologically. It is used for many purposes by using different part of the fenugreek. In this study, six morphological properties were examined, and relationship among these properties were determined by using correlation and PCA analysis in 75 different fenugreek genotypes and two cultivars. Morphological properties as 50% seedling days (7.68-38.68 days), first pod height (6.67-41.24 cm), pod thickness (1.13 2.83 mm), stem diameter (2.58-6.63), leaf length (1.33-4.09 mm) and leaf width (0.66-1.94) were measured. Correlation analysis was performed to determine the relationship among these properties and two highly significant positive correlations were found between leaf width and stem diameter (r=0.413) and leaf length (r=0.455). One positive correlation was found between stem diameter and leaf length (r=0.226). PCA analysis was carried to classify and characterize different origin fenugreek genotypes and cultivars depending on the six morphological properties. PC1 and PC2 explained 51.53% in total variations and it was divided three groups. First group occurred with stem diameter, leaf length and width. Second group consisted of 50% seedling days and pod thickness. The last group included only first pod height. It can be said that first pod height is effective on many of the fenugreek genotypes, and stem diameter and 50% seedling days are effective for the Çiftçi and Gürarslan cultivars, respectively.

Keywords: *Trigonella foenum graecum* L., principal component analysis, seedling days, first pod height.

Acknowledgments: This study was financially supported by Grant No: 2018.10.07.1400 of the Scientific Research Project Fund, Faculty of Agriculture, Bolu Abant Izzet Baysal University, Turkey.

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ORAL PRESENTATION

CYCLOOXYGENASE INHIBITORY ACTIVITY AND COMPOSITION OF *ECHINOPHORA TENUIFOLIA* L. SUBSP. *SIBTHORPIANA* (GUSS.) TUTIN L. ESSENTIAL OIL

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Abstract

Echinophora tenuifolia L. subsp. *sibthorpiana* (Guss.) Tutin L. (Apiaceae) is known as 'çörtük, turşu otu, tarhana otu' and used both food and folk medicine in Turkey [1-3]. The aim of this study was to determine the chemical composition and inhibitory activity of the essential oil on cylooxygenase enzymes of *E. tenuifolia* subsp. *sibthorpiana*. The plant was collected in May 2018, from Tokat province, Turkey. Aerial parts of the plant were subjected to hydrodistillation using Clevenger apparatus. Chemical composition of the oil was analyzed with GC-FID and GC-MS techniques. The effect of inhibition of essential oil on cyclooxygenase enzymes was investigated using the "COX Colorimetric Inhibitor Screening Test Kit" containing both ovine COX-1 and human recombinant COX-2 enzymes. Main components of the oil were found as methyl eugenol (45.5%), *p*-cymene (11.5%) and *δ*-3-carene (11.2%). The oil is exhibited inhibitory activity at 100 µg/ml on cyclooxygenase-1 (COX-1); 68,89±2,96 %, while no inhibition activity on cyclooxygenase-2 (COX-2). Rofekoksib and SC-560 were used as standard inhibitors for COX-2 and COX-1, respectively. The results showed that the essential oil may play an inhibitory role on COX-1 enzyme and inflammation relation diseases. To the best of our knowledge, this is the first contribution into the COX enzymes inhibition activity of *Echinophora tenuifolia* L. subsp. *sibthorpiana* (Guss.) Tutin L. essential oil.

Key Words: *Echinophora tenuifolia* subsp. *sibthorpiana*, essential oil, GC, GC/MS, COX

Acknowledgement: We are grateful to Prof. Dr. Yavuz Bulent Kose, Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey, for determination of the plant specimen.

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ORAL PRESENTATION

THE EFFECT OF HARVEST TIME ON SQUALENE CONTENT IN TWO OLIVE CULTIVARS' OILS

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Abstract

Olive (*Olea europaea L.*) is a significant crop especially in Mediterranean countries. Its fruit and oil have a major agricultural importance in Turkey. The oil obtained from olive fruits have been used not only nutrient and as a flavor enhancer in Mediterranean dishes, but also a medicine because of having essential roles for human health. The pharmaceutical value of olive oil is related to its biochemical content. Mainly four chemical sources: polyphenols, carotenoids, mono-unsatured fatty acids and squalene are functional bioactives in olive oil particularly for pharmaceutical affects. The present study was designed to examine changes of squalene contents of olive oil gained Kilis Yağlık and Gemlik olive cultivars' fruits harvested in tree different time along the two years. Squalene content in olive oils varied according to harvest time, cultivar and cultivation year. Accordingly, the variety affected the squalen content, in Gemlik cultivar squalene was more than Kilis Yaglık cultivar. In terms of its cultivated year, in the high yield year, an increase in squalen content was observed. In olive oil, squalen content decreased first then increased by the fruit maturation. Therefore, harvest time effected biochemical content of oil by the fruit ripening stages.

Key Words: *Olea europaea L.*, olive oil, squalen, harvest time, Kilis Yaglık, Gemlik

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ORAL PRESENTATION

COMBINED EFFECT OF TWO MEDICINAL PLANTS; FENUGREEK (*TRIGONELLA FOENUM*-GRAECUM L.), CUMIN (*CUMINUM CYMINUM* L.) WITH PROBIOTICS AGAINST *HELICOBACTER PYLORI*

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Abstract

Helicobacter pylori causes gastritis, gastric cancer and peptic ulcers and affects more than half of the world's population. Despite the fact that this infection can have serious implications, most infected people present moderate gastritis. While no new cure or remedy have been discovered, the present therapy still depending on a variety of known antibiotics and anti-secretory agents, with a standard triple therapy of two antibiotics and a proton-pump inhibitor suggested as the first-line regimen. Many determinants for prosperous therapy are elaborated, in particular individual primary or secondary antibiotic resistance, patient compliance, side-effect profile, mucosal drug concentration and also cost. In the present study, we discuss alternatives therapies for *H. pylori*, mainly phytotherapy and probiotics. The potential of combination between methanolic extracts of two medicinal plants; cumin (*Cuminum cyminum L.*), fenugreek (*Trigonella foenum-graecum L.*) and different strains of lactic acid bacteria ; *Bifidobacterium breve, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus rhamnosus LA80, Lactobacillus rhamnosus GG, Lactobacillus helviticus, Lactococcus lactis, Streptococcus thermophilus, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus fermentum and Lactobacillus casei* against *H. pylori* was investigated. *H. pylori* was inhibited by all combined mixtures of extracts and probiotics with different varying results, while cumin */ B. breve* and fenugreek */ B. breve* combinations exhibited the higher anti-*Helicobacter pylori* activities with DZI of 26 and 29 mm respectively. Preliminary studies on the mode of action of probiotics against *H. pylori* revealed that the inhibition may be due to lactic acid and bacteriocins. Also, it may be related to the presence of phenolic compounds in our studied plants such as gallic acid, caffeic acid, quercetine, and vanillic acid. This research demonstrates the synergic effect of cumin and fenugreek with beneficial lactic acid bacteria for *H. pylori* inhibition.

Key Words: *Helicobacter pylori*, fenugreek, cumin, probiotics, antibacterial effect

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ORAL PRESENTATION

GERMAN CHAMOMILE (*MATRICARIA RECUTITA* L.) IN UKRAINE AND ITS COMPOSITION OF ESSENTIAL OIL

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Abstract

Each The Ukraine traditional medicine comprises medical aspects of traditional knowledge that developed over generations within the folk beliefs of various societies before the era of modern medicine. Medicinal plants are used with the intention of maintaining health, to be administered for a specific condition, or both, whether in modern medicine or in traditional medicine. One of the more popular examples of a medicinal plant is the use of the chamomile flowers (Chamomillae anthodium) to treat respiratory infections such as a cold or mild flu. Today this drug is officially registered in the Ukraine and European Pharmacopoeia. Despite its economic importance, however, chamomile, *Matricaria recutita* L., is little known about the extent and nature of the essential oil variability and its composition of this species in Ukraine. Therefore, the information about extent of uses of various gene pools is extremely valuable for the development of future chamomile cultivation and breeding programs. The aim of the study was the analysis of differences among chamomile plant populations growing naturally in different sites in all parts of Ukraine. The subject was created in collaboration with the Botanical Garden of the Lviv University of Medical Sciences in Lviv, Ukraine. The quantities of essential oils in the present study were measured from 0.20 ± 0.05 % in Cherson to 0.85 ± 0.10 % in Chernihiv. The yield of volatile oil was depending on geography, altitude, and other factors, including stress influence on site (20) of plant population growth. Essential oil extracted chamomile inflorescences were recorded to have from 52 to 72 chemical components. It was found that /-/-α-bisabololoxides B and A was the major constituents in 16 samples collected from individual Ukraine sites and only 4 had dominant /-/-α-bisabolol (the most 55.17 % on site Katerinopols). The uniquely determined chemical type of chamomile wild populations in Ukraine is chemical type B (/-/-α-bisabololoxide A > /-/-α-bisabolol > /-/-α-bisabololoxide B) by a large majority.The most important result of our study is the creation of the new map of the plant population distribution in order to the chamomile species in Ukraine with their chemotype determination.

Key Words: Chamomile, chemical type, essential oil, GC/MS, sesquiterpenes, Ukraine

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ORAL PRESENTATION

QUALITATIVE AND QUANTITATIVE ANALYSIS OF HIGH-LEVEL CHEMICAL CONSTITUENTS OF CHAMOMILE AND PEPPERMINT GROWN IN EASTERN SLOVAKIA

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Abstract

Medicinal plant production is mainly depended on ecological conditions. *Matricaria recutita* L. and *Mentha* × *piperita* L. have been widely grown in Eastern Slovakia owing to substantial demands for not only culinary but also pharmaceutical industries. The objective of this study was to identify and quantitatively analyze the essential oil constituents derived from the selected medicinal plants namely *M. recutita* and *M. piperita* grown in the University of Presov, Slovakia. Soil chemical and climatic conditions were studied and maintained. After the four years of cultivated chamomile variety "*Lianka*", a diploid genotype, the essential oil compositions were reported to present an outstanding high content of two active substances, which were /-/-α- bisabolol and chamazulene belonging to a bisabolol chemotype. Besides, the essential oil extracted from peppermint variety "*Kristinka*", was consisted of 70 – 74 % menthol and only trace amounts of pulegone and carvone indicating an interesting chemotype. Both plant varieties were consequently certificated by the Community Plant Variety Office (CPVO) in Angers, France in 2017 respectively 2018. Therefore, *M. recutita* and *M. piperita* grown in Eastern Slovakia could be promising resources of bioactive substances for culinary and pharmaceutical products. However, a very important mission of the owner of varieties of medicinal plants - the University of Presov in Presov, Slovakia, is to maintain varietal diversity, balance and stability after receiving certificates for many years.

Key Words: Chamomile, composition, essential oil, Eastern Slovakia, fields, GC/FID, peppermint

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ORAL PRESENTATION

LC-MS/MS PROFILING AND BIOLOGICAL ACTIVITIES OF THE FLOWERS OF KILIS YAĞLIK AND GEMLIK OLIVE CULTIVARS

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Abstract

Olive tree (*Olea europaea* L., fam: Oleaceae) with organs (fruit, leaf, and stems), products (oil and olive) and by-products (olive milled waste, fruit pulp and seeds) is a valuable medicinal plant used in medicine, food additives, pharmaceuticals, nutraceuticals, cosmetic industry and aromatherapy. This research was undertaken for identification of phytochemical contents and determination of biological activities including anticancer, antiproliferative, antioxidant, anti-cholinesterase, anti-tyrosinase, and apoptotic DNA fragmentation. The flowers of 'Kilis Yağlık' and 'Gemlik' olive cultivars were collected from Kilis province, and crude extracts prepared by maceration method using different solvents. The activities of the extracts on the development of cancer, cell proliferation, apoptosis and DNA damage were analyzed towards MCF-7 (human breast cancer), HT-29 (human colorectal adenocarcinoma), and HepG2 (human liver adenocarcinoma) cells using MTT, trypan blue and immunological-based ELISA methods, respectively. Anti-cholinesterase and anti-tyrosinase activities of the extracts were examined with regards to their enzyme inhibition potentials using spectrophotometric microtiter assays, whilst in vitro DPPH, ABTS, FRAP and CUPRAC assays were conducted to evaluate antioxidant capacities of the extracts. Identification and quantification of phenolic constituents of the flowers were performed by using LC-MS/MS and GC-MS/FID analyses. The extracts obtained from the flowers of 'Kilis Yağlık' were found to have higher biological activities in almost all the tested assays, comparing the extracts of 'Gemlik' olive cultivars. As for phytochemical properties, LC-MS/MS screening indicated the presence of eight metabolites, including acetohydroxamic acid, caffeic acid, luteolin, naringenin, oleuropein, protocatechuic, quercetin, and thymoquinone. Of which, acetohydroxamic acid was determined only the flowers of 'Kilis Yağlık', the others were found in both of the olive cultivars. The amount of oleuropein and thymoquinone were higher in the flowers of 'Kilis Yağlık' than 'Gemlik' olive cultivars. Even if a huge number of studies have been focused on the evaluation of biological activities and pharmaceutical properties of the olive fruit, olive-oil and olive leaves, no research has been conducted to investigate biological activities of the flowers of 'Kilis Yağlık' and 'Gemlik' olive cultivars, and their phenolic constituents in comparison. Herewith this research could be considered as the first report for the scientific literature, however, detailed pharmacological and clinical studies are necessary to discover plant-based pharmaceutics.

Keywords: Bioactivity, Gemlik, Kilis Yağlık, LC-MS/MS, Olive flower, Phytochemicals
Acknowledgements: This work was financially supported by Scientific Research Projects Coordination Unit of Kilis 7 Aralik University, Kilis-Turkey (Project number: 20-12972MAP1).

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ORAL PRESENTATION

GENE EXPRESSION EVALUATION OF STAR IN TESTIS AND TSPO IN QUAIL OVARIES UNDER THE INFLUENCE OF VITAMIN E

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Abstract

Vitamin E is found in the oils of many plants and plays a significant protective and medicinal role. STAR and TSPO genes are involved in reproduction performance. In order to investigate the effect of different levels of vitamin E on STAR gene expression in testis and TSPO gene in ovaries of parent quail, 360 Japanese quails for ten weeks were assayed. Poultry was kept in 18 cages as a completely randomized design with three treatments, six replications, and 24 quails (16 Females and 8 males). Treatments included pure vitamin E levels (25, 50, and 100 IU per kg of diet) in the diet. The results showed that different levels of vitamin E did not affect STAR gene expression in the testis. Quails receiving 50 and 100 IU levels of vitamin E showed an increase in TSPO gene expression in the ovaries by 7 and 2 times, respectively ($P<0.01$). The results of reproductive performance showed that different levels of vitamin E had no significant effect on fertility percentage ($P<0.05$), but the percentage of quail hatching chicks was significantly higher in vitamin E (50 and 100 IU / kg) levels than The level of 25 IU per kg of vitamin E increased ($P<0.05$).

Key Words: Vitamin E, Medical role, Quail, Gene expression

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ORAL PRESENTATION

DETERMINATION OF SILAGE QUALITY OF DIFFERENT *PLANTAGO* SPECIES (*PLANTAGO MAJOR* L., *PLANTAGO LANCEOLATA* L. AND *PLANTAGO MEDIA* L.)

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Abstract

In this study, it was aimed to determine the silage quality of different *Plantago* species. *Plantago major, Plantago lanceolata* and *Plantago media* species were collected from the natural meadow area and their silages were made. Silage dry matter (KM) value was found to be higher in *Plantago media* herbage than the others (P<0.05). It was determined that the PH value of the silage of *Plantago lanceolata* species was lower than the other species (P<0.05). Silage inorganic matter level was higher in *P. major* silage (P<0.05). Crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) values of *Plantago lanceolata* and *Plantago media* silages were higher than those of *Plantago major* (P<0.05). *In vitro* ruminal total gas production, metabolic energy (ME) and net energy lactation (NE_L) values of *Plantago media* silage were higher than that of *Plantago major* silage. *In vitro* ruminal methane production was similar in *Plantago media* and *Plantago lanceolata* silages, with higher values than that of *Plantago major* silage. Ruminal ammonia-nitrogen concentrations were similar for all three *Plantago* species. The ratio of oleic acid, monounsaturated fatty acid (MCFA) and total w-9 fatty acids of *Plantago major* silage was higher than those of other silages (P<0.05). The w-3 fatty acid ratios of *Plantago lanceolata* silage and *Plantago media* silage were higher than that of *Plantago major* silage (P<0.05). Medium chain fatty acid (MCFA) ratio of *Plantago lanceolata* silage was higher those of other silages (P<0.05). Very long chain fatty acid (VLCFA) ratio of *Plantago media* silage was lower than those of *P. major* and *P lanceolata* silage (P<0.05). As a result, it was determined that good cell wall elements and *in vitro* ruminal digestion levels (only *P. major* silage had a lower value than the others), moderate CP and similar ruminal ammonia-nitrogen levels were found to be effective for rumen fermentation of *Plantago* silages. In addition, it was concluded that *Plantago major* silage had higher oleic acid and w-9 fatty acids levels than others, and *Plantago media* and *Plantago lanceolata* silages had a higher total w-3 and α-linolenic acid ratio than those of *P. major* silage.

Key words: *Plantago major*, *Plantago lanceolata*, *Plantago media*, silage, in vitro digestion, fatty acid.

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ORAL PRESENTATION

COSMECEUTICAL POTENTIAL OF *MANIHOT ESCULENTA* LEAF: UV FILTER, ANTIOXIDATION, ANTI-MELANOGENESIS, COLLAGEN SYNTHESIS ENHANCEMENT, AND ANTI-ADIPOGENESIS

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Abstract

Cassava leaf, *Manihot esculenta* (L.) Crantz in the Euphorbiaceae family, is a potential agroecomonic cassava root co-product. This work aimed to explore the cosmeceutical potential of a cassava leaf in *in vitro* models. The ethanolic cassava leaf extract (BM) was prepared and used in the experiments to determine the antioxidation using DPPH assay, anti-melanogenesis, anti-adipogenesis, and collagen synthesis enhancement using cell culture-based technique. Additionally, UV filter capacity and chemical composition were investigated using UV-visible spectrophotometry and HPLC-UV analysis. As a result, cosmeceutical potential of BM was significantly demonstrated in a manner of wide spectral effect. This was illustrated in reducing melanogenesis and adipogenesis as well as increasing the collagen content in a dose-dependent manner after BM treatment. Furthermore, desirable UV absorptivity covering UVA and UVB wavelength region. Rutin, Apigenin and Kaempferol content in BM were predominantly demonstrated. Conclusively, the cassava leaf was interestingly manifested as a potential natural agent possessing multiple cosmeceutical effects. This data will support the introduction of cassava leaf as a potential agroeconomic resource of nutrients and cosmeceutical agents. However, further research works vis-à-vis the safety and efficacy should be considered to rationale and sustainable applications for health and well-being.

Key Words: *Manihot esculenta*; cassava leaf; UV filter; flavonoids; melanin; collagen; adipogenesis.

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ORAL PRESENTATION

HERBAL COSMETIC FORMULATIONS WITH HONEY PLANT AS SKIN CARE PRODUCTS AND THEIR CLINICAL EVALUATION FOR COMMERCIALIZATION

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Abstract

There are various herbal skin care products available in the market which contains mostly poly herbal formulations. In the present study, Indian honey plant i.e., *Stevia rebaudiana* was used for preparation of moisturizer gel and vanishing cream and evaluated for safety and efficacy of the products for commercialization. Three different concentrations of Stevia extract (1.5%, 2.5% and 5.0%) was used for both the preparations. The gels were prepared by using Carbopol 934 and the creams were O/W emulsion-based formulations. Various physicochemical studies, stability studies were carried out. Preclinical and clinical studies were carried out for preliminary skin irritation tests with dermal toxicity study. Further formulated creams and gels were compared with market products by clinical studies with human subjects. Skin elasticity, skin pH, skin moisture, skin glow, sun protection effect, etc were evaluated and compared with market products. The result revealed that 2.5% Stevia extract contained both the formulations (moisturizer gel and vanishing cream) were better than other two formulations with respect to stability study, pH of the formulation, and results of human trials. Results further concluded that Stevia extract containing skin care products are effective and safe and to be used as herbal market products.

Key words: Clinical trial, efficacy, moisturizing gel, vanishing cream, *Stevia*, stability

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ORAL PRESENTATION

IS *IN VITRO* PLANT EXTRACTS' ACTIVITY CONDITIONED BY PHYLOGENETIC DIFFERENCES BETWEEN TARGET SPECIES?

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Abstract

On the phylogenetic scale, the immune system gained in complexity gradually, while species-specific diversification occurred. Although the immune system evolved from simple to complex, *Aves* and *Mammalia* share some of the complex response mechanisms to antigenic challenge. This study aimed at defining the potential differences in the cell-mediated responses as a measure of adaptive immunity and the dose-effect relationships for medicinal plant extracts in *Aves* and *Mammalia* classes. An *in vitro* blast transformation micro-method was performed on heparinized blood sampled from adult hens and rabbits to estimate the effects of alcoholic extracts of *Calendula officinalis*, *Vaccinium myrtillus*, *Echinacea spp.* and *Hippophae rhamnoides* using concentrations of 1.5% and 6.5%. Cell growth was quantified at the end of the incubation period (48h at 37°C for both species) by an orto-toluidine glucose consumption assay. The statistical significance of the differences between the variants was interpreted by Student's t test. In chickens, the plant extracts showed an activity depending on the active principles contained (i.e., 81.93 ± 18.74% and 74.70 ± 22.15% for *Vaccinum myrtillus* and *Calendula officinalis,* at 1.5% concentration, respectively). All extracts stimulated rabbit immune cells *Calendula* (80.72 ± 6.82% at a concentration of 6.5%) and *Echinacea* (68.67 ± 17.04% at a concentration of 6.5%), proving more stimulating than *Vaccinium* (65.06 ± 35.78% at a concentration of 1.5%). Both the hosts' phylogenetic position and the plant species influenced the *in vitro* immune responses in chickens and rabbits. While in birds some of the extracts showed a homeopathic effect, in rabbits the maximal effects were directly dose-dependent. The results support the importance of preliminary tests in choosing the vegetal extract to attain the strongest immune stimulation/modulation effect.

Keywords: Phylogeny, chicken, rabbits, medicinal plants, immunity, homeopathic effect

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ORAL PRESENTATION

EFFECT OF NEW SULFONYL HYDRAZONE COMPOUNDS AGAINST *CANDIDA* BIOFILMS

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Abstract

Objectives: Candidiasis is the most frequently encountered fungal infection, with ranges from mild superficial infections to disseminated candidiasis. Most manifestations of Candidiasis are associated with biofilm formation. *Candida* biofilms contributes to therapeutic failure due to resistance to antimicrobial agents and causes an increase in mortality rates. For these reasons, finding new drugs and antimicrobial agents are urgently needed. Sulfonyl hydrazones derived from sulfonamide, are well-known for its pharmacological effects, such as antifungal, antibacterial potential activity. This study aimed to investigate the anti-biofilm activity of the new sulfonyl hydrazone compounds (Anaf-Bsh, Anaf-Esh, Anaf-Psh) against *Candida* species. **Methods:** *Candida* biofilms was carried out in a 96-well flat-bottom plate. Biofilm biomass was measured using Crystal violet staining method. Forty-two isolates tested were classified according to their ability to produce biofilms and 10 isolates that have strong biofilms producer were selected. The inhibitory effects of each compound (0.5–256 µg/ml) on *Candida* biofilms were measured MTT assay. The total RNA was isolated from the control and treated biofilm cells using TRIzol reagent. Synthesis of cDNA from total RNA was carried out using Transcriptor High Fidelity cDNA Synthesis kit. Expression analysis of biofilm related key genes (*ALS3, HWP1, UME6, SAP5, ECE1*) was investigated with the RT-qPCR method. Fold changes were calculated by the $2^{-\Delta\Delta CT}$ method. The statistical analysis was performed using one-way ANOVA test. **Results:** In RT-qPCR experiments, changes in the expression levels of biofilm-related key genes have been observed after sulfonyl hydrazone compounds. **Conclusions:** These results indicate that sulfonyl hydrazone compounds may have therapeutic potential in the treatment and prevention of biofilm-associated *Candida* infections.

Key Words: Biofilm, biofilm related genes, *Candida*, sulfonyl hydrazone compounds.

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ORAL PRESENTATION

COMPARATIVE KARYOTYPE ANALYSIS OF POPULATIONS IN THE *MUSCARI MASSAYANUM* (ASPARAGACEAE) IN TURKEY

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Abstract

Muscari is a genus of bulbous plants belonging to the Asparagaceae family. The genus has a wide distribution in the Mediterranean basin, Central and Southwest Europe, the Caucasus, Southwest Asia and Central Asia. The species belonging to this genus have various medicinal and biological activities such as antioxidant, anti-inflammatory, emetic, diuretic, hypoglycemic and stimulating effects. In this study, the chromosome number and morphologies of four populations belonging to the endemic *Muscari massayanum* C. Grunert species were investigated. The detailed karyomorphologic features of the populations belonging to *M. massayanum* was reported and examined here for the first time. This work was carried out using the squashing method at the root tips and it was determined that all populations were diploid ($2n=2x=18$) and the basic chromosome numbers were $x=9$. Also, comparative karyotype analyzes showed that all populations have the common karyotype formula. According to asymmetry indices used in here Erzincan and Erzurum populations had the most asymmetric karyotype among all populations. These results indicate that the populations have the common chromosome number and a karyotype formula but display different karyo morphological features.

Key Words: Asparagaceae, endemic, karyomorphology, *Muscari*, Turkey.

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ORAL PRESENTATION

TREATMENTS TO OVERCOME SEED DORMANCY IN CAPER (*CAPPARIS SPINOSA*)

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Abstract

Caper (*Capparis spinosa* L.) is a perennial monoecious shrub which grows in hot and dry climates. This plant is medicinal plants with considerable tolerance to thermal and moisture stresses. It has variety products and can be economically valuable. Caper mass production is limited due to poor seed germination. The objective of this study was to investigate various seed treatments to overcome seed dormancy and improve seed germination of capper. Treatments were seed priming using Potassium nitrate (0.5, 1 and 2%), gibberellic acid (100, 200 and 400 ppm) combination of Potassium nitrate 2% and gibberellic acid 500 ppm, chemical and mechanical scarification and light treatment. This experiment was performed in factorial based on completely randomized design at seed technology laboratory of department of plant production and genetics, faculty of agriculture, Agricultural Sciences and Natural Resources University of Khuzestan during 2018. Germination percentage and seed vigor index were measured as the indicator of seed quality. The results of this experiment showed that application of potassium nitrate and gibberellic acid had significant effects on germinations characteristics and increased the germination percentage, rate of germination and seedling length. Seeds soaked in distilled water had not germination. The best time of soaking was 48 hours. The highest germination percentage and vigor obtained from hormone seed priming for 48 hours with 400 ppm gibberellic acid from mechanical scarified seeds.

Key Words: Caper, dormancy, priming, seed

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ORAL PRESENTATION

THIADIAZOLE DERIVATIVES as POTENTIAL EGFR TARGETED ANTICANCER AGENTS

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Abstract
Epidermal growth factor receptor (EGFR), one of the most important members of receptor tyrosine kinase superfamily, has been reported to act as a key mediator in tumor growth and progression including cell proliferation, impair of apoptosis, metastasis and angiogenesis. EGFR is frequently overexpressed in many human cancers such as non-small-cell lung carcinoma and glioblastoma [1]. Since 1,3,4-thiadiazole derivatives have been shown to display diverse biological activities including anticancer activity, previously, we synthesized a series of thiadiazole derivatives and reported their antileukemic effects [2]. In the current study, we investigated anti-lung and anti-glioma effects of the most effective anticancer derivatives (compounds **2**, **3** and **5**) on A549 human lung adenocarcinoma and U251 human glioblastoma cell lines, respectively. *N*-(5-Nitrothiazol-2-yl)-2-((5-((4-(trifluoromethyl)phenyl)amino)-1,3,4-thiadiazol-2-yl)thio)acetamide (**2**) was identified as the most potential anticancer agent against A549 cells with an IC_{50} value of 12.94±1.72 µM compared to erlotinib (IC_{50}= 25.33±3.54 µM), an approved EGFR inhibitor in lung cancer treatment. Compound **2** also showed significant cytotoxicity against U251 cells with an IC_{50} value of 19.00±3.15 µM. Further apoptotic and EGFR inhibitory effects of compound **2** were screened to gain a mechanistic insight. Compound **2** showed significant apoptotic effects in A549 cells compared to erlotinib. Besides, compound **2** inhibited EGFR significantly with an IC_{50} value of 35.80±4.32 µM compared to erlotinib (IC_{50}= 0.06±0.02 µM). Molecular docking studies, which were performed in the ATP binding site of EGFR (PDB ID: 4HJO) suggested that a crucial hidrogen bonding with Cys773 as similar with erlotinib could contribute to its potential inhibitory action, whereas the lack of hydrogen bonding with Met769 could diminish EGFR inhibitory effects of compound **2**. Besides, *in silico* predicted pharmacokinetic parameters of compound **2** indicated that this compound possessed anticancer drug-like properties. According to both *in vitro* and *in silico* studies, compound **2** draws an attention for further mechanistic studies.

Key Words: EGFR, non-small-cell lung carcinoma, glioblastoma, apoptosis, molecular docking, erlotinib

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ORAL PRESENTATION

MICROENCAPSULATION PROCESS AFFECTS AROMA PROFILE OF *HIBISCUS SABDARIFFA*

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Abstract

Roselle (*Hibiscus sabdariffa*) is a medicinal plant that belongs to the *Malvaceae* family. In traditional medicine, roselle is used for curing a wide variety of diseases. Besides, roselle calyxes have been used in the preparation of herbal drinks. Roselle tea is a rich source of bioactive compounds, especially anthocyanins. To improve the stability of anthocyanins, spray drying is one of the most common applications in the food industry. However, this process might lead to loss of aroma compounds. This study presented the stress-tolerance behavior to air inlet temperature of aroma compounds naturally found in Rosella during the conversion of its extracts into microcapsules form using spray drying. An optimization process was performed to maximize the total phenolic content, antioxidant activities, and total anthocyanin content in the brewing of roselle tea. The satisfying extraction conditions were at 54 °C for 120 min. Thereafter, microcapsules containing the extract were produced with help of maltodextrin as coating material by spray drying at different air inlet temperatures (165 and 200 °C). GC-MS analysis was applied to evaluate the effects of air inlet temperature of spray drying on the aroma profile of roselle extracts. The results were compared with those of plant, extract, and the mixture of extract and maltodextrin to exactly demonstrate the ability of the applied spray drying air inlet temperatures for increasing and/or decreasing the amount of aroma compounds. In general, the amount of aroma compounds diminished with incorporation of the extracts into the microcapsules. However, some of the compounds were detected only in the microcapsules. Changing the process of microcapsules resulted in varying effects on each aroma compound. The variables and scores were classified successfully by using two uncorrelated principal component analyses with 10.97% of loss variation.

Key Words: *Hibiscus sabdariffa* L, Response surface methodology, Microcapsules, Aroma compounds, Principal component analysis

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ORAL PRESENTATION

IN VITRO INHIBITION OF HUMAN BREAST CANCER ADENOCARINOMA CELL BY PROPOLIS VIA INDUCTION OF INTRINSIC PATHWAY OF APOPTOSIS

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Abstract

Breast cancer is considered as a major cause of cancer death in woman worldwide[1]. Conventional treatments such as chemotherapy associated to surgery are actually the main used treatments. However, they present several side effects and can induce the development of multidrug resistance[2,3]. A new approach has been developed in the last recent years, natural products, well known to possess anticancer activity, are investigated for a potential adjunctive complementary intervention to the actual conventional methods[2,3]. Propolis antitumor activity have become the subject of increasing research. Propolis was found to be effective on many types of cancer. We aimed in the present study to investigate the cytotoxic and anticancer effects of Algerian propolis on Human breast adenocarcinoma cells (MDA-MB-231) and intend to explain its mechanism of action. Cytotoxic activity was evaluated using MTT assay. Mechanism involved in the cytotoxic activity were also investigated. The MTT assay showed a significant antiproliferative activity. Algerian propolis was found to significantly increase mRNA levels of proteins associated with tumor suppression and apoptosis. The tested propolis probably induces intrinsic pathway of apoptosis through caspase cascade and activation of pro-apoptotic proteins. The presented findings suggest that Algerian propolis show potential in anticancer therapeutic strategy in particular in Human breast adenocarcinoma. Further investigation is in need to better understand the mechanism involved in the tested activity

Key Words: Algerian propolis, human breast adenocarcinoma cell, mechanismof action, apoptosis.

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ORAL PRESENTATION

CORRELATION BETWEEN ANTIBACTERIAL ANTIOXIDANT AND PHENOLIC AND FLAVONOID CONTENTS OF DIFFERENT ALGERIAN AND ANATOLIAN PROPOLIS

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Abstract

Propolis, one of the most studied bee product and recognized for its plethora of pharmacological activity is a complex mixture collected by honeybees. Chemical composition of propolis is quite variable according to geographical and botanical origin, bee species, time of collection. Because of their richness in wooded regions as well as their soils in endemic plants, Algeria and Turkey are likely to provide propolis different from those of other temperate and Mediterranean zones, especially from the chemical point of view. In the present work, several propolis samples collected from different part of Algeria and Turkey were investigated. MIC values against four bacterial strains (three Gram negative bacteria and one Gram positive bacteria) and FRAP assay were used for the determination of antibacterial and antioxidant activity. In addition, phenolic and flavonoids contents were also determined and Pearson's correlation test was used for the determination of correlation between the antibacterial and antioxidant activity with TP and TF. All tested propolis exhibited a pronounced antioxidant activity depending on the site of collection. However, antibacterial activity depends on both geographical origin and the tested bacterial strain with a more pronounced activity of Algerian propolis against *S. aureus ATCC 25923.* On the contract, Anatolian propolis samples were most active against *P. aeruginosa ATCC 27853.* MIC values were 0,04 ± 0.00-0.30±0.06 mg/Ml and 0.20±0.00-0.60±0.00 mg/Ml respectively. A strong positive correlation was detected between TP and TF and antioxidant activity of all tested propolis samples. All Anatolian propolis samples showed a positive correlation linking MIC, TP and TF. While the correlation observed for Algerian propolis was largely influenced by the tested bacterial strains. Our results indicated that Algerain and Anatolian propolis are a rich source of polyphenol and flavonoid components with a notable antibacterial and antioxidant activity. Both propolis could be useful in the food and pharmaceutical industries.

Key Words: Algerian propolis, Anatolian propolis, antibacterial activity, antioxidant activity, phenolic and flavonoid contents, correlation.

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ORAL PRESENTATION

THE STATUS OF HERBAL PRODUCTS IN PHARMACIES IN KAHRAMANMARAŞ/TURKEY

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Abstract

The rate of herbal products in Turkish pharmacies is quite low in parallel with the purchase of the public and the prescriptions of the physicians. However, there are regional differences according to the sociocultural and economic status of the people. In this study, it is aimed to obtain information about how much natural products are sold in pharmacies in Kahramanmaraş, how often they are bought by patients, how often doctors prescribe them, which ones are most preferred and which patient groups choose them.

A questionnaire consisting of 20 questions was prepared in order to understand the place of herbal products in pharmacies and it was filled by pharmacists. In the evaluation of the data, besides the classification of the answers given by the pharmacists to the questions, their own opinion on natural products were also determinative.

In this study, it was seen that pharmacists did not give enough importance to herbal preparations. It has been determined that pharmacists do not keep such natural products in their pharmacies much, either because they state that they do not care, or because patients do not buy them from pharmacies at all, or because they claim that they are sold by herbalists.

Key Words: Herbal teas, natural products, Kahramanmaraş, pharmacy, Turkey.

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ORAL PRESENTATION

THE PLACE OF PLANTS IN PROTECTING OUR HEALTH AND TREATING DISEASES

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Abstract

Spices and aromatic herbs have been used as preservatives, coloring and flavor enhancers since ancient times. Spices, which have long been the basis of traditional medicine in many countries, have also been the subject of scrutiny, especially by the chemical, pharmaceutical and food industries, due to their potential uses to improve health. Scientific studies have shown that these substances act as antioxidants, anti-inflammatory, digestive, regulating blood sugar and blood fats, have antibacterial, anti-inflammatory, antiviral and anticarcinogenic activities, and are very important in the balance and proliferation of our intestinal microbiota. Functional Medicine, acting on the principle that "nutrients are our medicine", uses these beneficial physiological effects both in the protection of health and in the treatment of diseases. The plants used by Functional Medicine, which focuses on finding and solving root causes in the treatment of diseases, must be fully known, toxin-free and pesticide-free, and must not be contaminated. Although it uses as supplements forms for the treatment of diseases, integrating these plants into the diet makes its health effects sustainable. Spices and aromatic plants are already an important part of the human diet and have a place in all cultures of the world. For this reason, it is necessary to know the plants, to learn the right combinations, to know how to use them without losing their useful properties in the kitchen, even by increasing them.

Keywords: Functional medicine, health, inflammation, herb, aromatic herbs, spices.

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ORAL PRESENTATION

A NOVEL HERBAL COFFEE: OLIVE STONE COFFEE

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Abstract

Herbal coffees, which refer to the coffee product enriched with herbs, spices or other herbal ingredients, have become of the most popular types of coffee generally accepted by consumers, as the consumer's perspective on consuming the coffee product has begun to change, not only for "pleasure" but also by considering its health benefits such as reducing high caffeine consuming. Terebinth coffee (*Pistachia terebinthus* L.), black cumin coffee (*Nigella sativa* L.), carob coffee (*Ceratonia siliqua* L.), date kernel coffee (*Phoenix dactylifera*), Gundelia coffee (*Gundelia tournefortii* L.), chicory root coffee *(Cichorium endivia*), and dandelion root coffee (*Taraxacum officinale* L.), are of the widely consumed herbal coffees as a caffeine-free and healthy substitute to regular coffee. Herbal coffees help prevent certain illnesses thanks to several health benefits including reduced inflammation, decreased blood sugar, improved digestive health, increased the metabolism, stimulated cell growth, weight loss etc.

Along with the interest in consuming of herbal coffees, there is currently a huge demand for producing products from industrial and agricultural wastes around the world. In this regard, olive stone, an important by-product produced in the olive oil extraction and pitted table olive industries, is an attractive source of bioactive and rich source of valuable compounds e.g., cellulose, hemicellulose, lignin, polysaccharides, polyphenols, protein, fat, minerals and fibers. Olive stone coffee is a product innovation that consists of a mixture of special ingredients such as carob (*Ceratonia siliqua* L.), terebinth fruit (*Pistacia terebinthus* L.), cinnamon (*Cinnamomum ceylanicum* L.), clove (*Syzygium aromaticum* L.), vanilla (*Vanilla planifolia*) , as well as olive kernel (*Olea europaea* L.). This coffee has flavorful aroma and rich nutritional value, and also provides many health benefits in reducing the risk of stomach pains, gastritis, ulcers, hemorrhoids, intestine disorders, diabetes and autoimmune diseases which are well known for using in Traditional Anatolian Medicine for olive kernel.

Keywords: Herbal coffee, olive stone, novel product, by-product, *Olea europaea*

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ORAL PRESENTATION

ANTIMICROBIAL EFFECTS OF PEA PROTEIN BASED EDIBLE FILMS WITH THE INCORPORATION OF THYME, EUCALYPTUS, LEMON AND NIAOULI OILS

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Abstract

Edible packaging has been used for years in foods and it plays an important role in the food packaging industry due to its advantages over synthetic films. Essential oils are a good source of bioactive compounds that possess antioxidative, pharmacological and antimicrobial properties, so in food products, they can be used to control the growth of pathogenic and spoilage microorganisms, and extend shelf-life. The aim of this study was to evaluate the antimicrobial properties of pea protein based edible films with the incorporation of thyme, eucalyptus, lemon and niaouli essential oils (in different concentrations as 1% v/v, 2% v/v, 3% v/v, 4% v/v, 5% v/v). The antimicrobial activity of edible films was measured using agar disc diffusion method. The antimicrobial activities of films were investigated for their inhibitory effects towards Gram-positive *Staphylococcus aureus*, Gram-negative *Escherichia coli* and fungus *Aspergillus niger*. According to the results, thyme oil showed antimicrobial effects against *E. coli, S. aureus* and *A. niger* at 2%, 3%, 4%, 5% v/v concentrations. Also, niaouli oil had antibacterial effect at 2% v/v against *S. aureus*. Further studies are necessary to evaluate the efficacy and applications of these oils on industrial scale due to their high antimicrobial activities.

Key Words: Edible films, essential oils, food packaging, antimicrobial activity

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ORAL PRESENTATION

EFFECTS OF IRRIGATION AND TYPE OF SUBSTRATE ON THE MORPHOLOGICAL AND PRODUCTION CHARACTERISTICS OF BIOTYPES OF *ROSMARINUS OFFICINALIS* L. GROWN IN POTS

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Abstract

Irrigation and growing medium are essential aspects of agronomic technique to obtain good productive and qualitative performance of the potted rosemary plants. In pot cultivation, the chemical characteristics, physical and biological substrate must be stable over time to allow regular plant growth. However, the effects of the cultivation on the characteristics of potted rosemary are little known. There peat is traditionally used as a biological substrate; however, despite the numerous advantages, its use has led to a degradation of the peat bogs northern hemisphere and an increase in greenhouse gases in the atmosphere. The purpose of the present study was to evaluate the effects of irrigation and alternative substrates to peat in terms of morphology, aesthetics and the production characteristics of the biotypes of Sicilian rosemary in pots with different types of habitus. They were used two years, two different irrigation levels, three alternative substrates to the peat and three types of rosemary. The habitat of the plants was tested in a project by split into two parts for a four-factor experiment. The results have showed that irrigation and substrate resulted in differences significant for all tested parameters. The rosemary plants showed the better performance when watering was more frequent; on the contrary, the content higher percentage in essential oil was obtained when the events of watering were less frequent. The chemical-physical characteristics of the substrates alternatives to peat have changed with decreasing peat content e the increase in compost content. The biotype of the erect habitat has been shown to have the best ability to adapt to various treatments. Our results suggest that irrigation and alternative substrates to peat affect in growth of rosemary plants significantly and should, therefore, be used in order to improve the cultivation of this species in pots a ornamental purpose.

Key Words: Aromatic species; alternative substrates; irrigation; plant habitus; sustainable cultivation; essential oil

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ORAL PRESENTATION

AUTUMN LEAVES FRAGRANCE OIL

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Abstract

In autumn the trees that bid farewell to their leaves, with their foliage release the seasonal scents. *Cercidiphyllum japonicum*, Cercidiphyllaceae and *Punica granatum*, Lythraceae release maltol giving the air a pleasant scent. Plants that undergo similar process, Ginkgoaceae and Caprifoliaceae leaves give the air completely opposite scent. There is a reason for that. The goal of the work was to compare the compounds relased from *C. japonicum* and *P. granatum* autumnal leaves with the compounds in the oils that coat *Gingko biloba*, Ginkgoaceae autumnal leaves. Supercritical fluid extractions (SFE), with carbon dioxide, were done with autumnal leaves of: *C. japonicum*, *P. granatum* and *G. biloba*. The SFE extracts were subject to the gas chromatography-mass spectrometry (GC-MS) analysis. The chromatograms obtained revealed the major compounds influencing the autumnal leaves scent. In *C. japonicum* and *P. granatum* major compounds present were: L-limonene, *trans*-anethole and maltol. In *G.biloba* major compounds identified were: borneol, bornyl acetate, phytol and neophytadiene. The major compound of autumnal leaves fragrance oil in *C. japonicum* and *P. granatum* is maltol. There are several compounds influencing the scent of autumnal *G. biloba* leaves.

Key Words: *Cercidiphyllum japonicum*, *Punica granatum*, *Gingko biloba*, SFE, GC-MS

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ORAL PRESENTATION

EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA ON BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS OF BASIL

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Abstract

Medicinal plants are natural reservoirs for of therupatic agents. Phytochemial contents of plants depend upon their cultivation conditions especially under various abiotic and biotic stresses. The new and advance strategy for the production of medicinal plant is the use of Plant Growth Promoting Rhizobacteria (PGPR) which not only modulate the growth and secondary metabolites of plants but also decrease the relince of chemical fertilizer, particularly under stressed conditions. Present study was carried out to determine the effect of Plant Growth Promoting Rhizobacteria (PGPR) on biochemical, physiological and morphological parameters of basil (*Ocimum basilicum* L). Seed inoculation was carried out by a consortium of four PGPR strains (*Bacillus* sp., *Azospirillum brasilense, Azospirillum lipoferum, and Pseudomonas stutzeri.* Seeds were surface sterilized and were soaked in the bacterium inoculums for six hours. Experiment was carried out in glass house of university campus. At vegetative stage drought condition was imposed by restricting water for ten days while control conditions received normal supply of water. Drought stress has significant effect on physiology of plants by reduction in leaf chlorophyll, relative water content and leaf water potential. In the same way, drastic effect on biochemical parameters of basil plant were observed in water deficit condition. Inoculation of plants significantly improved the germination parameters. PGPR inoculation in plants leads towards better stress tolerance by improving different morphological parameters (root and length, fresh and dry biomass), physiological parameters (water potential, solute potential, osmotic potential, membrane stability and chlorophyll content), biochemical parameters (soluble protein, proline content, sugar analysis, free amino acid, enzymatic and non enzymatic antioxisants) and plants secondary metabolites. The results of present study indicate that the inoculation with PGPR improved not only growth but also different properties of basil plants particularly under drought stress.

Key Words: Basil, medicinal properties, PGPR, Drought.

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ORAL PRESENTATION

EVALUATION OF ANTI-TYROSINASE AND ANTIOXIDANT ACTIVITIES OF *LAMIUM* L. TAXA GROWING IN TURKEY

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Abstract

The genus *Lamium* L., which belongs to the Lamiaceae family, is widely distributed in Europe, Asia, and North Africa. *Lamium* species have been reported to have various ethnopharmacological uses worldwide. In this study, the ethanol extracts of 31 *Lamium* L. taxa collected from Turkey were investigated for their antioxidant activity using DPPH radical scavenging and ferric-reducing antioxidant power (FRAP) methods. Besides the extracts were subjected to tyrosinase (TYR) inhibitory activity assay, which is an enzyme related to skin hyperpigmentation and formation of neuromelanin, which is also linked to pathophysiology of Parkinson's disease (PD). The studied *Lamium* species were collected throughout Turkey. The herba and root extracts of the plant samples were prepared separately with 96% ethanol. Antioxidant activity of the etanol extracts was investigated by microplate-modified DPPH radical scavenging and FRAP methods. Their *in vitro* inhibitory effects on TYR were determined using ELISA microtiter assays. Among the tested extracts, 42 extracts showed varying levels of DPPH radical scavenging activity (11.06±0.36 – 55.07±1.82%), whereas all extracts showed antioxidant activity with FRAP method. *L. cariense* R.R. Mill collected from Mount Ida showed the highest antioxidant activity in both methods with 55.07±1.82% by DPPH method and 1.143±0.045 by FRAP method. The highest TYR inhibitory activity at 200 µg/mL was found in the root exract of *L. pisidicum* R.R. Mill collected from Seydişehir to Manavgat (35.46±0.63%), while the inhibitory activity of alpha-kojic acid at 200 µg/mL, used as reference, was found 76.58±0.85 (IC_{50}= 52.42±2.67 µg/mL). As a result of our findings, although antioxidant activity of some *Lamium* species is variable, the species seem to be more active in FRAP method. However, their anti-TYR activity was observed to be low to moderate. Our further studies will focus on identification and isolation of compound(s) responsible for antioxidant and anti-TYR activities of *L. pisidicum*.

Key Words: *Lamium*, tyrosinase inhibition, DPPH, FRAP, antioxidant activity

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ORAL PRESENTATION

BIOACTIVE COMPOUNDS FROM *IPOMOEA PES-CAPREA* (L.) R. BR.: EXTRACTION METHODS, CHEMICAL CHARACTERIZATIONS, AND ANTI-INFLAMMATION OF TOXIC EFFECTS FROM THE JELLYFISH VENOMS

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Abstract: In Thailand, folk wisdom uses fresh leaves of *Ipomoea pes-caprea (L.) R. Br.* to treat inflammation of toxic effects from the jellyfish venoms and dermatitis. Various extraction methods are important to obtain effective bioactive compounds with high yield extraction and were analyzed types and content of the major substances in *Ipomoea pes-caprae (L.) R. Br.*This study aims to use different extraction methods: maceration assisted extraction, ultrasound-assisted extraction, microwave-assisted extraction, sub-supercritical fluid extraction, spray dry, and espresso machine for evaluating the yield of eugenol, β-damascenone, 3,5-Di-O-caffeoylquinic acid, quercetin, and quercetin-3-glucoside bioactive contents as well as determining antiinflammation from each part of *I. pes-caprae*. The extraction by reflux using six different methods such as maceration, microwave, ultrasound, sub-supercritical fluid, spray dryer, and espresso machine were used to extract the leaves. The phytochemical fingerprint of the bioactive profile of each extraction method was characterized using HPLC. The anti-inflammation activity was determined using the reduction of nitric oxide radical content in Raw264.7 cells. The results showed that spray dry of leaves was the highest amount of yield and bioactive compounds. The concentration of 250 µg/ml was able to both reduce the NO radical content in Raw264.7 cells well and no toxicity to Raw264.7 cells, Vero cells, Human Keratinocyte Cell Line (HaCaT cells), and Normal Human Dermal Fibroblast (NHDF) with the number of active substances were eugenol (100.17±0.38 µg/ml) quercetin-3-glucoside (54.09±0.94 µg/ml) and 3,5-Di-O-caffeoylquinic acid (28.61±1.39 µg/ml). The crude extract of the plant *Ipomoea pes-caprae (L.) R. Br.* with spray dryer showed the most compromising results in terms of an inhibitory effect on nitric oxide synthesis in vitro for potential anti-inflammation effect, and the best effective extraction of bioactive contents for relieving toxic effects from the jellyfish venoms.

Key Words: *Ipomoea pes-caprea (L.) R. Br.*, anti-inflammation effect, extraction methods, HPLC, 3,5-Di-O-caffeoylquinic acid, eugenol

Acknowledgements: The authors gratefully acknowledge the support of a research grant by the Thailand Science Research and Innovation (TSRI) and Burapha University.

ORAL PRESENTATION

BIOLOGICAL CHARACTERIZATION OF ANTHOCYANINS IN OXIDATIVE STRESS AND URINARY INFECTIONS

Oana Cioanca[1*], Cornelia Mircea[1], Remo Dorneanu[1], Ioana Humulescu[3], Ionut Iulian Lungu[1], Silvia Robu[2], Dana Tutunaru[2], Monica Hancianu[1]

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Abstract

The present study includes the evaluations of the antioxidant and antimicrobial properties of three plant sources *Aronia melanocarpa*, *Sambucus nigra*, and *Vaccinium myrtillus*, from which two types of extracts were obtained: ethanolic and acetonic, as selective fractions of anthocyanosides. By chemical analysis techniques, a series of anthocyanin compounds were identified, predominantly glycosides of cyanidol. Cyanidin-3-glucoside is found mainly in all samples included in the study, but even in this context interspecific variability is visible, cyanidol sambubioside exceeds its glucoside in elderberry extracts, and delfinidol glucoside and cyanidol galactoside have higher values in blueberry fruits. In each antioxidant test, the ethanolic extract was the most active, this being directly correlated with the amount of active principles in the extracts. For the stability of the erythrocyte membrane, the same tendency of the intensity of the effects was observed, which corresponds to the results from the evaluation of the antioxidant potential. The antioxidant and membrane stability tests show the best of effects for the aronia ethanolic extract. All samples have antimicrobial and antibiofilm potential, with MIC values below 10 mg/mL, the lyophilized ethanolic fractions are more advantageous for antimicrobial action. Aronia fruit extracts have a better potential to inhibit biofilm formation (MIC below 5 mg/ml, activity on 11 of the 18 tested bacterial strains). The antimicrobial action and the antibiofilm capacity are directly correlated with the concentration of total anthocyanosides. The synergistic effects with antibiotics were significant for the elderberry extracts and less so for aronia. The results showed that the investigated extracts have good biological properties that recommend them in subsequent studies to obtain pharmaceutical forms indicated in inflammatory or infectious pathologies.

Key Words: Anthocyanosides, antioxidant, antimicrobial, uropathogens

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ORAL PRESENTATION

INVESTIGATION of ANTIMICROBIAL ACTIVITIES of THE SPECIES BELONG to THE GENERA of *TYMBRA*, *THYMUS* and *ORIGANUM* in LAMIACEAE FAMILY from TURKEY

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Abstract: In this study, it was aimed to determine the antimicrobial activities and genotypic characterization of the species belong to the genera *Thymbra*, *Thymus,* and *Origanum* in the *Labiatae* family in Turkey. A total of 30 extracts were obtained from the plants in each species by 24-hours extraction process using 3 different solvents (hexane, ethanol, and chloroform) in the Soxhlet device. The antimicrobial activities of the extracts against to bacterial strains [*Eshericia coli* ATCC 25922, *Pseudomonas auriginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212] were determined using the disk diffusion method. It was found that antimicrobial active substances were obtained more effectively with ethanol and chloroform than hexane. The highest antimicrobial effect was observed on the bacterial strain *S. aureus* ATCC 29213, and in the second order on *E. facealis* ATCC 25922. Effectiveness levels in the antimicrobial activities according to the sigma values in ANOVA results indicated that the antimicrobial activities were significant in the species *Thymbra spicata* var. *spicata* L., *Thymus vulgaris* L., *Thymus citriodorus* (Schreb), *Thymus cilicicus* Boiss. & Bal., *Origanum syriacum* L., and *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart, while the antimicrobial activities were not significant in the species *Origanum minutiflorum* O. Schwarz & P.H. Davis, *Origanum onites* L., *Origanum saccatum* P. H. Davis, and *Origanum vulgare* L. ssp. *gracile* (C. Koch) Ietswaart. Molecular genotypes of the thyme species were characterized by using 10 ISSR primers. The *Pearson*'s correlations result between the antimicrobial effects of the extracts and the ISSR loci showed very strong positive correlations. In addition, ISSR markers were successful in clustering the thyme species analyzed in this study according to their genus in general. Consequently, it was observed that different thyme species had different levels and effective antimicrobial activities.

Keywords: ISSR, antimicrobial activity, *Thymus*, *Origanum*, *Thymbra,* disc diffusion method

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ORAL PRESENTATION

UTILIZATION OF POTATO PEEL EXTRACT AS A SOURCE OF NATURAL ANTIOXIDANT AND INCORPORATION INTO VALUE ADDED PRODUCTS

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Abstract

Food processing industries are emerging rapidly and are one of the most important industries across the globe. Most of the byproducts are organic in nature and must be disposed properly to avoid environmental hazards. Some byproducts that are disposed as wastes are actually rich sources of bioactive compounds namely- antioxidants, dietary fibers, minerals, etc. and hence can be reused. Natural antioxidants have gained interest in recent years for their role in food preservation and nutrition. In the present study, potato peels that are by-products of various potato-based industries were chosen. Potato peels contain high natural antioxidant activity as well as nutritional value. Potato peels were collected, dried and their total chemical composition (Carbohydrate, Protein, Mineral content etc.) was determined. Ethanol and methanol extracts (10%) were prepared and the total phenolic content measured using Ferric reducing Antioxidant Activity (FRAP) and radical scavenging activity by 1,1-Dipyridyl-2 picrylhydrazyl (DPPH). Total phenolic content in methanol extract and ethanol extract was found to be 4.5g of Gallic acid/ 100g potato peels and 3.64 g of Gallic acid / 100g potato peels. Methanol extracts were found to have the best radical scavenging activity (85.6%) when compared to ethanol extracts (20%). Parameters that were used to assess the antioxidant activity were free fatty acid, Peroxide value and Thiobartituric acid (TBA). Results obtained indicate that potato peels extract exhibit strong antioxidant activity. The dried potato peels as well as their extracts would be added to various food products like chips, nachos etc. thereby enhancing their nutritive value and antioxidant levels. Such measures would not only enhance the nutritive value of products but are also environment friendly and help in solid waste management.

Key words: Potato; Potato peel extract; antioxidant activity; phenolic content; lipid oxidation.

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ORAL PRESENTATION

EFFECT OF INORGANIC FERTILIZER AND MICROBIAL CONSORTIUM TO GROWTH AND PRODUCTION OF SWEET BASIL (*OCIMUM BASILICUM* L.)

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Abstract

Sweet basil, belonging to the Lamiacea family, is of economic importance culinary aromatic herbs and is cultivated throughout the world, and its aromatic leaves are used fresh or dried as a flavoring for foods, confectionery products and beverages. The study assessed possible effects of NP (100 kg ha^{-1} N and 100 kg ha^{-1} P) and P fertilizer (100 kg ha^{-1}) mineral fertilizer, two commercial liquid bio-fertilizer and inoculation with multi-traits bacteria based three consortium in three strains combinations (*Pseudomonas fluorescens* RC512 + *Bacillus licheniformis* RC601 + *Bacillus subtilis* RC210, *Bacillus megaterium* RC16 + RC512 + RC210, and RC512 + RC601 + RC16) on the growth, yield, and oil content in various sweet basil (Ispir, Yusufeli and Kayseri) populations in field conditions of Çanakkale. The experiment was arranged as a completely randomized block design with eight treatments and three replicates. The biofertilizers and bacterial consortia inoculation involved dipping the root system of the seedling into a suspension of each formulations for 60 min, prior to planting. Inoculations of basil with RC512+RC601+RC210, RC512+RC210+RC16, and RC512+RC601+RC16, BMusaGreen, and BMusaVita and gave increases over control respectively of by 16.9, 19.1, 22.9, 18.1, and 20.7 % in plant height, by 14.1, 20.4, 24.9, 25.3, and 25.9 % in branch number per plant, by 7.2, 13.3, 16.4, 13.5, and 14.0% in fresh herba yield per plants, by 9.6, 18.1, 24.7, 17.9, and 19.5 % in dried leave yield per plants and by 15.3, 18.5, 17.7, 20.2, and 18.1 and 2.6 % in essential oil ratio. NP and P applications, however, increased height of plant up to 22.9 and 13.1%, branch number per plant by 24.8 and 6.0 %, fresh herba yield of plants by 18.4 and 4.0 %, yield of dry leaves by 22.1 and 4.4 % and essential oil ratio by 18.1 and 2.5%, respectively.

Key Words: *Ocimum basilicum* L., fresh herb yield, dry leaf yield, Co-inoculation, bio-fertilizers, essential oil content

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ORAL PRESENTATION

AN IMPORTANT NATURAL RESOURCE FOR HEALTH AND NUTRITION: OLEASTER (*Elaeagnus angustifolia* L.)

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Abstract

Elaeagnus angustifolia L. belongs to the family of Elaeagnacea and is an important medicinal plant associated with numerous pharmacological activities, which can be used as natural antioxidant source. Oleaster is an herbal resource with the same name for both its tree and fruit. The roots, leaves, flowers, fruits, fruits peel, and seeds of the oleaster tree are used in food, medicine, pharmacy, and perfumery. It has ecological and economic use, suitable for adverse conditions such as drought, salinity, rocky, and alkalinity. It has diuretic and antipyretic effects. Fruit extracts can be used in traditional medicine as anti-inflammatory and analgesic. It is stated that it provides body resistance, protects against diseases such as cold and flu, and relieves cough and diarrhea. Oleaster consumption is recommended because smelling its flowers provides mental clarity and refreshment and reduces the possibility of developing cancer. Its flowers are used as a nectar source for bees and as a flavoring agent in liqueur production. Oleaster fruits are rich in nutritional value and contain natural antioxidant, phenolic and flavonoid compounds. Since it contains various compounds from different phytochemical categories, it could be proposed for the discovery of new drugs. Oleaster is rich in protein, sugar, amino acids, various vitamins (tocopherol, carotene, vitamin C, thiamine), mineral substances (calcium, magnesium, potassium, iron, zinc and manganese) and fatty acids. For this reason, it is seen that it is used as an additive to food products in recent studies. Although it has high nutritional value and many beneficial effects on health, its consumption is not common. Therefore, in recent years, research has focused on its use in various food formulations and successful results have been obtained. In this study, the subject will be explained in detail.

Key Words: *Elaeagnus angustifolia* L., oleaster, Russian olive, phenolic compounds, antioxidants, nutritional value.

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ORAL PRESENTATION

APPLICATION OF INORGANIC FERTILIZER PROMOTES NUTRIENTS IN THE GINGER RHIZOME AND SOIL ENZYMES ACTIVITIES

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Abstract

Ginger is used as one of the important ingredients in traditional as well as modern medicine besides as a spice. It boosts immunity and is a rich source of many biologically active substances and minerals. Although it is a medicinally important crop, its productivity is, however, affected due to poor nutrient management and therefore it requires an adequate supply of nutrients in the form of inorganic fertilizers or organic manuring, or a mixture of both. In this context, the present study was aimed to investigate the effect of mineral fertilizers on the content of mineral elements in the ginger rhizome, on soil enzyme activity, and soil properties. Lysimeter experiments were conducted at the Institute of Genetics and Plant Experimental Biology, Kibray, Tashkent region, Uzbekistan. The experiment comprised of four treatments T1 – Control, T2 - $N_{75}P_{50}K_{50}$ kg/ha, T3 - and T4 - $N_{100}P_{75}K_{75}$+$B_3Zn_6Fe_6$ kg/ha. The results showed that the application of $N_{125}P_{100}K_{100}$ kg/ha increased rhizome K content by 49%, P content by 20%, and Na content by 58% as compared to control without fertilizer. While the application of $N_{100}P_{75}K_{75}$+$B_3Zn_6Fe_6$ kg/ha showed a significant enhancement in rhizome K, Ca, P, Mg, Na, Fe, Mn, Zn, Cu, Cr, Mo, and Si contents over the control. This treatment also improved active P content by 29%, total P content by 80%, total K content 16%, and N content by 33% content, and the activities of urease, invertase, and catalase activities as compared to control of without mineral fertilizer and control respectively. Thus, the application of NPK+BZnFe at the rate of 100:75:75:3:6:6 kg/ha helps in improving macroelements and microelements in the ginger rhizome and activities of soil enzymes that helps in mineral nutrition of the rhizome.

Keywords: Ginger, mineral fertilizers, rhizome nutrients, soil nutrients, soil enzymes

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ORAL PRESENTATION

DETERMINATION OF SOME MORPHOLOGICAL, PHYSIOLOGICAL AND QUALITY PROPERTIES OF BAY LAUREL (*Laurus nobilis* L.) POPULATIONS

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Abstract

This study was carried out on single plants belonging to the laurel (*Laurus nobilis* L.) population in the experimental area of Ege University Faculty of Agriculture Department of Field Crops in Bornova. In the study, 40 laurel plants (12 females and 28 males) selected from the population in the experimental area were used as plant material. Leaf samples were taken from the lower, middle and upper parts of the plants for five months (May, June, July, August and September) in 2019 and some morphological, physiological and quality characteristics were determined in these samples. Parameters determined in the research were; plant height, leaf width and height, leaf fresh and dry weight, moisture content, leaf chlorophyll value (SPAD), leaf area, specific leaf area (SLA) and essential oil ratio. As a result of the study, the average width of the leaf was 28,16 mm, while the average leaf length was 70.23 mm. The average leaf fresh weight was determined as 0.33 g, the average leaf dry weight was 0.19 g, and the average fresh and dry weight ratio was 1.72. Moisture content of the leaves were between 34.55%-49.48% and 41.16% on average, and SPAD value was between 40.99-46.76% with an average of 43.32. As the months progressed from May to September, it was observed that there was a decrease in parameters such as fresh weight, moisture content and SPAD value from upper parts to lower parts of the plants. The average leaf area was 13.9 cm2, and the specific leaf area average was 0.73 dm2. The average essential oil ratio was 1.53% while, the values varied between 0.94% and 1.96%.

Keywords: Bay laurel, *Laurus nobilis* L., essential oil, leaf chlorophyll content, SPAD value.

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ORAL PRESENTATION

PHARMACOLOGY AND MOLECULAR BASED BIOINFORMATICS ANALYSIS ON CARVACROL

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Abstract

Pharmacology and molecular based bioinformatics approaches are an emerging discipline to elucidate potential molecular mechanisms and pharmacological properties of natural compounds. Therefore, target genes, proteins, and molecular pathways modulated by carvacrol in human genome and proteome were aimed to identify in this work. Network-based bioinformatics analyses was performed by using ChEBI database, DIGEP-Pred, GeneCards database, STRING and KEGG enrichment database in the current research. A total of 17 proteins including CASP8, CAT, CCL2, CD14, CD83, COL1A1, ESR2, FLT1, HMOX1, KLK3, MDM2, PPARA, RAC1, RARA, TIMP1, TNFRSF1A, and VDR effected by carvacrol were determined by gene set enrichment analysis. In addition, multiple molecular pathways such as cancer pathway, transcriptional misregulation in cancer, tuberculosis, human cytomegalovirus infection, Chagas disease, HIF-1 signaling pathway, p53 signaling pathway, and TNF signaling pathway were also detected to be regulated by carvacrol. This research demonstrated that carvacrol exhibits highly active pharmacological activity as an anti-inflammatory, antimicrobial, chemoprotective and neuroprotective agent. However, biological activities and pharmacological properties of carvacrol have already been determined, molecular signaling pathways, gene targets, and pharmacological properties based on bioinformatics analyses have not been fully revealed. Consequently, this work is network-based scientific research that will be very useful in understanding the biological, molecular and pharmacological properties of carvacrol for clinical applications.

Keywords: Carvacrol, network-based pharmacology, molecular pathway, bioinformatics, gene database, protein-protein interactions

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ORAL PRESENTATION

INVESTIGATION OF THE EFFECT OF SIX PLANT SPECIES ON ENZYMES ASSOCIATED WITH DIABETES AND OBESITY

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Abstract

In this study, the inhibitory effect of six plant species on enzymes that associated with carbohydrate and lipid metabolism were investigated. Their antioxidant activities, total phenol and flavonoid contents were also evaluated. Methanol and water extracts were prepared from aerial parts of *Centaurea solstitialis* L. subsp. *solstitialis, Convolvulus arvensis* L., *Crocus ancyrensis* (Herb.) Maw, *Marrubium vulgare* L., *Sideritis caesarea* H. Duman, Aytaç & Başer and *Thymus praecox* Opiz. All extracts were evaluated for α-amylase, α-glucosidase and pancreatic lipase inhibitory activities in vitro. In addition, their total phenol, flavonoid contents and antioxidant/radical scavenging activities by different methods (metal chelating, super oxide dismutase, reducing power, ABTS, CUPRAC, DPPH) were also determined [1-6]. Methanol extracts of *M. vulgare* and *T. praecox* came into prominence with their high α-glucosidase inhibitory activities (IC50: 163.80 and 268.03 µg/mL, respectively). Only *C. ancyrensis* methanol extract displayed moderate α-amylase inhibitory activity (38.54%); while the methanol extracts of *C. solstitialis* subsp. *solstitialis*, *C. arvensis* and *M. vulgare* exhibited moderate pancreatic lipase inhibitory activity (23%). Generally, all the studied extracts showed moderate to high antioxidant activity in CUPRAC, ABTS, SOD, metal chelating and DPPH methods. The highest total antioxidant activity with phosphomolybdenum assay was observed by *C. ancyrensis* water extract. Among the studied plants, methanol extract of *M. vulgare* attracted attention with its high α-glucosidase and pancreatic lipase inhibitory activity; while the methanol extracts of *C. ancyrensis* came into prominence with its α-amylase inhibitory activity. The obtained results reveal the necessity of doing more detailed studies on the mentioned extracts.

Key Words: *Marrubium vulgare*, *Crocus ancyrensis*, α-glucosidase, α-amylase, pancreatic lipase, antioxidant.

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ORAL PRESENTATION

THE PERFORMANCE OF BIOAPIFIT® ANTI ATOPIC OINTMENT IN THE TREATMENT OF *PSORIASIS VULGARIS*

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Objective / Purpose: *Psoriasis vulgaris* is chronic relapsing inflammatory skin disorder characterized by the presence of pink to erythematous plaques with overlying silver hyperkeratotic plaques. The purpose of this study was evaluation of the performance and safety of Bioapifit® anti atopic ointment compared to cream base in the treatment of the patients suffering from Psoriasis vulgaris for 3 to 15 years. **Materials and methods:** The study included 100 patients with *Psoriasis vulgaris* according to predefined inclusion and exclusion criteria. The study involved both women and men older than 18 years of age. 50 participants were treated with Bioapifit® anti atopic ointment which was applied onto all affected parts of the body three times daily for 28 days. Another 50 of them were treated with cream base which was applied onto all affected parts of the body three times daily for 28 days. Bioapifit® ointment consisted of Glycerin, *Prunus Amygdalus* Dulcis oil, Mel (certified organic), *Cera flava*, *Helichrysum italicum* flower extract, *Nigella sativa* seed oil, *Oenothera biennis* oil oil, *Argania spinosa* kernel oil, *Theobroma* Cacao, *Calendula officinalis* flower extract, *Matricaria chamomilla* flower extract, *Lavandula officinalic* flower extract, *Achillea millefolium* extract, *Salvia officinalis* extract, *Plantago major* leaf extract, *Olea europaea* leaf extract, *Melaleuca alternifolia* leaf oil, *Thymus vulgaris* ct. thymol oil, *Origanum vulgaris* oil. **Results:** The treatment of the patients with Psoriasis vulgaris with Bioapifit® anti atopic ointment resulted with decrease of total Psoriasis Area and Severity Index (PASI) score from 77.3 to 98.1% (88.4±4.1%). In the end of the treatment the total PASI score decreased from the baseline value of 37.4±12.2 to 3.2±1.1. In the control group the mean total PASI score compared to baseline was reduced for 14.3±27.1%. In the experimental group the mean scores for the Head/neck, upper limbs, trunk and lower limbs decreased for 89.5±10.4%, 88.4±9.1%, 52.1±8.0% and 91.4±11.4%, respectively. In the case of the control group the score for Head/neck decreased 8.5±34.3%, upper limbs for 14.3±34.8%, 17.6±32.3% for the trunk and 17.6±29.1% in the case of lower limbs. None of the patients experienced any adverse effect during the treatment and follow up period. **Conclusion / Discussion**: Bioapifit® anti atopic ointment is safe and clinically efficient in alleviating the symptoms of chronic, inflammatory skin diseases like Psoriasis with clinical cure rate of 70% thanks to its moisturizing, humectants, emollient,coating, soothing, and pH adjusting effect.

Key words: anti atopic ointment, Bioapifit, Psoriasis vulgaris, inflammatory skin disease

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ORAL PRESENTATION

MERGING THE MULTI-TARGET EFFECTS OF KLEEB BUA DAENG, A THAI TRADITIONAL HERBAL FORMULA IN UNPREDICTABLE CHRONIC MILD STRESS-INDUCED DEPRESSION

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Abstract

Major depressive disorder (MDD) is a common and debilitating psychiatric disease characterized by persistent low mood, lack of energy, hypoactivity, anhedonia, decreased libido, and impaired cognitive and social functions. However, the multifactorial etiology of MDD remains largely unknown due the complex interaction between genetics and environment involved. Kleeb Bua Daeng (KBD) is a Thai traditional herbal formula that has been used to promote brain health. It consists of a 1:1:1 ratio of the aerial part of *Centella asiatica, Piper nigrum* fruit, and the petals of *Nelumbo nucifera*. According to the pharmacological activities of the individual medicinal plants, KBD has good potential as a treatment for MDD. The present study investigated the antidepressant activity of KBD in an unpredictable chronic mild stress (UCMS) mouse model. Daily administration of KBD to UCMS mice ameliorated both anhedonia, by increasing 2% sucrose intake, and hopeless behavior, by reducing immobility times in the forced swimming test (FST) and tail suspension test (TST) without any effect on locomotor activity. The mechanism of KBD activity was multi-modal. KBD promoted neurogenesis by upregulation of brain-derived neurotrophic factor (BDNF) and cyclic AMP-responsive element binding (CREB) mRNA expression in the frontal cortex and hippocampus. Daily treatment with KBD significantly reversed UCMS-induced HPA axis dysregulation by upregulating the glucocorticoid receptor (GR) while downregulating serum- and glucocorticoid-inducible kinase 1 (SGK1) and FK506 binding protein 5 (FKBP5) mRNA expression. KBD treatment also normalized proinflammatory cytokine expression including tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-1β and IL-6. KBD and its component extracts also exhibited an inhibitory effect in vitro on monoamine oxidase (MAO) A and B. The multiple antidepressant actions of KBD emphasize its potential as an effective, novel treatment for MDD.

POSTER PRESENTATIONS

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POSTER PRESENTATION

EFFECT OF SEVERAL PARAMETERS ON THE CHEMICAL COMPOSITION OF ESSENTIAL OILS AND SECONDARY METABOLITES EXTRACTED FROM AERIAL PARTS OF THREELAMIACEAE SPECIES

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Abstract

Background: *Thymus* numidicus, *Lavandula stoechas* and *Origanum glandulosum* are a member of Lamiaceae that considered as a rich source of essential oils and secondary metabolites, which are widely used in pharmaceutical industries and food production. The objectives of the current study were to evaluate effect of several parameters, such as harvest location and period, on the chemical composition of essential oils and secondary metabolites extracted from these species.
Methods: The aerial parts of three species were subjected to hydrodistillation to produce essential oil. Chemical identification of the oil composition was conducted by GC-MS analyses. The extraction of secondary metabolites (flavonoids and tannins) was carried out by solid-liquid extraction followed by liquid-liquid extraction using suitable solvents.
Results: GC / MS analysis of the essential oils show that variation in the chemical composition is affected by species of the plant, the locality of harvest as well as the geostrategic and meteorological conditions. LC / MS analysis of the alcoholic extracts (flavonoids and tannins) revealed the presence of nine compounds for the methanolic extracts obtained on the *Origanum* glandulosum and *Thymus* numidicus, one compound for those of *Lavandula* Stoechas and three compounds for the n-butanol fraction (flavonoids) extracted from *Thymus numidicus* and *Origanum glandulosum*.
Conclusion: The results suggest that the three plant species are rich in bioactive substances of therapeutic interest. It remains important to use these plant resources for economic development of pharmaceutical and cosmetic industries.

Keywords: Chemical composition, Essential oil, Secondary metabolite, *Thymus numidicus*, *Lavandula stoechas*, *Origanum glandulosum*

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POSTER PRESENTATION

ECOLOGY OF THE MEDICINAL PHYTODIVERSITY IN CENTRAL NORTHERN SAHARA (REGION OF OUARGLA)

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Abstract

The study concerns the ecological aspect of the medicinal phytodiversity at the level of the region of Ouargla, being situated in central northern Sahara. We proceeded to the ecological analysis of the phytodiversity and its potential ethnobotanic use. Indeed, the application of the subjective sampling in the different ecosystems (sebkha, chott, reg, erg, hamada, oued and daya), followed by ethnobotanic inquiries led with the autochthonous population allowed to list 34 species. They belong 22 families but the most involved in the regional pharmacopoeia are *Apiaceae, Amarenthaceae, Poaceae and Fabaceae.* The category of the long-lived is dominant with 25 species. They belong to diverse biological types with the dominancy of chamaephytes (38.23 %). *The class of the arido-active long-lived species is the most dominant* with 61.76 %. The most represented phytochores is the element Saharo-sindienwith 15 species (44.1 %), followed by the endemic set with 8 species (23.52 %). They are *Ammodaucus leucotrichus, Ferula vesceritensis, Oudneya africana, Cleome arabica, Urginea noctiflora, Euphorbia Guyoniana.* On the ethnobotanic plan, the affections handled by these plants are important of what we note approximately 55. The most frequent diseases are the rheumatism with 6 species (17.64 %), the dermatosis with 4 species (11.6 %) as well as the digestive diseases with 4 species (11.76 %). As a conclusion, it is advisable to note that the biological diversity and that phototherapeutic show themselves well. Also, the adaptation and the endemism characterizing these species seem to explain their tolerance to the hyper- aridity of the region of Ouargla.

Keywords: Flora, biology classes, herbal medicine, Ouargla.

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POSTER PRESENTATION

KNOWLEDGE AND ACCEPTANCE OF ALGERIAN PHYSICIANS TOWARD PHYTOTHERAPY AND HERBAL REMEDIES

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Abstract

Plants have been the first and main therapeutic tool for many centuries and in many civilizations. Today, the practice of herbal medicine is still relevant in developing countries like Algeria. Unfortunately, the use of medicinal plants in Algeria is done in an anarchic, uncontrolled and unregulated way and, consequently, serious consequences can affect patients and compromise their good care, especially since this use is often associated with the lack of training and knowledge of a large part of Algerian doctors on the benefits and risks of medicinal plants. This study aims to determine the relationship between the knowledge of Algerian physicians on medicinal plants and their acceptance of the practice of herbal medicine. A cross-sectional study was conducted in different health institutions in two regions (Tlemcen and Ain-Témouchent). The main tool that was used to collect the required data was a self-administered questionnaire that was specifically developed and approved by the researchers to meet the study objectives. The data collected was processed by two statistical software programs: SPSS and R++.

The study revealed that 54.85% of the participating physicians had below average knowledge about plants, and more than half of them acquired their knowledge through self-training. In addition, the results show that 30% of physicians tend to use herbal medicines, while only 12% prescribe them to their patients. Interestingly, a large majority (81.4%) of the participants expressed a desire to improve their knowledge of herbal medicines and there was a highly significant relationship between physicians' knowledge and their acceptance of herbal medicine (Spearman's test: $p=0.00066$). Almost all physicians (87.55%) agreed that knowledge of medicinal plants is important to them and should be included in the general medical curriculum.

Key Words: Physicians, Herbal medicine, Knowledge, Acceptance, Algeria.

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POSTER PRESENTATION

PLANT-DERIVED NATURAL COMPOUNDS AND CORONAVIRUS: A REVIEW

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Abstract

Coronaviruses are responsible for a growing economic, social and mortality burden, as the causative agent of diseases such as severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and recently COVID-19. Naturally existing compounds provide a wealth of chemical diversity, including antiviral activity, and thus may have utility as therapeutic agents against coronaviral infections. The main objective of this study is to review the currently available scientific literature on natural substances of plant origin that have promising antiviral effects against Coronavirus. The PubMed, Science Direct and Biomed Central databases were searched for papers including the keywords "*Coronavirus*", "*SARS-CoV-2*" as well as "*Alkaloids*", "*Polyphenols*", "*Phytosterols*", "*Terpenes*" and "*Secondary metabolites*" with 148 research articles selected. The majority of the studies on natural substances acting against Coronaviruses were carried out in the last two years: 2020 (38.6%) and 2021 (55.6%) coinciding with the emergence of the new coronavirus SARS-CoV-2. Most of the studies were performed by *in silico* methods with a percentage of 83%, 16.9% by *in vitro* methods and only 0.10% by *in vivo* tests. Our research has established a list of 964 natural substances of plant origin tested against Coronaviruses. Polyphenols represent the most tested secondary metabolites against Coronaviruses, followed by terpenes then alkaloids. Taking into account the frequency of citation in the studies, we ranked 63 most cited substances in descending order such as: Quercetin, Catechin, Glycyrrhizin, Kaempferol, Rutin, Curcumin, Myricetin, Apigenin, Hesperidin. In the future, we hope that the active ingredients of medicinal plants can be used to treat SARS-CoV-2 infection in humans.

Key Words: SARS-CoV-2, COVID-19, Natural coumpounds, Secondary metabolites.

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POSTER PRESENTATION

BIOLOGICAL ACTIVITIES OF A POLYPHENOLIC ENRICHED EXTRACT OF *ROSMARINUS OFFICINALIS* L.

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Abstract

Rosemary (*Rosmarinus officinalis* L.) is one of the most well-known and used species worldwide, having both culinary and therapeutic properties. Its most important biological activities are the antioxidant, antiinflammatory, antimicrobial, antiseptic, hepatoprotective, diuretic and antispasmodic ones. The aim of the present study was represented by the evaluation of the *in vitro* biological activities of a polyphenolic enriched extract obtained from *R. officinalis* on human hepatocellular carcinoma cells (HepG2) viability and on three bacterial strains, *Staphylococcus aureus*, methicillin resistant *Staphylococcus aureus* (MRSA) and *Escherichia coli*. The polyphenolic enriched extract of *R. officinalis* was obtained by solid phase extraction on a LiChrolut RP-18 E column and its phytochemical analysis was performed by a HPLC-MS method. The cytotoxicity against the HepG2 cells was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay compared to two normal cell lines: oral tissue derived mesenchymal stromal cells (pMSCs) and human foreskin fibroblast cell line (Hs27). The antimicrobial potential was investigated by the diffusion method, with the minimum inhibitory and bactericidal concentrations determined by the broth microdilution method. Furthermore, the antibiofilm activity was assessed by using the crystal violet test. The tested sample displayed significant cytotoxic properties on HepG2 cells ($p<0.05$) compared to the control and did not affect the normal cells' viability. No statistically significant ($p>0.05$) differences between the values determined by the five distinct concentrations were noticed, indicating that the cytotoxic activity against the tumoral cell line is not dose dependent. Antimicrobial activity was recorded against the selected bacteria, with the most intense effect towards the Gram-positive species. Results obtained hereby bring further arguments in order to sustain the antiproliferative and antimicrobial activity of *R. officinalis*.

Key Words: *Rosmarinus officinalis* L., polyphenolic enriched extract, human hepatocellular carcinoma cells (HepG2), viability, antimicrobial activity

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POSTER PRESENTATION

YIELD AND ESSENTIAL OIL COMPOSITION DIVERSITY OF DIFFERENTBASIL GENOTYPES

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Abstract

Sweet basil (*Ocimum basilicum* L.) is an annual plant which belongs to the Lamiaceae family. Genetic factors are one of the most important crucial parameters to influence the yield and biological values of basil besides environmental and agronomic factors. Essential oil composition reveals the quality of basil. The present study was conducted to determine the yield, essential oil content and compositions of different origin basil genotypes. Dino cultivar, PI 531396, PI 174284 and PI 253157 genotypes had the highest total fresh herb yield. The mean essential oil content changed between 0.67-1.19% and the highest essential oil contents were found from PI 379412 (1.19%) and Ames 29184 (1.18%) genotypes. The total essential oil compositions ranged from 60.25% to 97.66%. The major essential oil compositions were determined as estragole, linalool, citral, β-citral, methyl eugenol, α-bergamotene and δ-Cadinene.In conclusion, plant chemotypes were significantly differences in different origin basil genotypes and large essential oil composition variabilities were observed. It was thought that basil genotypes can be used for the desired properties in terms of yield, essential oil content andcompositions because of including wide variations.

Keywords: Sweet basil, essential oil, yield

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POSTER PRESENTATION

MORPHOLOGICAL, YIELD AND ESSENTIAL OIL CONTENT OF SAGE GROWN WITH DIFFERENT ORGANIC MANURE

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Abstract

Organic manures are alternative sources as environmentally friendly to recovery infertile soils in intensified agricultural applications. This study was conducted to determine the effect of organic manures on the quality of sage to find out the better sustainable fertilization practice for sage cultivation. The study was split-plots, arranged in randomized complete blocks with three replications. Main plots including different organic manures and different four doses of organic manures were allocated to sub plots. In this context, three different organic manures as sheep (500, 750, 1000, 1250 kg/da), chicken (500, 750, 1000, 1250 kg/da) and vermicompost (50, 100, 150, 200 kg/da) with a control (no manure) and conventional fertilizer were used. Our results showed that conventional fertilizer increased the values of all morphological and yield parameters of sage except for essential oil content in first harvest. These values showed differences in second harvest. Essential oil content was found higher in vermicompost manure than other manures in every two harvests. In conclusion, 150 kg/da vermicompost, 750 kg/da chicken and 1000 kg/da sheep manures had positive effects on yield values of sage. As a result of the study, vermicompost and chicken manure were found more useful than sheep manure for sage production.

Keywords: Sage, Salvia sp., Manures, Productivity

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POSTER PRESENTATION

EVALUATION of PLASTOQUINONE ANALOGS as ANTI-COLORECTAL and ANTI-BREAST CANCER AGENTS

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Abstract

1,4-Quinones such as plastoquinones (PQs) attract a great attention due to their potency as anticancer hit molecules. Based on the importance of PQs in anticancer drug design and discovery, somechlorinated PQs were synthesized and their anti-leukemic effects were reported by our group previously [1]. Among these PQs, the National Cancer Institute (NCI) selected three PQ analogs (**PQ11**, NCI: D-827199/1, **PQ12**, NCI: D-827200/1, and **PQ15**, NCI: D-827201/1) for preliminary *in vitro* anticancer screening at a single dose (10 µM) including the panel of 60 human cancer cell lines originating from nine cancer types such as colorectal and breast cancers. Results indicated that 2- chloro-5,6-dimethyl-3-((3-(trifluoromethyl)phenyl)amino)-1,4-benzoquinone (**PQ12**) displayed notable growth inhibition against HCT-116 colorectal and MCF-7 breast cancer cell lines with inhibition percent values of 66.14% and 64.64%, respectively. Besides, **PQ12** was analyzed for five- dose screening in both HCT-116 and MCF-7 cell lines and **PQ12** showed significant antiproliferative activity with 1.93 µM and 1.71 µM GI_{50} values, respectively. The cytotoxic effects of **PQ12** on HCT- 116 and MCF-7 cell lines were further investigated using MTT assay. It was determined that **PQ12** showed promising anti-colorectal and anti-breast cancer activity with IC_{50} values of 5.11±2.14 µM and 6.06±3.09 µM, respectively when compared with cisplatin (IC_{50}= 23.68±6.81 µM for HCT-116 cells; 19.67±5.94 µM for MCF-7 cells). Due to the promising anticancer data of **PQ12** on colorectal and breast cancer cells, the apoptotic effects of **PQ12** on both cell lines were assessed by annexin V/ethidium homodimer III staining method. According to the results, **PQ12** significantly induced apoptosis in HCT-116 and MCF-7 cells compared to cisplatin. Overall, **PQ12** stands out as a potent anti-colorectal and anti-breast cancer agent for future mechanistic studies.

Key Words: Colorectal cancer, breast cancer, plastoquinones, National Cancer Institute (NCI), apoptosis, cisplatin

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POSTER PRESENTATION

CYTOTOXICITY EVALUATION OF *INULA HELENIUM* AND *GENTIANA ASCLEPIADEA* ETHANOLIC EXTRACTS ON RATINTESTINAL EPITHELIAL CELLS

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Objective: *Inula helenium* L. (elecampane) and *Gentiana asclepiadea* L. (willow gentian) are known for their wide therapeutic effects such as antimicrobial, anthelmintic, antioxidant and anti- inflammatory [1, 2]. These effects are attributed to their most important active constituents, alantolactones and isoalantolactone for *I. helenium*, and secoiridoid glycosides and xanthones for *G. asclepiadea*. This study was carried out to determine the cytotoxic potential of 70% ethanolic extract of elecampane and willow gentian rhizomes and roots on rat intestinal epithelial cell culture. **Methods:** The cytotoxic potential of the extracts was determined using MTT assay. In addition, total polyphenolic (Folin Ciocalteu method), flavonoid content, and antioxidant capacity (FRAP, DPPH) of extracts were examined. **Results:** The results of MTT assay showed that inhibitory concentration 50% (IC50) was 13.43 ± 2.32 µg/ml for *I. helenium*, and 26.29 ± 4.36 µg/ml for *G. asclepiadea*, both plants showing insignificant cytotoxic activity on intestinal epithelial cells at all concentrations tested. However, at higher concentrations, both tested extracts influenced the morphology of the intestinal cells, causing them to become rounded and flatter, without affecting their viability. The total polyphenolic content of *I. helenium* and *G. asclepiadea* was 3.066 and 2.144 mg GAE/g of plant material respectively. Total flavonoid content was 0.602 mg RE/g of plant material for *I. helenium* and 0.280 mg RE/g of plant material for *G. asclepiadea*. The results of the FRAP test showed that *I. helenium* extract has higher antioxidant capacity compared to *G. asclepiadea*, with values of 629.04 and 145.23 µM trolox equivalent/100 ml of extract. Results of the DPPH test revealed moderate to low antioxidant activity of extracts, value for *I. helenium* being 173.2 µg/ml and 36.4 µg/ml for *G. asclepiadea*. **Conclusion:** The results of the present study indicate that both *I. helenium* and *G. asclepiadea* extracts could be considered safe for further *in vivo* studies.

Key Words: cytotoxicity, MTT, *Inula helenium*, *Gentiana asclepiadea*, ethanolic extract

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POSTER PRESENTATION

PHENOLIC PROFILE AND EVALUATION OF BIOACTIVE PROPERTIES OF TWO HALOPHYTHES

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Abstract

Taking in consideration of the stimulation of synthesis and accumulation of polyphenols and possible high antioxidant capacity of halophytes to cope with salt stress in the soil, in this study, phenolic profile and *in vitro* antioxidant and antimicrobial activities of two halophytic species growing on salty steps of inner Anatolia, *Limonium caspium* (Willd.) Gams. and *Frankenia hirsuta* L. were evaluated. Phenolic compounds of methanolic extracts prepared from aerial parts of both species were qualified and quantified by a LC–MS/MS analysis. In addition, total phenolic compound, *in vitro* antioxidant and antimicrobial activities of methanol extracts and their hexane, dichloromethane, ethyl acetate and water fractions were evaluated. LC–MS/MS analysis indicated that tannic acid was the most abundant phenolic acid in both species (6316.55±322.14 µg/g extract in *L. caspium* and 7596.32±387.41 µg/g extract in *F. hirsuta*), whereas hyperoside was the most abundant flavonoid (633.15±31.02 µg/g extract in *L. caspium* and 2628.94±128.94 µg/g extract in *F. hirsuta*). The highest total phenolic contents were determined 678.82±7.52 mg GAE/g extract in *L. caspium* and 652.55 mg GAE/g extractin *F. hirsuta*. The antioxidant capacity was evaluated by DPPH and total antioxidant capacity (TAC) assays and stronger antioxidant activity in ethyl acetate fractions were highlighted (DPPH: IC_{50}=16.008±0.32 µg/mL in *L. caspium* and 12.748 µg/mL in *F. hirsuta*; TAC: 0.353±0.001 mM UAE, 771.733±2.565 µM CRE in *L. caspium* and 0.698±0.014 mM UAE, 1527.36±30.49 µM CRE in in *F. hirsuta*). In conclusion, especially high phenolic content with interesting antioxidant activities as well as antimicrobial potential obtained in this study indicated that *L. caspium* and *F. hirsuta* are promising plants that can be evaluated in pharmaceutical, cosmetics and food industry.

Key Words: *Limonium caspium*, *Frankenia hirsuta*, LC-MS/MS, antioxidant, antimicrobial

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POSTER PRESENTATION

PHYTOCHEMICAL COMPOUNDS and ANTIOXIDANT CAPACITYOF *CISTUS* L. SPECIES

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Abstract

The genus *Cistus* (Cistaceae) is represented by five species in the flora of Turkey. These species are *C. creticus* L., *C. laurifolius* L., *C. monspeliensis* L., *C. parviflorus* Lam. and *C. salviifolius* L. (1). The aim of this study was to determine phenolic compounds and antioxidant capacity of *Cistus creticus*. The phytochemical compositions and antioxidant capacity of extracts were to determine HPLC-PDA and differential pulse voltammetric method, respectively. In this study, (+)-catechin, epigallocatechin gallate and rutin, were detected in 5 *Cistus* species extracts by HPLC analysis. *C. creticus* extract was found to have the most antioxidant activity. The ACA values calculated as a resultof the antioxidant capacity were 0.4764, 0.452, 0.052, 0.0172 and 0.0068 for *C. creticus*, *C. parviflorus, C. monspeliensis*, *C. salviifolius* and *C. laurifolius*, respectively

Key Words: *Cistus*, HPLC analysis, antioxidant

Acknowledgements

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POSTER PRESENTATION

CYTOTOXICITY EVALUATION OF *MARRUBIUM VULGARE* L. FROM DIFFERENT POPULATIONS OF TURKEY

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Abstract

Marrubium vulgare L. (Lamiaceae) is used for respiratory and gastrointestinal system disorders in Anatolian traditional medicine. The plant is also used in beverage and pharmaceutical industries and cultivated in different countries. Identifications and standards of the plant are described in many monographs and pharmacopoeias, such as European Pharmacopoeia. In present study, general secondary metabolites (phenolics, flavonoids) of *M. vulgare* from three locations in Aegean province of Turkey were analyzed quantitively by UPLC and cytotoxic potentials were evaluated.

In the samples, forsythoside B, arenarioside, verbascoside and apigenin-7-O-glucoside were determined in different ranges. When the sample from Izmir, west of Aegean province showed specifically cytotoxicity against human neuroblastoma (SH-SY5Y) cell line (IC_{50}=59.80 µg/mL), it has no effect non-cancerous NIH-3T3 cells.

This study will be contributed to limited knowledge of chemical profiles and cytotoxic effects of *M. vulgare* originated from Turkey.

Key Words: Lamiaceae, verbascoside, forsythoside B, cytotoxicity.

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POSTER PRESENTATION

TYROSINASE, ACETYLCHOLINESTERASE and BUTYRYLCHOLINESTERASE ENZYME INHIBITORY ACTIVITIES of *CISTUS CRETICUS L.*

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Abstract

The genus *Cistus* (Cistaceae) is represented by 21 taxa in the world and represented by 5 taxa including *Cistus creticus* L., *Cistus laurifolius* L., *Cistus monspeliensis* L., *Cistus parviflorus* Lam. and *Cistus salviifolius* L. in the flora of Turkey (1,2). In traditional folk medicine, *Cistus* species are used various medical purposes such as rheumatism, stomach ache, upper respiratory tract infections, skin diseases, burns, wounds and diabetes (3-5).

In this study, 80% ethanolic extract was prepared from the leaves of the *Cistus creticus* and tyrosinase,acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzyme inhibitory activities of extract were investigated. *C. creticus* extract showed potent activity with the IC_{50} values as 206, 164 and 19 ug/ml respectively.

This is the first study where the tyrosinase, acetylcholinesterase and butyrylcholinesterase inhibitory activities were investigated of *Cistus creticus* distributed in Turkey.

Key Words: *Cistus*; *Cistus creticus*; tyrosinase; cholinesterase.

Acknowledgements

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POSTER PRESENTATION

PHENOLIC COMPOUNDS AND ENZYME INHIBITORY POTENTIALSOF *MARRUBIUM PEREGRINUM* FROM TURKEY

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Abstract

Marrubium L. (Lamiaceae) has 40 taxa in the world and represented by 25 taxa in Anatolia. Different *Marrubium* species ere used for eczema treatment, antipyretic and expectorant in traditional medicine. Phytochemical investigations on this genus are generally revealed of phenolics, flavonoids and labdane diterpenes. In present study, phenolics and flavonoids of, were analyzed quantitively by UPLC. Additionally, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzyme inhibition potentials were evaluated. The plants were extracted with methanol in ultrasonic bath. Modified Ellman's method was used for their enzyme inhibition activities.

Phytochemical studies showed, verbacoside (acteoside), forsythoside B, arenarioside (forsythosideF), kaempferol-3-*O*-*β*-D-glucoside (astragalin) were found in various ranges. *M. peregrinum* inhibited AChE and BuChE in rates of 58.06% and 69.52%.

Key Words: Phenolic, flavonoid, *Marrubium*, UPLC, enzyme inhibition.

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POSTER PRESENTATION

AN ETHNOBOTANICAL STUDY OF MEDICINAL HALOPHYTES in KEŞAN (TURKEY)

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Abstract

In Turkey, as in several other countries, drought and salinity constitute the two major constraints responsible for the limitation of crop productivity and the deterioration of vegetation cover. This ethnobotanical study was carried out in Keşan province, which is a town of Edirne city located in the north west part of Turkey, near the North Aegean Sea, depending on details of halophytic plants used infolk medicine. Beside other economic uses, halophytes adapted to grow in saline soils of arid/semi-aridregions could be a potential source of natural antioxidants. Due to the extreme stress that the halophytes native to the harsh environment experience, they are likely to be superproducers of protective compounds. For his reason, several salt plants have traditionally been used for medicinal and nutritional purposes. This study focused on the potential uses of halophytes for food and therapeutic purposes by local people, as well as their impacts on the environment and societies, and aims to record information on traditional herbal medicine.

In this work, an assessment was carried out of the halophyte species native to the Saros Gulf region, collected in the summer along the coastal areas of Keşan, Edirne, Turkey. The samples of *Salicornia europae* L., *Salsola kali* L., *Plantago lagopus*, *Limoniastrum monopetalum* (L.) Boiss., *Suaeda maritima* (L.) Dumort., *Xanthium strumarium* L., *Eryngium maritimum* L., *Limonium narbonense* Mill., *Anchusacalcarea* Boiss. were characterized in order to evaluate their suitability for medicinal and nutritional purposes. Most of the characterized plant species were members of Chenopodioideae, Amaranthaceae,Plumbaginaceae families.

In conclusion, amounts of antioxidant compounds were analyzed, in an aim to find alternative raw materials for possible drug production. The present study highlighted the potential of medicinal halophytes as a source of natural antioxidants, valuable phytochemicals, and essential nutrients for pharmaceutical, nutraceutical, and chemical industries.

Key words: Ethnobotany, Medicinal Halophytes, Salinity, Antioxidant

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POSTER PRESENTATION

EFFECTS OF WALL MATERIALS ON AROMATIC PROFILE OF SPRAY DRIED CLARY SAGE OIL (*Salvia sclarea*)

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Abstract

Clary sage (*Salvia sclarea*) oil is a valuable essential oil due to its aromatic properties, bioactive constituents and beneficial health effects. Spray drying is the most common method used for microencapsulation of essential oils and aromas providing the opportunity to differentiate the taste and aroma of foods, to mask undesired tastes and odors, to ensure the stability of food components and to increase their bioavailability. The most important point to be considered in the encapsulation process of essential oils is the preservation of the natural composition of the essential oil. The aim of this study was to maintain flavor retention of spray-dried clary sage oil. Therefore, effects of wall material composition (80-100% maltodextrin, 0-10% gum Arabic, and 0-10% modified starch-EmCap®) on encapsulation of clary sage oil were investigated. The D-optimal design was used with 3 factors and 2 levels. Spray dried samples were characterized in terms of retention rates of volatile compounds in the clary sage oil using GC-MS. The retention rates of linalyl acetate (LA), linalool (LO) and α-terpineol (α-T) the main constituents of sage oil in powder products were compared. Encapsulation efficiency, moisture content, water activity (a_w), and glass transition temperature (T_g) were determined as well. The main volatile organic compounds (VOCs) of clary sage oil were identified as LA, LO, and α-T which constituted ~85% of the oil. Encapsulation efficiency of spray dried samples varied between 94.1 %- 98.6% whereas their T_g ranged from 54.9°C to 87.6°C. Moisture content values varied between 0.8 and 1.7%, while water activity (a_w) values were in the range of 0.11-0.20. According to the retention rates of linalyl acetate, linalool, α-terpineol, as well as the ratios of LA/LO and LA/α-T, a mixture of 95:5 maltodextrin:gum Arabic could be suggested as the most suitable wall material for microencapsulation of clary sage oil, which could best preserve the natural aromatic composition of clary sage oil.

Key Words: Clary sage, essential oil, spray drying, maltodextrin, gum Arabic

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POSTER PRESENTATION

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITYOF ESSENTIAL OILS AND HYDROSOLS FROM *SALVIA OFFICINALIS* L.

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Abstract

Sage (*Salvia officinalis* L.) is an aromatic and medicinal plant of the mint family (*Lamiaceae*), originated from the Mediterranean region and well known by its therapeutic effects. Many research has been carried out on essential oils (EO) extracted from this plant, while, studies on its hydrosols (HD) are very limited.

The objective of our research was to extract EO and HD from dried sage leaves which were collected from the region of Tiaret (Algeria) by using hydrodistillation and to evaluate their antioxidant and antibacterial activity.

The results of the phytochemical analysis showed that EO is richer in secondary metabolites namely polyphenols, flavonoids, condensed and hydrolysable tannins, than HD. By using DPPH method, ithas been shown that the percentage of inhibition of EO exceeded 65% with an IC_{50} of 2.61mg/gagainst 30% for HD which does not allow having an IC_{50}. The antibacterial activity was evaluated by Aromatogram (agar diffusion disc) against four pathogenic bacterial strains. EO exhibited strong antibacterial activity compared to HD, the most sensitive strain for HE was *Staphylococcus aureus* with a DZI of 25.5 mm and *Staphylococcus epidermidis* with DZI of 16 mm for HD. On the other hand, the EO incorporation test recorded a MIC of 1000µl / ml for *Staphylococcus aureus* and *Staphylococcus epidermidis* and 500 µl / ml for *Escherichia coli.*

This study confirmed the effectiveness of the essential oil of sage leaves. Indeed, the main goal of this study was the valorization of the hydrosol of this plant which is treated as a byproduct of the essential oil distillation process.

Key Words: *Salvia officinalis* L., Sage, Hydrosol, Essential oils, Antibacterial activity, Antioxidant activity.

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POSTER PRESENTATION

FORMULATION AND EVALUATION OF HERBAL ORAL GEL WITH *MATRICARIA RECUTITA*

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Abstract

Objective: To prepare and evaluate an herbal oral gel which contains essential oil extract of *Matricariarecutita.*
Introduction: *Matricaria recutita*, otherwise known as chamomile is a member of the Astaraceae family and is vastly grown in Europe and western Asia. Chamomile is one of the most widely used andwell-studied herbs in the world. Its floral part has been found to have several pharmacological activities which include; anti-inflammatory, antibacterial and antifungal properties. Chamomile extract hascompounds such as flavonoids, chamazulene, αbisabolol, bisabolol oxid A and B terpenes as well as theflavonoids are some of the many substances responsible for those pharmacological activities.
Methods: Chamomile essential oil was extracted from the aerial parts of *Matricaria recutita* and the analysis of the constituents was performed by Gas Chromatography Mass Spectrometry. Preformulation studies was carried out to decide the most suitable gel base depending on the physicochemical properties of the essential oil extract. Four placebo gel formulations were made using Carbopol 940P and Hydroxypropylmethyl cellulose were used in concentrations 0.5% or 1% and 2% or 2.5% (w/w) respectively. The evaluation of all placebo formulations was done by the analysis of different parameters such as organoleptic properties (colour, odour), pH, and viscosity.
Results: Compounds such as α-bisabolene oxide A and chamazulene was detected. Placebo formulations with 1% Carbopol 940P and 2.5% Hydroxypropylmethyl cellulose showed good consistency, appearance, and no separation with the pH within the desired range. Both gels showed shear thinning ability; as the shear stress increased, the viscosity decreased.
Conclusion: Results from the preformulation studies suggest that both placebo formulations mentioned above are suitable to produce an herbal topical gel, which will be further evaluated for its anti- inflammatory and anti-bacterial activities. It is anticipated that the next research stage will address the its anti-inflammatory and anti-bacterial activities produced by the gels containing chamomile essential oil.

Keywords: *Matricaria recutita,* formulation, oral gel, anti-inflammatory.

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POSTER PRESENTATION

ESSENTIAL OILS AS FOOD PRESERVATIVE: ENCAPSULATION, BIOLOGICAL ACTIVITIES, AND SENSORY IMPACT

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Abstract

This work evaluates the nanoencapsulation effect on essential oil potential to ameliorate food conservation. After extraction by hydrodistillation, tested essential oils *(Syzygium aromaticum, Cinnamomum zeylanicum*, and *Lavandula stoechas)* were analyzed by GC-MS, then mixed and encapsulated into nanoemulsion-based delivery system. The encapsulation effect has been evaluated, firstly *in vitro*, based on the antioxidant and antibacterial activities of the mixture. Then, deliberately contaminated milk (with *Staphylococcus aureus*) was chosen to evaluate the essential oilencapsulation effect in a real food model *via* the enumeration of surviving bacteria. Finally, a hedonic sensory survey studied the encapsulation effect on consumer acceptability of essential oil enriched milk. GC-MS obtained results showed a significant variability of volatile compounds depending on essential oils. Eugenol (75%), cinnamaldehyde (89%) and camphor (35%) were distinguished as majorcompounds of studied essential oils, respectively. The study of the *in vitro* encapsulation effect revealed that the nano encapsulated mixture had a lower DPPH inhibiting capacity, as compared to the bulk mixture. Indeed, for the same concentration (0.5 mg/ml) the inhibiting capacity significantly decreased from 65 to 61% (for bulk and nano encapsulated essential oil, respectively). Interestingly, the encapsulation has significantly improved the efficiency of the mixture for the iron reducing power test. Concerning the evaluation of antimicrobial activity, results revealed promising potential against *Escherichia coli* and *Staphylococcus aureus* with improved efficacy of the nano-encapsulated mixture. The study of the effect of enriching contaminated milk with bulk or nano-encapsulated mixture revealed a significant decrease in the bacterial load, compared with the negative control. The sensory evaluation revealed similar acceptances for control milk with the one treated bynanoemulsion, while milk supplemented with bulk essential oil was found unacceptable by panelist. In conclusion, gathered results conclude that the essential oil nanoencapsulation not only ameliorate its potential as natural food preservative, but also its sensorial acceptability.

Key Words: Essential Oils, Nano-encapsulation, Anti-oxidant Activity, Anti-bacterial Activity, Milk

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POSTER PRESENTATION

CINNAMOMUM ZEYLANICUM AND *MYRTUS COMMUNIS* ESSENTIAL OILS: CHEMICAL INVESTIGATION AND BIOLOGICAL POTENCIES

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Abstract

Essential oils have been widely used for medicinal, cosmetic, pharmaceutical, agricultural and food applications. Thus, the aim of the present work was to assess myrtle and cinnamon essential oils for their chemical composition then for their presumed antioxidant and antimicrobial activities. The two essential oils volatile components were analyzed by GC and GC/MS apparatus. Antioxidant activities were assessed through the antiradical activity and the reducing power test and the antibacterial potentials were evaluated against fourteen bacteria, generally implicated in food poisoning, including Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and S*erratia marcescens*) and Gram-positive ones (*Enterococcus*, *Staphylococcus aureus* and *Bacillus licheniformis*). The results revealed the presence of variable and large number of volatile compounds depending on the studied essential oil. In fact, lavender EO was more diversified, containing over than 40 molecules, with Alpha-Pinene (75%) and Cinnamaldehyde (89%) as major ones while clove essential oil major compounds were eugenol (75.5%). Considering the antioxidant activities, *S. aromaticum* EO was significantly the most efficient with IC_{50} values equals to 1.5 $\mu g.ml^{-1}$ and 1.3 $mg.ml^{-1,}$ for the DPPH and FRAP tests, respectively. Finally, and as for the antioxidant activities, clove EO was more active than that of *Lavandula stoechas* as it exhibited the highest inhibition diameter (ID) against the 14 tested germs (ID always superior to 15 mm). Gathered together, these results suggest the possible use of these two EOs, especially *Syzygium aromaticum* one, as a natural food preservative.

FULL PAPERS

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FULL TEXT–ORAL PRESENTATION

ANTIBACTERIAL ACTIVITY TESTING OF NATURAL EXTRACTS; ESSENTIAL OIL, PHENOLS AND ALKALOIDS OF SOME MEDICINAL PLANTS AND FUNGUS AGAINST MULTIRESISTANT DRUG BACTERIA

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Abstract

In order to search for new antibacterial molecules, different studies are targeting herbal medicine, hence the biodiversity of medicinal plants and the large number of secondary metabolites extracted. This research was done to find out which compounds had antibacterial activity, as well as to determine the activity using minimum inhibitory concentrations of biologically active metabolites. Essential oils were extracted using a Clevenger apparatus, phenols were obtained by ethanolic and methanolic maceration followed by evaporation with a Rotavap and alkaloids were obtained utilizing a very exact process. The alkaloid extracted from *Ruta graveolens* and *Rhanterium adpressum* essential oil were tested on three bacteria isolated from Laghouat hospital, *Staphylococcus aureus*, *Aeromonas hydrophilia, Klebsiella pneumoniae*. Antibacterial activity was determined using the disk diffusion method, which involved performing a dilution series (neat, 1/2, 1/10) to test sensitivity and the liquid microdilution procedure to determine minimum inhibitory concentrations. By opening the discs for the pure concentration without dilution of the extract, the alkaloid of *Ruta graveolens* inhibits the two bacteria *Klebsiella* and *Aeromonas*; nevertheless, the bacterium *Staphylococcus aureus* is resistant to the alkaloid. The microdilution method revealed the confirmation, with the Minimum inhibitory concentrations being the initial concentration for the two bacteria, with a value of 54.2 mg / ml. In fact, *Rhanterium adpressum* essential oil has a 10 mm inhibition zone and a minimum inhibitory concentration of 397.1 µg / ml against *Staphylococcus. Ruta graveolens* alkaloid and *Rhanterium adpressum* essential oil may be suitable for treating multidrug-resistant Enterobacteriaceae and Methicillin-resistant *Staphylococcus aureus.*

Keywords: *Ruta graveolens* alkaloid, multidrug resistant bacteria, antibacterial activity, inhibition, *Rhanterium adpressum* essential oil.

1. Introduction

Nosocomial infections cause a major health problem, especially in hospitalized patients where they risk being infected with bacterial strains that can pose a risk of vitality [1]. This is due to the increased ineffectiveness of antibiotics used in hospital settings day after day [2].

In order to find new antibacterial compounds, researchers are focusing on herbal medicine, which explains the diversity of medicinal plants and the high number of natural chemicals extracted. Traditional medicine recognizes medicinal plants for their antibacterial properties due to secondary metabolites [3]. It was for this reason that this study on the antibacterial activity of natural extracts was conducted. We've chosen essential oils from *Rhanterium adepressum,* phenols from *Inonotus hispidus, Ruta graveolens,* and alkaloids from *Haloxylon scoparium,* which are utilized as antiseptics in traditional medicine.

Indeed*, Inonotus hispidus* (Basidiomycetes) is a parasitic fungus preferably living on Pistacia atlantica tree in Algeria. It synthesizes a yellow–brown pigments, hispidin, bis-noryangonin and hypholomin B [4]. This fungus is known by its antiviral activity. *Haloxylon scoparium* is a desert plant classified among the family of Chenopodiaceae. The plant is widely as an anti-diabetic and an antibacterial plant [5-7]. *Rhanterium adpressum* (Asteraceae) is a spontaneous plant; endemic in arid regions of North Africa. It shows an interesting antimicrobial and antioxidant avtivities [8].

Ruta graveolens L. (Rutaceae) or common rue, native of the Mediterranean region. The whole herb is abortifacient, anthelmintic, antidote, antispasmodic, carminative, emetic, brings relief from giddiness and nervous headaches, strongly stimulant and mildly stomachic [9]. The goal of this work was to find a promising antibacterial activity of natural extracts from Laghouat against multidrug-resistant clinical bacterial pathogens.

2. Materiels and methods

2.1. Plants collection

Inonotus hispidus and *Haloxylon scoparium* were gathered from Hassi remel Laghouat, while *Rhanterium adpressum* was harvested from Siddi mekhlouf Laghouat in March 2020.

2.2. Essential oil, phenols and alkaloids extraction

This research was carried out at Laghouat University's research laboratory. The extraction of *Rhanterium* essential oil was carried out by hydrodistillation using the Clevenger as a device, with a mass yield of 0.24%. The extraction of the phenolic compounds of *Inonotus hispidus* was carried out by maceration of 10g in absolute methanol and ethanol of plant powder in cold and for 48 hours, then filtration and evaporation to dryness by the Rotavapor device at temperature 42° C. The extracts are dissolved in DMSO 10% in a volume of 10 ml. The methanolic extract of *Inonotus* has a yield of 7.19%, and the ethanolic extract of 9.38%. The alkaloids were extracted by macerating 5g of powder in 50ml of absolute ethanol for 24 hours

in the dark and at room temperature, then evaporating to dryness using the Rotavapor at 42° C. After a series of liquid liquid extractions in a separating funnel under the hood, the organic phase is eliminated by mixing 50ml chloroform with 50ml 5 percent HCl, then adding 50ml dichloromethane. The addition of 28% ammonia was carried out in the aqueous phase which allows the appearance of the other two phases by eliminating the aqueous phase this time. The addition of sodium sulfate anhydride followed by filtration then removing the solvents by evaporation to dryness in the Rotavap at 55° C. The alkaloid salts are dissolved in 10% DMSO and stored at 4° C until they are used.

2.3. Bacterial strains

Three bacteria were recovered from the pus and urine of hospitalized patients in Laghouat hospital at the level of internal medicine; *Staphylococcus aureus* as MRSA, *Klebsiella pneumonieae* and *Aeromonas hydrophilia* as multidrugs resistant bacteria pathogens.

2.4. Antibacterial activity

Antibacterial activity is measured by the disk diffusion method where a dilution series was done (neat, 1/2, 1/10) to measure sensitivity, and the liquid microdilution technique to determine the MIC [2]. Minimal inhibitory concentrations (MIC) were determined using 96-well microtiter plates.The volume of the inoculum deposited in the wells is 5 µl, the volume of liquid MH is 70 µl and the volume of the extract is 25 µl. The microplate was incubated at 37 ° C for 24 hours [10].

3. Results and Discussion

The alkaloid of Ruta graveolens gives zones of inhibitions for the two bacteria *Klebsiella* and *Aeromonas* by opening the discs for the pure concentration without dilution of the extract, on the other hand, the methanolic and ethanolic extract of Inonotus hispidus and *Haloxylon scoparium* alkaloids does not give any antibacterial activity against multiresistant isolates (Table 1).

In addition, the essential oil of *Rhanterium adpressum* was effective on multi-resistant *Staphylococcus aureus* gram positive cocci by giving zones of inhibitions of 10 mm by pure oil, 8 mm by a dilution of 1/2 and resistant with the dilution 1/10 (Figure 1, Table 1).

Table 1. Antibacterial activity testing of *Ruta graveolens* alkaloids, *Inonotus hispidus* methanolic and ethanolic extracts and *Rantherium adpressum* essential oil against *Klebsiella pneumoniae, Aeromonas hydrophilia and Staphylococcus aureus by* disk diffusion method.

Bacteria	***Ruta graveolens* alkaloids (inhibition zones mm)**	***Inonotus hispidus* methanolic and ethanolic extracts (inhibitions zones mm)**	***Rantherium adpressum* essential oil (inhibitions zones mm)**	***Haloxylon scoparium* Alkaloids mm**
Klebsiella pneumoniae	09	/	/	/
Aeromonas hydrophilia	09	/	/	/
Staphylococcus aureus	08	/	10	/

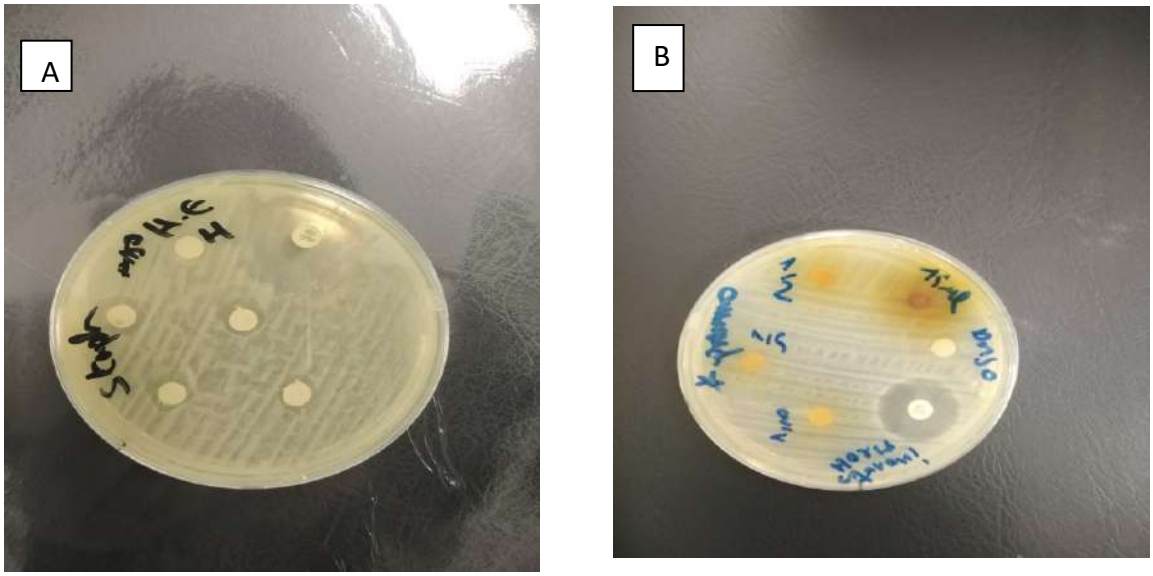

Figure 1. (**A**): Antibacterial activity of *Rhanterium adpressum* essential oil against *Stahpylococcus aureus,* (**B**): No antibacterial activity of *Inonotus hispidus* methanolic extract against *Klebsiella pneumoniae*.

The use of gentamicin as a positive control makes it possible to make a comparison between the diameters of inhibitions of the different extracts (Figure 2).

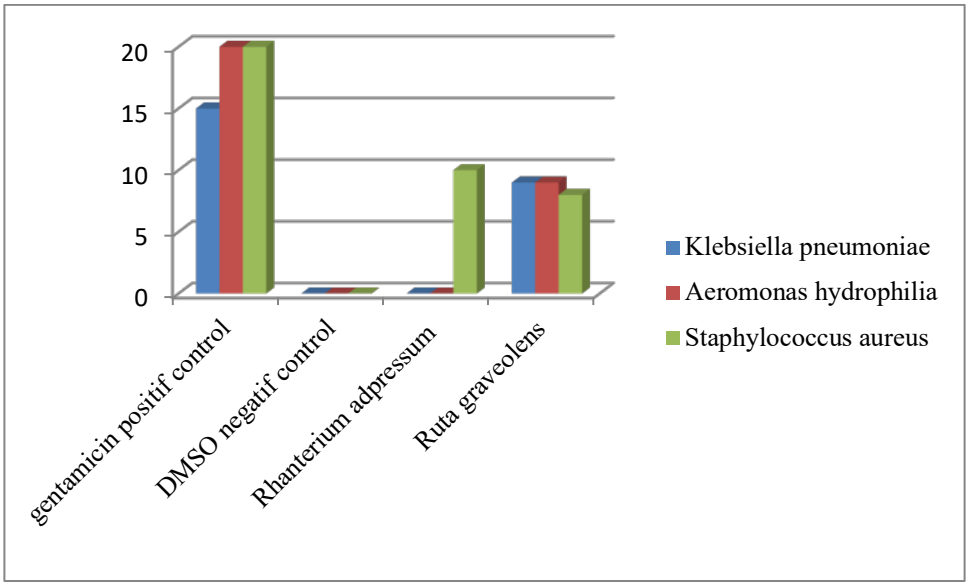

Figure 2. Evaluation of antibacterial activity of *Rhanterium* essential oil and *Ruta* alkaloid against multiresistant strains.

3.1. MIC determination

For Ruta graveolens extract, the bacterium *Staphylococcus aureus* is resistant to the alkaloid. The confirmation was well visualized by the microdilution method where the MIC is the first concentration for the two bacteria *Klebsiella* and *Aeromonas* with a value of 54.2 mg / ml. For *Rhanterium adpressum*, the MIC is 397.1 µg / ml against *Staphylococcus aureus* (Table 2).

Table 2. MIC of *Rhanterium adpressum* essential oil and *Ruta graveolens* alkaloid against multidrugs resistant strains.

Bacteria	Minimal inhibitory concentration Rhanterium adpressum essential oil µg / ml	Minimal inhibitory concentration Ruta graveolens alkaloid mg / ml
Klebsiella Pneumoniae	/	**54.2**
Aeromonas Hydrophilia	/	**54.2**
Staphylococcus aureus	**397.1**	/

Several studies have been made to target new antibacterial molecules of natural origin. The objective of this study is to test the antibacterial activity of some different natural extracts to rule out plants which do not have an activity, also to prove which of the secondary metabolites has antibacterial activity compared to the others. The recent study has proved an important antibacterial activity of *Rhanterium* essential oil and *Ruta* alkaloid, unlike phenolic compound which generally, do not have this activity. *Rhanterium* essential oil showed an antibacterial

activity against gram negative cocci, unlike the alkaloid where it showed an antibacterial activity against gram negative bacilli represented by enterobacteria. It is compared with some studies that have shown an antimicrobial activity of Ruta, because of the présence of several bioactive molecules inhibit and destroy several types of pathogenic microorganisms [11]. The Essential oils extracted by hydrodistillation also showed antibacterial and antifungal activities against *Staphylococcus aureus* in some studies [12].

4. Conclusion

Plants possess several types of secondary metabolites such as essential oils, phenolic compounds and alkaloids possessing biological activities can be used in medical and pharmaceutical fields. *Ruta graveolens* and *Rhanterium adpressum* have been used in phytomedicine in multiple pharmacological activities including recently an antibacterial effect that's proven with this study. The recent study opens à door in the field of antibiotics where multiresistant bacteria can pose a risk of vitality for hospitalized and immunocompromised patients.

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Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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FULL TEXT–ORAL PRESENTATION

GENE EXPRESSION EVALUATION OF STAR IN TESTIS AND TSPO IN QUAIL OVARIES UNDER THE INFLUENCE OF VITAMIN E

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Abstract

Vitamin E is found in the oils of many plants and plays a significant protective and medicinal role. STAR and TSPO genes are involved in reproduction performance. In order to investigate the effect of different levels of vitamin E on STAR gene expression in testis and TSPO gene in ovaries of parent quail, 360 Japanese quails for ten weeks were assayed. Poultry was kept in 18 cages as a completely randomized design with three treatments, six replications, and 24 quails (16 Females and 8 males). Treatments included pure vitamin E levels (25, 50, and 100 IU per kg of diet) in the diet. The results showed that different levels of vitamin E did not affect STAR gene expression in the testis. Quails receiving 50 and 100 IU levels of vitamin E showed an increase in TSPO gene expression in the ovaries by 7 and 2 times, respectively ($P<0.01$). The results of reproductive performance showed that different levels of vitamin E had no significant effect on fertility percentage ($P<0.05$), but the percentage of quail hatching chicks was significantly higher in vitamin E (50 and 100 IU / kg) levels than The level of 25 IU per kg of vitamin E increased ($P<0.05$).

Key Words: Vitamin E, medical role, quail, gene expression

1. Introduction

As a high-yield and profitable activity, Raising quail has attracted considerable attention. Traits such as rapid growth, short generation interval, precocious maturity, and high laying rate have made quail special among poultry breeders. Quail are hardy, durable, and well-built and can adapt to different environments. It grow quickly and reach maturity in 6 weeks. Within 50 days, it is considered a laying herd (Sahin et al., 2008). The lifespan of a bird is about 2 to 2.5 years (Randall and Bola, 2008).

Rapid growth and high metabolic rate in birds can stimulate free radicalization and enhance oxidative stress. The body of birds or any other living organism has a defense system called the antioxidant system, which is developed within the body's tissues to fight free radicals. Oxidative stress occurs when active oxygen exceeds the ability of an organism's antioxidant system (Surai et al., 1997). There are several antioxidants of internal and external origin protect cell components against free radicals. These compounds are divided into three main groups based on how they work. A) Antioxidant enzymes, which include catalase, glutathione peroxidase, glutathione reductase, and superoxide dismutase. B) Proteins that bind to metals such as ferritin, transferrin, lactoferrin, and ceruloplasmin. C) Antioxidants that stop chain reactions, which are two classes. 1) Antioxidants acting in the lipid phase include vitamin E, carotenoids, flavonoids, and coenzyme Q. 2) Aqueous phase antioxidants include vitamin C, uric acid, bilirubin bound to albumin, and proteins containing thiols such as glutathione (Young and Woodside, 2001).

Vitamin E is the prevalent fat-soluble antioxidant (Morrissey et al., 1994). This vitamin is located inside the cell and in the fat layer of the cell wall and protects the body's cells against destruction. Birds cannot synthesize this vitamin and therefore depend on it for food (Tengerdy and Nockels, 1973). Testicular tissue, despite its low oxygen concentration, is susceptible to oxidative stress due to the presence of high levels of unsaturated fatty acids and the oxygen-producing system (ROS) (Turner and Lysiak, 2008). Defects in the antioxidant system and decreased testicular testosterone and plasma testosterone concentrations have been reported as reasons for reduced fertility of a male bird after peak production (Surai et al., 2000). Acute steroid regulating protein (STAR) and translucent protein (TSPO) play an essential role in the synthesis of steroid hormones (Kostic et al., 2011).

STAR is a carrier protein that regulates cholesterol transport in the mitochondria, which acts as a rate-limiting step in producing steroid hormones. STAR promotes cholesterol transfer from the outer mitochondrial membrane to the inner mitochondrial membrane where P450scc (the enzyme that acts in the first step and speed regulator in steroidogenesis) is located and serves as a major determinant in steroidogenesis. It works and shows an important role in the early stages of steroidogenesis (Stocco and Clark, 1996).

TSPO is generally expressed in the mitochondrial inner membrane of steroidogenic tissues such as the ovaries, testes, and other tissues. Although TSPO is present in many tissues, it is naturally expressed at the highest levels in the tissues in which steroids are synthesized. It plays vital role in the transport of cholesterol into the mitochondria of steroid-producing cells (Kostic et al., 2011). According to the cholesterol transport mechanism, evidence suggests that TSPO interacts with STAR to transport cholesterol to the mitochondria, both of which are essential components of the Steroidogenesis pathway, and are highly expressed in the testes and ovaries (Bogan et al., 2007). These two genes play a carrier role, transporting cholesterol into the cell,

and involved in two enzyme systems that elaborate in the enzymatic conversion of cholesterol to progesterone. When cholesterol enters this mechanism, the side branch of cholesterol must be removed by the enzyme. This side branch contains six carbons, which, if removed, converts the cholesterol molecule to pregenolone. This enzymatic phase has a slow reaction rate, so it is a controlling phase and regulates progesterone production (McCune et al., 1970).

2. Material and Methods

This study was performed in the livestock station of Agricultural Sciences and Natural Resources University of Khuzestan with 360 Japanese quails for ten weeks in a completely randomized design with three treatments, six replications, and 24 parent quails (16 females and 8 males) in 18 cages. Treatments included pure vitamin E levels (25, 50, and 100 IU per kg of diet). Diets were adjusted based on the values of the Laying Quail Tables (NRC) for the laying period. The birds were exposed to sixteen hours of light and eight hours of darkness during the day and night. At the end of the last week, six quails (3 males and 3 females) from each treatment were randomly slaughtered, and the testes and ovaries were quickly separated and transferred to a freezer at -80 °C, protected by liquid nitrogen.

2.1. RNA extraction and cDNA synthesis

In this study, RNA extraction from 100-150 mg of testicular and ovarian tissue was performed using Trizol reagent solution produced by Invitrogen, life technology. Samples were stored in a freezer at -80 ° C until cDNA was fabricated. RNA concentration was calculated using the absorbance reading of the samples at 260 nm at the Nanodrop spectrophotometer (Thermo Scientific NanOdrOp. 2000C. USA). If the adsorption ratio of 260/280 was above 1.8, the samples were used to synthesize cDNA. To construct cDNA, a cDNA synthesis kit made by Sinaclone was used. Primer design for quantification by polymerase chain reaction for the genes in question was performed using the Primer Quest program on the IDT (Integrated DNA Technologies) site. The sequence and specificity of the specific gene primer are presented in Table 1.

2.2. qPCR real-time method

A Step one plus device (Applied Biosystem company) was used to perform the qPCR real-time technique. Reactions were performed on a final volume of 25 µl of ABI Master Mix SYBER Green. The PCR technique was performed according to the protocol in Table (2) in 40 cycles. The method of studying the gene expression in this study was ΔΔCT (comparative threshold) method. The housekeeping genes were beta-actin (BTA) and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). In the relative comparison method, the relative difference of the tested sample against the control sample is calculated with the formula $2^{-\Delta\Delta CT}$.

Table 1. Real-time qPCR perimers information

Gene	Primers Sequence	Product size (bp)	Annealing temperature (C)
β-actin	F: TGACCGCGGTACAAACACAG R: CATACCAACCATCACACCCTGA	167	62
TSPO	F: GCAACTTCATTCAGAGGAAGAAATC R: CAGCTGCAGTGAGACCATAAA	121	61
GAPDH	F: CAGTTCTGTTCCCTTCTGTCTC R: CAGATCAGTTTCTATCAGCCTCTC	98	60
STAR	F: TTGCTGAAGACTCCAAGAGATG R: AGCAGGTGCAGTGTATGTATG	124	60

Table 2- Real- time qPCR protocol

Number of cycle	Time	Temperature	Description
1	10 min	95	Initiate denaturation
40	15 s 30 S 45 S	95 60-61 72	denaturation annealing extension

3. Results and Discussion

After RNA extraction, the concentration of extracted RNA and its purity were evaluated. The results of Nanodrop showed that the quality of the extracted RNAs is suitable for qPCR real-time experiments. PCR reaction was performed to evaluate the temperature properties and specificity of the primers. The results of PCR product electrophoresis for GAPDH, beta-actin, STAR, and TSPO are shown in Figure 1. After confirmation of amplification of the desired fragments, the qPCR real-time technique was performed to investigate the expression of the desired genes.

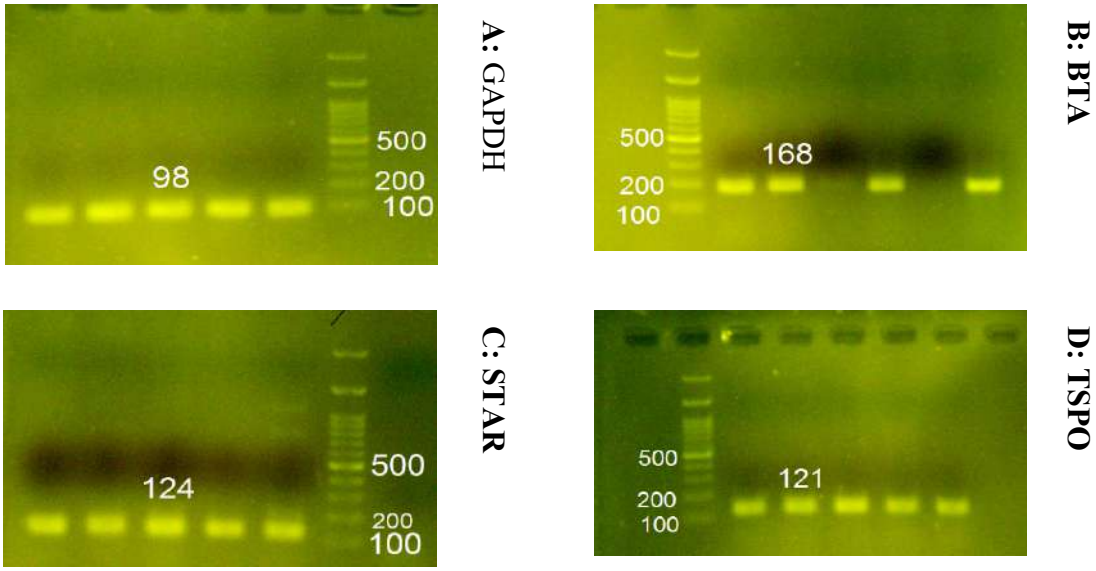

Figure 1. Electrophoresis of the polymerase chain reaction product of GAPDH (A) genes, beta-actin (B), STAR (C), TSPO (D) on agarose gel (1%)

3.1. Effect of vitamin E on gene expression and reproductive performance

The effect of different levels of vitamin E on STAR gene expression in male parent quail testes is presented in Table 3. The results showed that different levels of vitamin E did not affect STAR gene expression in the testis.

The effect of different levels of vitamin E on TSPO gene expression in female parent quail ovaries is presented in Table 3. The results show that the mean changes of TSPO gene expression in the group receiving 50 and 100 IU levels compared to the control, increased by 7 and 2 times, respectively ($P < 0.01$). The highest level of TSPO gene expression is related to the 3rd treatment, and this shows that the addition of 100IU of vitamin E to the diet has caused a significant increase in the expression of TSPO gene. In this study, vitamin E was added as a substance to reduce oxidative stress influence on the expression of TSPO protein gene in ovarian tissue. Reports have shown that TSPO regulates mitochondrial energy by modulating the oxidation of fatty acids in steroid-producing cells (Tu et al., 2016). This protein is needed to transport cholesterol and regulate the production of steroids (Khorasani et al., 2014). In animals and humans, TSPO plays a crucial role in inflammatory, immune, and stress responses (Papadopoulos et al., 2006). Vitamin E helps maintain the stability and natural function of many fatty acid-derived substances by preserving the structure of fatty acids and preventing their peroxidation (Tengerdy and Nockels, 1973). Biswas et al. (2007) reported that diets containing vitamin E will significantly increase quail hatching and fertility. On the other hand, according

to Lin et al. (2004), vitamin E significantly increased the fertility of laying hens. Kling and Soares (1980) suggested that very low levels of vitamin E in the diet were insufficient to support natural reproduction in quail.

The results of reproductive performance showed that different levels of vitamin E had no significant effect on fertility percentage ($P<0.05$), but the percentage of quail hatching chicks was significantly higher in vitamin E (50 and 100 IU / kg) levels than the level of 25 IU per kg of vitamin E increased ($P<0.05$).

Table 3. The effect of different levels of vitamin E on the expression of STAR and TSPO genes‡

	vitamin E			
	25 IU	50 IU	100 IU	
STAR (Testicles)	1	0.8866	0.3292	($\Delta\Delta C_T$)
TSPO(Ovary)	1	1.1121	1.9977	($\Delta\Delta C_T$)
STAR(Testicles)	1	0.5409	0.7960	($2^{-\Delta\Delta C_T}$)
TSPO(Ovary)	1	0.4626	0.2504	($2^{-\Delta\Delta C_T}$)
STAR(Testicles)	1	0.5409	0.7960	(fold change)
TSPO(Ovary)	1^{a}	7.6470^{c}	2.4284^{b}	(fold change)

‡Calculations have been performed for the control treatment (level of 25 IU of vitamin E)

4. Conclusion

We can conclude that different levels of vitamin E had no effect on STAR gene expression in the quail testis. Certain levels of vitamin E increased the expression of the TSPO gene in the ovaries. Finally, the percentage of quail hatching chicks increased significantly at certain levels of vitamin E consumption.

Acknowledgements

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Conflict of Interest

No conflict decleard by all authors.

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FULL TEXT–ORAL PRESENTATION

TREATMENTS TO OVERCOME SEED DORMANCY IN CAPER (*CAPPARIS SPINOSA*)

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Abstract

Caper (*Capparis spinosa* L.) is a perennial monoecious shrub which grows in hot and dry climates. This plant is medicinal plants with considerable tolerance to thermal and moisture stresses. It has variety products and can be economically valuable. Caper mass production is limited due to poor seed germination. The objective of this study was to investigate various seed treatments to overcome seed dormancy and improve seed germination of capper. Treatments were seed priming using Potassium nitrate (0.5, 1 and 2%), gibberellic acid (100, 200 and 400 ppm) combination of Potassium nitrate 2% and gibberellic acid 500 ppm, chemical and mechanical scarification and light treatment. This experiment was performed in factorial based on completely randomized design at seed technology laboratory of department of plant production and genetics, faculty of agriculture, Agricultural Sciences and Natural Resources University of Khuzestan during 2018. Germination percentage and seed vigor index were measured as the indicator of seed quality. The results of this experiment showed that application of potassium nitrate and gibberellic acid had significant effects on germinations characteristics and increased the germination percentage, rate of germination and seedling length. Seeds soaked in distilled water had not germination. The best time of soaking was 48 hours. The highest germination percentage and vigor obtained from hormone seed priming for 48 hours with 400 ppm gibberellic acid from mechanical scarified seeds.

Key Words: Caper, dormancy, priming, seed

1. Introduction

Capper (*Capparis spinosa*) is a well-adapted plant to arid and semi-arid regions (Mercati et al. 2019). This crop has a high potential valued for medicinal qualities and its derivatives are widely used in pharmaceutical and cosmetic industries (Pascual et al. 2004). Increasing demand for healthy medicinal products originated from plants globally raised during recent years. Despite of high medicinal and food potential application of caper, the production of this plant is limited due to low seed germination and weak seedling vigor (Labbafi et al. 2018). Seed priming is an effective method to enhance seed germination and increase over all quality of seed germination (Rakshit and Singh 2018). The objective of present study was to alleviate seed dormancy and improve seed germination and early seedling vigor of caper using seed priming technique.

2. Material and methods

Samples of caper landrace seed were collected in the State of Khuzestan, Iran, and evaluated in the present study. Seeds were first subjected to mechanical scarification using sandpaper and then primed with potassium nitrate (0.5, 1 and 2 %) and Gibberellic acid: GA_3 (100, 200 and 400 ppm). No primed seed were considered as controls. Treated seeds were palced on the top of two-layer watman No.1 filter paper inside the 10 cm Petri dishes and allowed to germinated at 25^0C incubator. Germinated seeds were examined every 24 hour for 21 days. Seeds with at least 2mm radicle length were considered as germinated ones. Seedling vigor was determined using following Equation (Eq) 1 ((ISTA 2012)):

Eq 1: Final seed germination × Seedling length

The experiment was conducted as factorial based on the complete block design with three replications. Data analyzed according to two-way analysis of variance (ANOVA) assumptions using Minitab software and graphs were plotted using Microsoft excel.

3. Results and discussion

Results of ANOVA revealed that seed priming treatments resulted significant impact on the germination and dormancy alleviation of caper. The duration of seed priming was not significant for KNO_3 while Gibberelline treatments were significantly affected by the durations of seed priming ($P \leq 0.01$). Our results also showed that mechanical scarification is mandatory to enhance impact of seed priming. It was found that KNO_3 increase seed germination and seedling vigor compared to control but there was no significant in seed germination percentage between KNO_3 at 1% and 2% (Figure 1). The maximum seed vigor was obtained form soaking seeds for 48 hour at 400 ppm GA_3 (Figure 2). Our results are in agreement with (Rehman and Park 2000) who reported that no seed germination was observed in no scarified seeds of *Koelreuteria paniculata* Laxm.

The maximum seed germination obtained from scarified primed seeds with GA_3 (Figure 3). However, there was no significant difference between 0.5, 1 and 2% KNO_3 for seed vigor. It was also revealed that the was no significant interaction effect of KNO_3 and scarification on seedling vigor of caper however, seed priming using GA_3 significantly enhanced seed vigor of scarified seeds compare to no scarified seeds (Figure 4). Previous report suggested that poor seed germination of caper is partially due to hard seed coat (Bahrani et al. 2008).

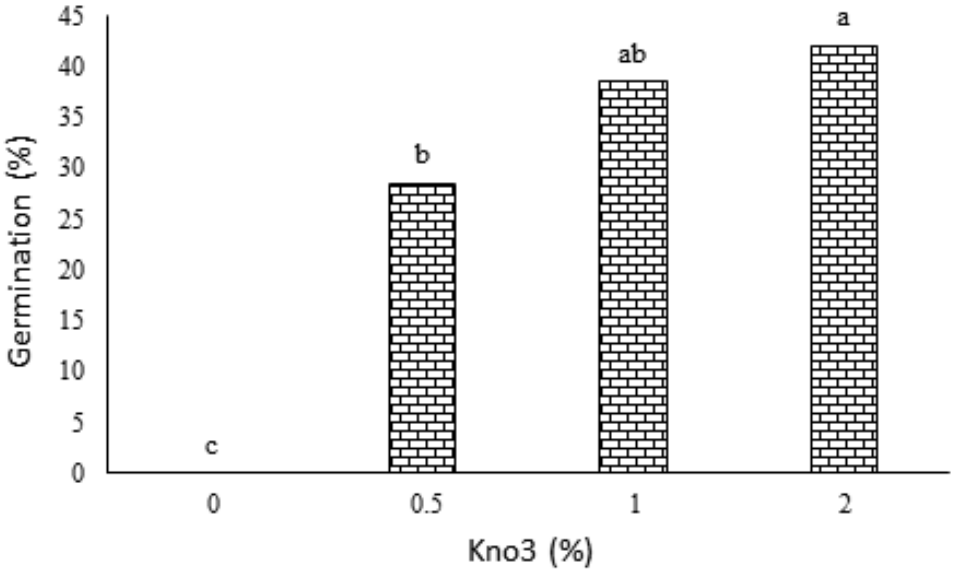

Figure 1. Effect of seed priming with KNO_3 on seed germination of caper

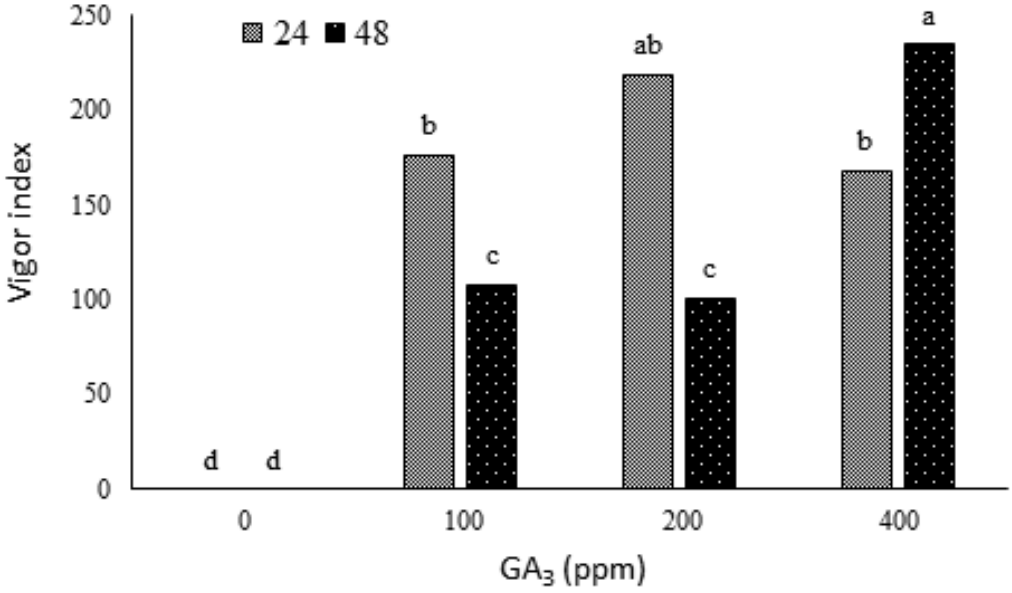

Figure 2. Effects of seed priming with GA_3 on seed vigor of caper

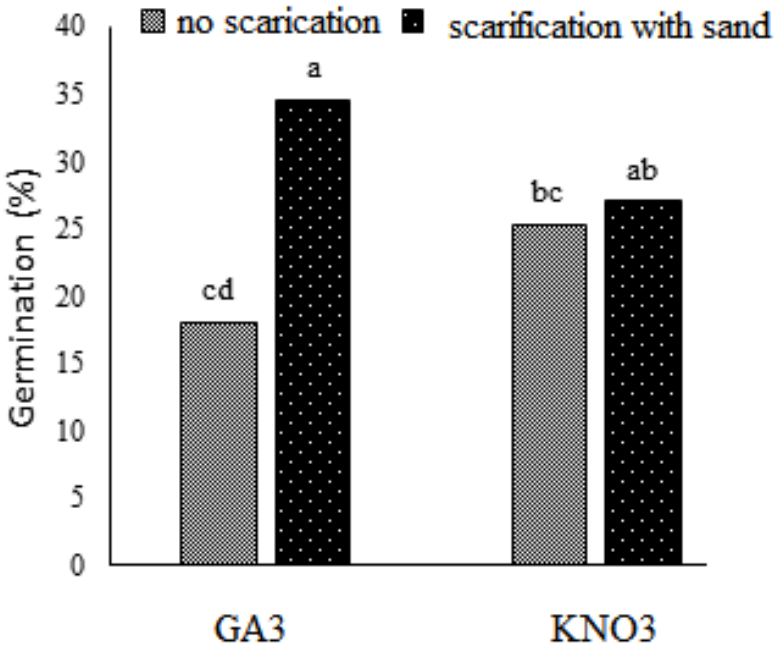

Figure 3. Interaction effect of scarification and seed priming on caper seed germination

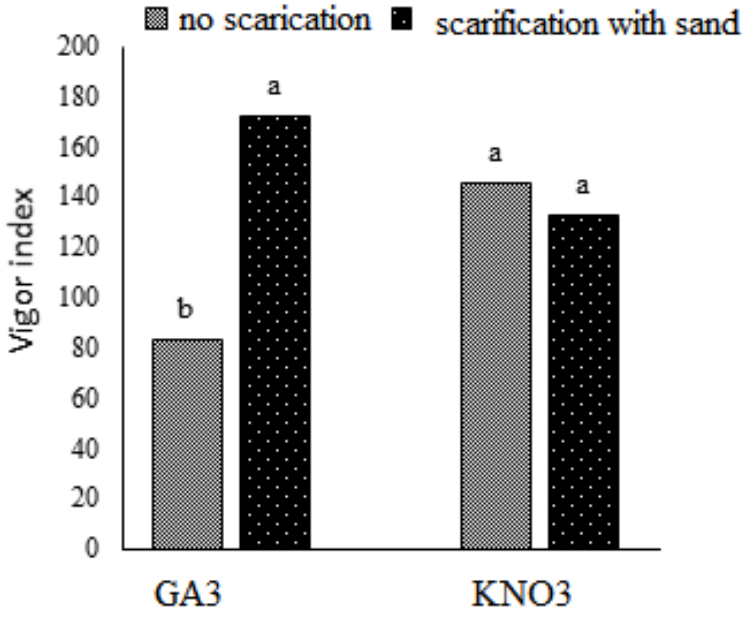

Figure 4. Interaction effect of scarification and seed priming on caper seed vigor

4. Conclusion

Caper seed dormancy is mainly due to existence of hard and impermeable seed coat while there are also some physiological mechanisms that inhibits rapid seed germination. Therefore, not only mechanical scarification is mandatory to initiate seed germination of caper, but also it is significantly advised to use GA_3 to overcome hindering physiological inhibitors.

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Conflict of Interest

Authors declare any conflict of interest in this research.

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FULL TEXT–ORAL PRESENTATION

THREE VALUABLE BY PRODUCTS: GRAPE, FIG AND POMEGRANATE KERNELS AS SUSTAINABLE RAW MATERIALS FOR COSMETIC INDUSTRY

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Abstract

Demand for efficient use of resources by minimization of waste products in cosmetic industry are increasing, because they are valuable raw materials for sustainability. For the production of safe and environmentally friendly cosmetic products, the sustainability effects are a consideration at all stages of the life cycle of cosmetic products, and therefore the choice of raw materials deserves more attention. In the cosmetics industry, there has been increasing interest in formulating cosmetic products using alternative ingredients that are considered more sustainable. Thus, this review was purposed to explain the relationship between sustainability and the cosmetics industry, emphasizing the importance of using grape (*Vitis vinifera* L.), fig (*Ficus carica* L.) and pomegranate (*Punica granatum* L.) seeds as sustainable raw materials in the cosmetics industry. *'Sustainability can be beautiful and beauty can be sustainable.'*

Keywords: Sustainability, Raw Material, Kernel, Environment Friendly, Green Cosmetics, Innovation

Introduction

Cultivating agricultural raw materials requires a lot of land and has a huge impact on people, animals and nature. For this reason, people should use natural resources sparingly and establish fair relations with farmers, workers and producers. Depending on the type of production, it is necessary to establish and implement sound sustainability standards and industry solutions to minimize the negative impact on people and the environment (Dobras, 2021; Morea et al., 2021). Recently, sustainability in the cosmetics industry has received increasing attention by consumers, cosmetics industries and organizations as well as academics from various disciplines. Growing concern about the safety of cosmetics, environmental impacts such as deforestation, and social impacts from unfair trade have intensified the attention given to this issue. The choice of raw materials deserves more attention, as sustainability effects occur at all stages of the cosmetic product lifecycle and information about it remains scattered and disorganized. It becomes mandatory to formulate it with alternative ingredients that are considered to be more sustainable in the production of cosmetic products. (Skorek et al., 2015; Górnaś and Rudzińska, 2016; Kalli et al., 2018).

This article discusses the relationship between sustainability and the cosmetics industry, the factors driving developments in this field, the need to evaluate them and available tools, as well as the sustainability impacts produced throughout the entire product lifecycle. This article will highlight the importance of using grape (*Vitis vinifera* L.), fig (*Ficus carica* L.) and pomegranate (*Punica granatum* L.) seeds as sustainable raw materials in cosmetics industry. In addition, it is aimed to reveal the factors affecting the developments in the field of green cosmetics using plants and the sustainability factors produced throughout their life cycle in the presented review.

Figure 1. Sustainability in the workplace

Sustainable Raw Materials for Cosmetic Industry

Sustainability in the cosmetics industry is the most important issue worldwide, and it has been recently focused on the production of natural and herbal products, also called green cosmetics. Therefore, consumers, cosmetics industries and organizations, and academics from various disciplines are now becoming more and more aware of the sustainability of green cosmetics and a clean ecosystem. Accordingly, the demands for the use of green and herbal cosmetics are increasing day by day and consumers are updated by taking into account the latest trends, natural ingredients, health benefits of herbal recipes and their effects on mother nature. Sustainability is defined as taking into account the environmental, social and economic dimensions of beauty and cosmetic products throughout their life cycle, including the production and post-production chains (Pereira de Carvalho and Barbieri, 2012; Fonseca-Santos et al., 2015; Bom et al., 2020).

Figure 2. Sustainability factors in the cosmetic industry

Cosmetic innovation is a main factor supporting sustainability and environmental protection, such as the environmental friendliness of packaging, the biodegradability of ingredients, healthy and natural formulations, and the use of waste using non-edible food and fruit waste. Cosmetic products, which are currently produced with a sustainable approach at both the formulation and production level, are becoming an increasingly important choice for many consumers who are environmentally friendly and carbon neutral. As a result, sustainable and environmentally friendly innovations in green cosmetics is a complex and multifaceted issue that cannot be assessed alone, but can be assessed with an integrated evaluation of environmental, social and economic dimensions, final product quality and performance. Grape (*Vitis vinifera* L.), fig (*Ficus carica* L.) and pomegranate (*Punica granatum* L.) kernels can cause sustainable problems, and therefore, new products can be produced that provide environmental, social and economic advantages thanks to their reuse (Bom et al., 2020; Leal et al., 2020).

Pomegranate (*Punica granatum* L.) Kernels and Cosmetic Industries

Pomegranate (*Punica granatum* L.) has known as a medicinal fruit that can be used universally for thousands of years. It grows in a large part of Asia, the Middle East and Latin America. The fruits of the pomegranate tree are used both in the treatment and prevention of various diseases. The seed of pomegranate include a long, soft, light-colored seed in their middle. It is about 3-5 mm long and surrounded by a glassy fruit rind filled with dark red juice. It is also known that that a pomegranate contains about 400 seeds. Due to the oil content of pomegranates is relatively low, all the oil-soluble active substances are stored in the kernel. For the production of 1 kg of oil, 10 kg of dry seeds, that is, 200-250 kg of pomegranate, are required (Belsito et al, 2019; Slaga and Snyder, 2020).

Pomegranate seed exhibit remarkable benefits for human body e.g., increasing blood flow and erectile response, reducing collagen-induced arthritis and join pain, fighting cancer cells and reducing risk of developing cancer, promoting cardiovascular health, stimulating probiotic bacteria, thus enhancing its beneficial effects at fighting microbial infections, reducing plaque in the carotid artery, lowering blood pressure and LDL oxidation, improving memory and brain function, and helping overcome depression (Górnaś and Rudzińska, 2016; Rodrigues et al., 2018).

Pomegranate seed extract shows sebum-regulating properties and is also astringent and antibacterial, thus it has the potential to be used in cosmetic products to treat acne, blackheads, oily skin, combination skin and dandruff. Pomegranate seed also has benefits for skin as listed below (Skorek et al., 2015; Michailidis,et al., 2021);

⇒ Naturally brightens the skin
⇒ Provides skin with many antioxidants
⇒ Moistures and softens dry skin
⇒ Soothes skin inflammations
⇒ Stimulates collagen production
⇒ Rehydrates skin and makes it plump
⇒ Prevents aging signs and regenerates the skin
⇒ Keeps skin healthy, youthful and growing

Figure 3. Pomegranate (*Punica granatum* L.) and kernels

Fig (*Ficus carica* L.) Kernels and Cosmetic Industries

Fig seeds obtained from *Ficus carica* L. possess a light brown color, depending on the variety of the fig fruit. Figs are among the oldest cultivated plants cultivated since ancient times and cultivated in large fields in the Mediterranean region. Fig cultivation is also quite developed in the latitudes of Turkey. It is a fruit tree that can reproduce by seeds or cuttings, and figs can be planted in our homes in a garden, or pots, and even on the balcony. In general, approximately 15 kg of dried figs are required to obtain 1 kg of fig seeds. The seeds have characteristic features including rich in antioxidants, binds free radicals, and strengthens the immune system. The general therapeutic properties and health benefits of the fig are given below (Oliveira et al., 2009; Michailidis,et al., 2021);

⇒ Helps get rid of sleep disorders e.g., insomnia
⇒ Protects against breast cancer
⇒ Reduces inflammation
⇒ Prevents cancer causing substances
⇒ Promotes bone density
⇒ Helps to treat diabetes, sore throat, and chronic constipation

They can be used in food industries as a dietary supplement, coffee-like drink, a topping, and an ingredient for cooking and baking. Besides usage for food industries, they can be also used for cosmetics such as care products e.g., face and body peeling, face and body mask u.v.m. The powder obtained from fig seeds is rich in vitamins E, D, A, K, C, B1, B2, B6, collagen u.v.m. Additionally, fig seed powder is rich in antioxidants and therefore has a wonderful physical effect in protecting against free radicals. In other words, fig seeds have numerous benefits for beauty and body. The kernels of fig prevent aging of the skin while providing a slight hydration of the skin. They exhibit also powerful effect on nourishing the skin for providing shiny and healthy skin. In addition, they have property of restoring the body for use in peelings and removing dead cells (Oliveira et al., 2009; Mawa et al., 2013; Ghimeray et al., 2015; Michailidis,et al., 2021).

Figure 4. Fig (*Ficus carica* L.) and kernels

Grape (*Vitis vinifera* L.) Kernels and Cosmetic Industries

Grapes (*Vitis vinifera* L.) and its seeds include many important compounds such as vitamins, minerals, lipids, proteins, carbohydrates, and complex of polyphenol compounds, particularly catechin monomers, or dimers, trimers, and oligomers mainly known as proanthocyanidins. Phenolic compounds are reported that the most important components found in grape seeds. It

is also reported that these phenolic compounds obtained from standardized grape seed extracts are 92-95% oligomeric proanthocyanidins. The structures of proanthocyanidins vary depending on the source of the building blocks (monomer units) of the flavanol(s), the presence of modifications of the 3-hydroxyl group (such as esterification), and the degree of oligomerization (how many flavanol repeat units). Catechin, epicatechin and taxifolin are the main flavanols found in grape seeds. Heating the oligomeric proanthocyanidins under acidic conditions leads to the release of anthocyanins and flavanols. Accordingly, the length of oligomeric proanthocyanidins and the concentration of flavanols in grape seed extracts are highly dependent on the used extraction techniques (Shi et al., 2003, Antignac et al., 2011; Leal et al., 2020; Unusan, 2020).

Studies have shown that grape seed extract significantly suppresses melanin pigment formation, as demonstrated in UV-induced hyperpigmentation guinea. The pigmentation process of the skin occurs in the basal layer of the epidermis, which contains melanocyte cells involved in the formation of melanin pigment. Melanin is formed by the oxidation process and is enzymatically catalyzed by the enzyme tyrosinase. Polyphenolic compounds in grape seed extract help to prevent tyrosine from penetrating into the basal layer of the epidermis to prevent the oxidation process of tyrosine to dopaquinone, thereby reducing the risk of hyperpigmentation. Grape seed extracts are classified as antidandruff, antimicrobial, antioxidant, antiaging, anti-inflammatories, oral care, skin protecting and UV absorber (Winter, 2009; Surini et al., 2018).

Grape skin extract (enocianina) is an approved food coloring additive by Food Drug Administrative (FDA), exempt from batch certification. The FDA states that the color additive contains common components of grape juice for example; anthocyanins, tartaric acid, tannins, sugars, and minerals. A small amount of residual sulfur dioxide may be present following aqueous (aq.) extraction in the presence of sulfur dioxide. The grape anthocyanins are usually either monoglycerides or diglycosides. Melatonin (N-acetyl-5-methoxytryptamine) is present in grapes, depending on variety and location, and levels of melatonin in grape skin have ranged from 0.005-1.2 ng/g. Recent studies have indicated that melatonin may also be present in the flesh and seeds of grapes (Bergfeld et al., 2005; Fiume et al., 2014; Dixon et al., 2015; Soto et al, 2015).

Figure 5. Grape (*Vitis vinifera* L.) and seeds

Grape-derived ingredients included in the safety assessment are reported to have many possible roles in cosmetic formulations. As stated in the International Cosmetic Ingredient Dictionary and Handbook, grape seed extract is declared to function as anti-caries, anti-dandruff, anti-fungal, anti-microbial, antioxidant, flavoring, mild. stabilizer, oral care agent, oral health care drug, and sunscreen agent. In addition, the other grape ingredients are also reported to have various functions as skin care agents, and antioxidants. Five of the ingredients of *Vitis vinifera*

L. including seed extract, fruit powder, fruit juice, juice extract, and skin extract are stated to act as flavoring agents, and four of these (except seed extract), along with skin powder are known to function as colorants. The FDA assembles data from manufacturers about the use of individual ingredients in cosmetics as a function of cosmetic product category in the Voluntary Cosmetic Registry Program (VCRP). According to VCRP data from the FDA in 2011, Vitis vinifera seed extracts is used in 463 cosmetic formulations, grape fruit extract used in 219 cosmetic formulations, and Grape Leaf Extract used in 78 cosmetic formulations as well. Accordingly, Vitis vinifera (grape) ingredients are reported to be mostly used in permanent products (Belsito et al., 2012; Soto et al, 2015; Surini et al., 2018; Bosso et al., 2020).

Conclusion and Future Recommendation

Sustainability is a buzzword in the corporate world in the midst of a scenario where competition between companies in supply chains is increasing in a model where goods age faster or offer a shorter market life cycle. Thanks to sustainability in the raw materials, it is aimed to increase material and energy consumption globally, and to intensify the amount of post-consumption waste. Regardless of the consumption debate, generating solutions in global sustainability is of critical social and environmental importance from the beginning of a product's innovation projects to the end of its useful life. This perspective requires supply chain management to approach the lifecycle management of a product from cradle to grave, with innovation reaching beyond the inter-organizational environment and reaching direct and indirect members of the supply chain collaboratively to seek solutions.

Declaration of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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FULL TEXT–ORAL PRESENTATION

THE PERFORMANCE OF BIOAPIFIT® ANTI ATOPIC OINTMENT IN THE TREATMENT OF *PSORIASIS VULGARIS*

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Abstract

Objective / Purpose: The purpose of this study was evaluation of the performance and safety of Bioapifit® anti atopic ointment compared to cream base in the treatment of the patients suffering from Psoriasis vulgaris between 3 and 15 years. **Patients and methods:** The study included 100 patients of both gender older than 18 years of age with moderate to severe Psoriasis vulgaris. 50 participants were treated with Bioapifit® anti atopic ointment which was applied onto all affected parts of the body three times daily for 28 days. Another 50 of them were treated with cream base which was applied onto all affected parts of the body three times daily for 28 days. The severity and the extent of psoriasis prior/following the treatment was assessed by Psoriasis Area and Severity Index (PASI) score **Results:** The treatment of the patients with Psoriasis vulgaris with Bioapifit® anti atopic ointment resulted with decrease of total PASI score between 82.2% and 99.4% (91.5±4.9%). In the end of the treatment the total PASI score decreased from the baseline value of 32.8±13.1 to 2.9±1.2. In the control group the mean total PASI score compared to baseline was reduced from 38.2±9.2 to 30.1±6.8 which accounted to 17.8±24.3% decrease compared to initial values. None of the patients experienced any adverse effect during the treatment and follow up period. **Conclusion / Discussion**: Bioapifit® anti atopic ointment is safe and clinically efficient in alleviating the symptoms of chronic, inflammatory skin diseases like Psoriasis vulgaris with clinical cure rate of 70% thanks to its multi-component composition that resulted with moisturizing, humectants, emollient, coating, soothing, and pH adjusting effect.

Key Words: anti atopic ointment, Bioapifit, Psoriasis vulgaris, inflammatory skin disease

1. Introduction

Psoriasis is chronic, relapsing imunologicaly mediated inflammatory skin disease of unknown ethiology which affect up to 3% of world population (Li et al., 2017). The peak incidence of the disease is bimodal and most often appears between 16th and 22nd or between 57th and 60th year of age. However, the disease can occur at any age. Although the etiology is unknown,

psoriasis is thought to be a multifactorial disease caused by a complex interaction of genetic, environmental, and immune factors. Stress is considered to be the most important and strongest provoking factor influencing the onset and worsening of existing psoriasis symptoms.

It was shown that the CW6, B13, B17 human leukocyte antigens (HLA) are associated with psoriasis (Henseler, 1997). It is a system located on the shorter arm of chromosome 6 that contains more than 200 different genes whose products, HLA antigens, are found on different cells in the body (Henseler, 1997). Immunologicaly associated psoriasis (psoriasis type I) is inherited, affects younger population (usualy starst before age 40), it is more severe, and is associated with a significantly higher incidence of HLA CW6 antigen. In the case of psoriasis type II the disease begins later (between the ages of 50 and 60), occurs sporadically, shows no association with HLA genes, and has a milder clinical course.

Psoriasis vulgaris (PV) is the most common clinical form of the psoriasis. It is manifested by the appearance of solitary erythematosquamous plaques in predilection sites that can last for months or years in the same places. However, after some external or internal stimulation, a sudden spread of the lesion is possible.

Although to date it has not been precisely clarified how the psoriatic process begins, it is assumed that psoriasis is a genetic disorder of keratinocyte proliferation mediated by T lymphocytes (Krueger, 2002).

Monoclonal antibodies were found to contain inflammatory cell infiltrate in the active psoriatic foci predominantly CD4+ T helper lymphocytes and in the regression phase cytotoxic CD8+ T lymphocytes in the immediate vicinity of dendritic shoots of Langerhans cells (Krueger, 2002; Kirby and Griffiths, 2001). Upon activation, T-lymphocytes secrete numerous cytokines (IL-2, IL-4, IL-6, IL-8, IFN-γ) that act autocrine and paracrine (Krueger, 2002). In addition to activation an increasing number of lymphocytes, they also stimulate the proliferation and activation of keratinocytes. It is also known that keratinocytes secrete cytokines (IL-1, IL-6, IL-8, TNF-α) and autocrine support their own hyperproliferation (Krueger, 2002; Kirby and Griffiths, 2001). At the same time, they stimulate the expression of adhesion molecules for T lymphocytes on endothelial vascular cells and other keratinocytes, which further enhances the influx of lymphocytes into the epidermis and closes the circle. The immune response thus initiated triggered by a bacterial superantigen or epidermal autoantigen does not cease, but continues and supports chronic psoriatic inflammation. Some authors point to the significant role of keratinocytes in the pathogenesis of psoriasis. It was shown that transcriptional activity is several times higher in epidermal than in dermal cells of the psoriatic focus (Bata-Csorgo et al., 1995). It is assumed that keratinocytes, after expressing their own gene mutation, activate and secrete cytokines that act autocrine, stimulating their own proliferation, ie paracrine,

causing activation and accumulation of T cells, macrophages, as well as numerous other intracellular metabolic changes. The genetic mutation appears to affect the mechanisms of keratinocyte growth regulation, causing either over-activation of growth-promoting factors (tumor necrosis factor-α, TNF-α) or inactivation of growth-inhibiting factors (IFN-γ) (Teriu et al., 1987).

Hyperproliferation and defective keratinocyte differentiation could decrease epidermal barrier function and progression of the disease (Montero-Vilchez et al., 2021). Consequently, the treatment associated with improved epidermal barrier function may lead to positive treatment outcomes of inflammatory skin disorders (Oreščanin, 2016).

Herbal based topical treatments were found effective in alleviating the symptoms of PV (Li et al., 2017; Yan et al., 2015). Moreover, our previous study with similar formulation using vegetable oils, oil macerates, glycerin, beeswax and honey was found highly efficient in alleviating the symptoms of chronic inflammatory skin disorder (Oreščanin, 2016).

The severity of PV is determined using the Psoriasis Area and Severity Index (PASI). PASI evaluates the area of skin affected by psoriatic lesions and the degree of erythema, infiltration and scaling of psoriatic lesions. A 75% improvement in the initial PASI score is considered an acceptable goal in the treatment of psoriasis.

The purpose of this paper was assessment of the performance and safety of multi-component Bioapifit® anti atopic ointment in the treatment of moderate to severe Psoriasis vulgaris during 28 days of topical application.

2. Material and Methods

2.1. Study design

The study was conducted at FINDRI GUŠTEK HEALTHCARE INSTITUTION, Ninska 5a, Sesvete, Croatia. The investigator recruited the patients based on their medical history, following the predefined inclusion and exclusion criteria. The study protocol was approved by the Ethics Committee of Findri Gustek Health Care Center with EudraCT number 2019-001380-79. The study was designed as single blind, randomized control trial. 100 patients that met inclusion/exclusion criteria with Psoriasis vulgaris were randomly selected according to the randomization code into experimental and control group. Inclusion criteria were: the patients who are ≥18 years of age, the patients with PASI score ≥10, non-existence of any other inflammatory skid disorder, in the investigator's judgment, the patients should receive local treatment only and signed informed consent. All the participants signed informed consent and completed the demographic questioner. The experimental group was treated 28 days with

Bioapifit anti atopic ointment (Apiherbal, Zagreb, Croatia) applied three times per day on all affected body area. The participants of the control group were treated 28 days three times a day with Belobaza cream base (Belupo, Koprivnica, Croatia) which was applied three times a day on all affected body parts. The severity of the disease was assessed on day 0 and day 29 for both groups using Psoriasis Area and Severity Index (PASI) tool (Ashcroft et al., 1999; Gourraud et al., 2012). Calculation of PASI score is presented in Table 1.

Table 1. The procedure for determining Psoriasis Area Severity Index (PASI) score

Severity of psoriatic lesions (0, slight; 1, mild; 2, moderate; 3, severe; 4, very severe)				
	Head and neck	**Trunck**	**Upper limbs**	**Lower limbs**
Erythtema (redness)	0 - 4	0 - 4	0 - 4	0 do 4
Induration (thickness)	0 - 4	0 - 4	0 - 4	0 - 4
Desquamation (scaling)	0 - 4	0 - 4	0 do 4	0 - 4
Total (1)	Σ	Σ	Σ	Σ
Area affected by psoriasis				
nil (0), 1-9% (1), 10-29% (2), 30-49% (3), 50-69% (4), 70-89% (5) or 90-100% (6)				
Degree of extent (2)	0 - 6	0 - 6	0 - 6	0 - 6
Multiplied by 1 x 2	1 x 2	1 x 2	1 x 2	1 x 2
Correction factor for the affected area = 3	0.10	0.30	0.20	0.40
1 x 2 x 3	A	B	C	D
Total PASI= A+B+C+D				

2.2. Description of the investigational product

Bioapifit® anti atopic ointment, a class IIa medical device is homogeneous, greasy, viscous mass of characteristic herbal odor and light brown color packed in 50 mL box. It is composed of consisted of Glycerin, *Prunus Amygdalus* Dulcis oil, Mel (certified organic), *Cera flava*, *Helichrysum italicum* flower extract, *Nigella sativa* seed oil, *Oenothera biennis* oil oil, *Argania spinosa* kernel oil, *Theobroma* Cacao, *Calendula officinalis* flower extract, *Matricaria chamomilla* flower extract, *Lavandula officinalic* flower extract, *Achillea millefolium* extract, *Salvia officinalis* extract, *Plantago major* leaf extract, *Olea europaea* leaf extract, *Melaleuca alternifolia* leaf oil, *Thymus vulgaris* ct. thymol oil, *Origanum vulgaris* oil.

2.3. Statistical analysis

For statistical evaluation Statistica 11.0 software package was employed. The description of the treated population was done by basic statistics and frequency tables. Statistical significance was

set to $p<0.05$ in all the tests performed. The differences in the mean values of each parameter prior and after the therapy as well as between the groups were assessed by Newman-Keuls test.

3. Results and Discussion

3.1. Description of the population

Both groups consisted of 25 males and 25 females. Age range for the experimental group participants ranged from 23 to 62 years (44.3±12.4). Similar age range was also found for the control group (24 to 63 years; 43.8±14.5). The patients of the experimental group suffered from PV from 4 to 15 years (8.1±3.3) and control group from 3 to 13 years (6.7±3.7). According to the results of t-test there was no significant difference between those two groups relating age ($p=0.9347$) and the duration of the disease ($p=0.3791$).

3.2. Treatment efficiency

The severity of the disease was determined by total Psoriasis Area Severity Index (PASI) score which ranged from 11.0 to 54.9 (32.8±13.1) in the experimental group and between 26.8 and 52.6 (38.2±9.2) in the control group (Table 2). The score for each body part was also presented in Table 2. The initial values for head and neck area ranged from 1.2 to 7.2 and from 1.8 to 7.2 for control and experimental group, respectively.

In the experimental group the scores between 1.8 and 10.0, 4.8 and 16.5 and 3.2 and 24.0 were obtained for upper limbs, trunk and lower limbs, respectively. Very similar range was also obtained for the control group ranging between 4.2 and 11.0, 7.2 and 15.0 and 10.8 and 22.0 for upper limbs, trunk and lower limbs, respectively.

Table 2. Basic statistical parameters for the total Psoriasis Area Severity Index (PASI) score and the scores for each body part at baseline (initial value) and following 28 days of the treatment for experimental group treated with Bioapifit anti atopic ointment and control group treated only with cream base. X-mean value; SD-standard deviation

Variable	Stat. parameter	Experimental group		Control group	
		Initial value	Final value	Initial value	Final value
Total PASI score	$\bar{X}$±SD	32.8±13.1	2.9±1.2	38.2±9.2	30.1±6.8
	Min.-Max.	11.0-54.9	1.2-4.8	26.8-52.6	18.5-41.8
Head/neck	$\bar{X}$±SD	4.1±2.1	0.3±0.1	5.2±1.9	4.2±2.1
	Min.-Max.	1.2-7.2	0.0-0.6	1.8-7.2	1.2-7.2
Upper limbs	$\bar{X}$±SD	6.6±2.8	0.6±0.2	7.8±2.5	6.3±2.4
	Min.-Max.	1.8-10.0	0.0-0.8	4.2-11.0	3.6-11.0
Trunk	$\bar{X}$±SD	10.7±3.8	1.3±0.7	10.9±3.3	8.2±1.6
	Min.-Max.	4.8-16.5	0.6-2.7	7.2-15.0	5.4-10.5
Lower limbs	$\bar{X}$±SD	11.3±6.0	0.7±0.6	14.6±4.1	11.4±4.0
	Min.-Max.	3.2-24.0	0.0-1.2	10.8-22.0	3.2-18.0

Following 28 days of the treatment the total PASI score decreased between 82.2 and 99.4% (91.5±4.9%) in the experimental group treated with Bioapifit anti atopic ointment decreasing total PASI score from 32.8±13.1 to 2.9±1.2. In the same time, control group showed only slight decrease of the total PASI score from 38.2±9.2 to 30.1±6.8 which accounted to 17.8±24.3% decrease compared to initial values (Table 2).

When looking at each body part of the experimental group the highest decrease was observed for lower limbs score (91.4%) and the lowest for trunk (52.1%). In the case of the control group the highest decrease was observed for the trunk and lower limbs (17.6%) and the lowest for head/neck area (8.5%). Control group showed higher standard deviations compared to the experimental group.

Based on the results of Newman-Keuls test (Table 3) for the total Psoriasis Area Severity Index (PASI) score and the scores for each body part it was obvious that there was no significant difference in the initial values between experimental and control group. Moreover, there was no significant difference for neither of the tested variables of the control group between initial (baseline values) and those obtained following the treatment with cream base. However, statistically significant difference was obtained for the total Psoriasis Area Severity Index (PASI) score and the scores for each body part between initial (baseline) values and those obtained following the treatment with Bioapifit anti atopic ointment.

Table 3. The results of Newman-Keuls test for the total Psoriasis Area Severity Index (PASI) score and the scores for each body part between each pair of the groups. E-I –experimental group at baseline; E-F-experimental group following 28 days of the treatment with Bioapifit anti atopic ointment; C-I-control group at baseline; C-F-control group following 28 days of the treatment with cream base;*-Marked effects are significant at $p < 0.05$

	E-I	**E-F**	**C-I**	**C-F**
TOTAL PASI SCORE				
E-I		0.000127*	0.177480	0.496856
E-F	0.000127*		0.000159*	0.000121*
C-I	0.177480	0.000159*		0.112222
C-F	0.496856	0.000121*	0.112222	
		Head/neck		
E-I		0.000145*	0.362292	0.990170
E-F	0.000145*		0.000160*	0.000194*
C-I	0.362292	0.000160*		0.180282
C-F	0.990170	0.000194*	0.180282	
		Upper limbs		
E-I		0.000128*	0.226642	0.794782

E-F	0.000128*		0.000159*	0.000122*
C-I	0.226642	0.000159*		0.306470
C-F	0.794782	0.000122*	0.306470	
		Trunk		
E-I		0.000127*	0.881214	0.039850*
E-F	0.000127*		0.000159*	0.000122*
C-I	0.881214	0.000159*		0.071065
C-F	0.039850*	0.000122*	0.071065	
		Lower limbs		
E-I		0.000122*	0.185722	0.948710
E-F	0.000122*		0.000159*	0.000129*
C-I	0.185722	0.000159*		0.092315
C-F	0.948710	0.000129*	0.092315	

The clinical cure rate for both groups was defined as more than 90% decrease of total PASI score. In the experimental group, clinical cure was observed in 70% of the patients while the outcome in another 30% of the patients was classified as clinical improvement. None of the result was classified as clinical failure. In the control group none of the case was classified as clinical cure, 25% was classified as clinical improvement (decrease of total PASI score more than 25%) while 75% of the cases was classified as clinical failure.

3.3. Treatment safety

At Visit II the respondents using medical device or standard treatment (either ointment or cream base) are asked by the principal investigators if they experienced any side effects or adverse reaction after the first application of the ointment/cream base as well as throughout the course of the application. Furthermore, a complete examination was performed to determine the possible occurrence of adverse reactions (irritation, allergic reaction), worsening of the existing or the occurrence of new symptoms. Patients were asked to describe in their own words the feeling after the application of the ointment/suppositories. None of the patients from either Bioapifit or cream base group experienced any discomfort or adverse effect including allergic reaction, worsening of the existing or the occurrence of new symptoms during the treatment. This was also confirmed by Principal investigators by the examination. Based on the above facts obtained from the patients or by direct examination by Principal investigators it is possible to conclude that tested medical device does not cause any adverse effect and is safe for local administration onto affected skin area three times per day for the period of 28 days.

4. Conclusion

Bioapifit anti atopic ointment was found highly efficient in alleviating the symptoms of Psoriasis vulgaris following four weeks of the application with average decrease of the total

PASI score for 91.5±4.9%. The ointment performed significantly better compared with the cream base used under the same condition which accounted for average decrease of total PASI score for only 17.8±24.3%. Significantly better results obtained with Bioapifit anti atopic ointment compared to cream base could be linked to its complex composition combining vegetable oils, plant macerates, glycerin, beeswax and honey that all together resulting in moisturizing, humectants, emollient, soothing, coating and pH adjusting effect. The product removes the scaly surface of the skin by the emollient action of macerates and vegetable oils. The humectant, soothing and emollient effect of glycerin and honey in the combination with vegetable oils and oil macerates relieves flaking, burning, redness and itching. Moreover, the ointment promotes the restoration of epidermal barrier function by optimizing skin hydration, preventing trans-epidermal water loss due to its coating effect, and lowering skin pH toward a healthy range.

Conflict of Interest: None.

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FULL TEXT–ORAL PRESENTATION

JOJOBA CULTIVATION AND INDUSTRIAL USE CASE

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Abstract

Jojoba is an industrial plant in the form of a greyish-green bush, perennial and evergreen. The seeds of jojoba (*Simmondsia chinensis* Link), a member of the Simmondsiaceae family known for its superior quality oil seeds, contain oil ranging from 45% to 60%. The feature that distinguishes it chemically from other vegetable oils is that it has a liquid structure called wax although oils produced from jojoba seeds are triglycerides. Jojoba, which is not cultivated economically in Turkey, has an average of 300 ha cultivation area and 150-ton production in recent years in the world. Although jojoba is a plant grown both by seed and vegetatively, it has been stated that vegetative cultivation is suitable for superior yield. Jojoba can easily grow in barren, arid, saline and sloping lands where other plants do not grow, and thanks to this feature, it is a promising plant. Since jojoba has a very strong and deep root system, it also minimizes the loss of landslides exposed to erosion. Some researchers in Turkey have been determined that the commercial cultivation of jojoba can be carried out in the entire Mediterranean coastline, Southeastern Anatolia and the microclimate areas of the Aegean. Jojoba oil is mostly used in the machinery industry, leather production, cosmetics and pharmaceutical industries. Besides, it is used in wax, disinfectants, detergents, floor polishes, shoes, cars and for the preparation of a large number of different substances. Jojoba, one of the main plants used as a raw material in biodiesel studies because it is a sustainable and renewable energy source. The versatile use of jojoba oil, the need for bioactive substances in the pharmaceutical industry and the fact that the raw materials used in the production of biodiesel are plants that are used as food add another dimension to the subject. Considering that today's rapid population growth, nutrition and health problems come to the fore, it would be appropriate to state that oil production should be prioritized, the bioactive material requirements should be given importance and jojoba cultivation should be supported.

Key Words: Jojoba, *Simmondsia chinensis* Link, industrial plant

1. Introduction

Jojoba (*Simmondsia chinensis* L.) is a perennial desert plant with stunted, bushy, bluish-green leaves. The homeland of jojoba, which is in the Simmondsiaceae family of the dicotyledons subclass, is the Sonora Desert located between California, Arizona and Mexico. It has been documented that the natives of the California-Baja Island used jojoba oil for medicinal purposes such as cooking, hair care, wound treatment, hair strengthening, and induction of labor pains (Naqvi and Ting, 1990). In addition, evidence has been found that jojoba oil was used in the manufacture of perfumes and paints to be used in religious ceremonies in ancient times (Mejia et al., 2013). Jojoba plant is also known as goat nut (Akdeşir, 2001). The value of the jojoba plant was realized in early 1969. After the studies showed that the oil obtained from jojoba seeds can be used instead of sperm whale oil, commercial cultivation of jojoba plant has started in some other countries, especially in America. Since this date, thanks to jojoba oil, the slaughter of sperm whales for oil has been prevented (Naqvi and Ting 1990). In order to protect the dwindling world population of sperm whales and seven other whale species, the United Nations added sperm whales to its endangered species list in 1970 and banned the importation of oil, meat and other produce from whales. There is ample evidence that jojoba oil is an alternative to sperm whale oil.

Compared to sperm whale oil, jojoba oil provides advantages such as not having a fishy odor, not requiring any processing for some industrial purposes, and not blackening in the sulphurization process (Wisniak, 1994).

2. Situation of the jojoba plant in the world

Jojoba plant is grown commercially in many countries such as Mexico and Israel, especially in America and Argentina. It has been determined that the global jojoba oil market has reached a volume of 16,318 tons according to 2020 data, and it is estimated that this rate will reach 25,000 tons in five years (FAOSTAT, 2021). The export volume of jojoba oil in the world was 4382974 thousand dollars in 2020, and the countries that exported the most jojoba oil were India, America, Mexico, Netherlands and France, respectively. The import value was 4673644 thousand dollars in 2020, and the most importing countries were America, China, Germany, France and the Netherlands, respectively. Turkey has exported 86008 thousand dollars of jojoba oil in 2020 and Libya was in the first place with an export value of 36456 thousand dollars. The import value for 2020 was 49356 thousand dollars, and America took the first place among the importing countries with 14207 thousand dollars (Comtrade, 2021). The European market is geographically the leader, followed by North America. This situation can be attributed to the developing cosmetics industry in the mentioned regions. The current production (15000 tons) in the world does not meet the demand. Therefore, new production areas are needed. Jojoba

cultivation in Turkey is not done economically at the moment, but especially the coastal part of the Mediterranean region and most of our South East Anatolia region are suitable for jojoba cultivation. As a result of the studies carried out in Turkey, it is expected that jojoba cultivation will make a great contribution to the economy (Al-Obaidi et al., 2017). Jojoba has potential value in combating desertification and land degradation in arid and semi-arid areas. The subject of Jojoba plant, which was the subject of the issue number 7285 on 09 October 2007 of the General Directorate of Afforestation and Erosion Control of the Ministry of Environment and Forestry, has been included in the scope of combating erosion and desertification. Jojoba is a very high quality oil plant that can withstand drought very well and can grow in calcareous soils that can adapt to the Mediterranean Aegean Southeast Anatolian climate. Agricultural production in arid and semi-arid lands is minimal due to the severe environmental conditions and most of these lands are not used. In this sense, jojoba is a promising oilseed crop for the economic development of arid and semi-arid soils all over the world.

3. Jojoba Cultivation

Jojoba plant naturally spreads in the region between 25°-34° north latitude and 109°-117° west longitude at 0-1500 m altitude, on the slopes of mountains and valleys (Gentry, 1958). Jojoba occurs naturally on sloping lands in well-drained soils containing clay and silt in the lower soil layers. In cultivated areas, some genotypes grow better in sandy soils and some in alluvium-rich soils. Very clay soils may be suitable in some places, but good drainage is essential. For the production of jojoba, it can be grown in hot and dry climates at temperatures between -5 and 35 ºC. 500 mm of precipitation per year is sufficient. The cultivation of the Jojoba plant in Turkey is generally more suitable for the Mediterranean climate. Because summers are hotter and drier in the Mediterranean climate, and this plant is easier to adapt because it has high temperature and thirst resistance. The jojoba plant can adapt very well to the semi-desert climate and the Mediterranean climate. Therefore, seed propagation is not used in commercial cultivation (Thomson, 1982).

Jojoba, which can be produced with seeds and cuttings, starts to bear fruit from the 2nd or 3rd year when it is obtained from seed, and gives fruit in the same year when propagated from cuttings (Eed and Burgoyne, 2015). Seeds planted in the soil germinate in 2-4 weeks under ideal conditions. Approximately 50% male or female plants are formed from the seeds. The sex of the plants is understood from the 2nd or 3rd year. Since the jojoba plant is pollinated by allogam plants, a high rate of opening is seen in the seeds sown. In vegetative propagation, the genetic structure of the propagated plant is the same as the plant from which the propagation material was taken. Although the cost of reproduction per plant in vegetative propagation is higher than in propagation by seed, uniform plants with high seed yield are obtained (Hogan et al., 1981). Reproduction of jojoba plants with superior characteristics allows the establishment

of plantations, which is an example of growth and yield (McKeon, 2016). It stated that large numbers of male and low-yielding female plants in naturally grown jojoba populations can be cutting for grafting from high-yielding female plants. According to Thomson (1982), 2-3 year branches of jojoba plants can be cutting for grafting between February and April. Eed and Burgoyne (2015) reported that the rooting potential of the cuttings varies according to the season and the genotype of the plant from which the cuttings were taken. Also, they showed that the rooting potential is high in the spring and summer periods when the growth is active. Thomson (1982) determined that the best cutting time is between the end of March and the end of August and the rooting rates decrease in cuttings taken after August. Also, researcher explained the reason as the plant begins to rest and spends its energy on the ripening of the fruit. Palzkill (1988) stated that cuttings taken in the hot months of the year rooted better, but between November and March rooting was close to optimum. Ayanoğlu (2001) investigated the optimum cutting time for female jojoba plants by taking cuttings in 8 different periods starting from April 20, 20 days apart, and determined that the most suitable cutting time was mid-July. Thomson (1982) applied 4000 ppm IBA to 10-15 cm tall cuttings taken from the jojoba plant in July and August. He observed that the cuttings took root at a rate of 30-70% and the plants that were transplanted into the tubes could reach the size to be planted in the field the next spring. An average of 4-5 kg of seeds are obtained from a 12-year-old plant that gives full yield, in this case, 125-450 kg of seeds can be taken per decare (Ayanoğlu, 2001). Planting distance between plants varies according to ecology, harvesting method used and cultural processes. It is planted with an average spacing of 1.5 m between rows and 3.0-4.5 m between rows (Hogan et. al., 1981). The distance between the female plants and the pollinator male plants should not be more than 27 m. In the early stages of jojoba cultivation, weed control is important for the performance of plants (Hogan et.al., 1981). Root rots caused by factors such as Verticillium, Phymatotrichum and Macrophomina are seen in plants that are over-watered and exposed to ground water. Although aphids and some leaf eaters appear on the plants, there is no economic damage (Sharma and Singh, 2011).

Since jojoba seeds do not mature at the same time, they can be harvested at different times. Harvesting of ripening fruits is done in the period from mid-July to September, depending on the types. The hot and dry air in the summer causes the seeds to fall to the ground by cracking the green capsules in which the seeds are located. While the harvesting of seeds is done by hand-picking the spilled seeds by laying a cover under the plants in small plantations, it is done with specially developed vacuum harvesters in large plantations. Pruning of the lower branches can provide convenience in hedge or garden-type plantations. Machine harvesting is done with machines that pour the seeds on the ground by giving air from the top and collect the spilled seeds from the bottom with vacuum (Mejia et al., 2013). Leveling, planting spacing and pruning should be done in accordance with machine harvesting in the field where machine harvesting will be done (Ash et al., 2005). Post-harvest seeds can maintain their viability for a long time

when stored under suitable conditions. It has been reported that the viability of jojoba seeds stored at room temperature decreases by 40% at the end of the second year, but they maintain their viability for ten years when stored under low humidity and low temperature (3 °C) conditions (Liorente et al., 2013).

4. Botanical Description

This plant has perennial, evergreen and bluish green oval leaves (Akdeşir, 2001). Its height is usually 1.5-2 meters, with good irrigation it can reach 4-5 meters (Pehlivanlı, 2010). The root of the plant goes 9-10 m deep, so groundwater can be used. Although 80% of the roots are within the first 80 cm from the surface of the soil, it can reach 4-5 meters in a year or two. In some mature plants, the roots can go 13 meters deep (El-anany, 2007). With this feature, it is an excellent plant in the fight against erosion. Jojoba is a dioecious plant. Male and female flowers are on separate plants. To determine the sex, the first flower buds must be seen and 1 to 4 years must pass within this period (Jangra et al., 2014). The male plant produces pollen and has only yellow flowers with stamens. They are found on the plant in small bunch. The female plant has flowers with an ovary with three egg cells, and the female flowers are small, odorless or with a scent gland, no nectar. When the flowers of female plants are pollinated (usually by the wind), they give fruit containing oil-containing, brown and approximately hazelnut-sized seeds. Mature plants have a yield of 1.5 -5.5 kg of seeds (dry weight) per year, with an average annual yield of around 2.3 kg. The plant begins to bear fruit after about 5 years. The life span of a plant that is not seriously damaged by disease or insects varies between 50-200 years (Rosengarten, 2004). Flower buds usually grow in summer and autumn and open in spring. In general, each capsule contains one seed, while in some capsules this number may increase to two or three (Thomson, 1982). The weight of 1000 grain seeds is 900-1200 g. Although it varies according to the environmental conditions and plants in which it is grown, 50% of the volume of the seed is oil. The rate of male or female emergence from seeds is approximately 50% (Ayanoğlu, 2001).

5. Characteristics of its oil

Jojoba oil has superior physical and chemical properties that it is in the form of an odorless and colorless liquid wax. Jojoba seed contains 60% oil and 26-33% protein. Hence, jojoba oil is the main product derived from jojoba seeds. It is composed of long straight chain fatty acids and alcohol esters with a high number of carbons, in contrast to tri-glyceride containing oils from other plants. 87% of these esters are combinations of fatty acids and alcohols, each having 20-22 valence carbon atoms with a double bond. Glyceride esters, which are predominant in other animal and vegetable oils, are not found in jojoba oil, and the fatty acids in them are usually 16-18 carbons. The oils obtained from the seeds of all other plants are triglycerides

(combination of a molecule of glycerol with three fatty acids). Waxy oils such as jojoba and whale oil are wax esters (esterification of a long-chain alcohol molecule with a long-chain fatty acid). Similar to whale oil being different and unique among animal oils, jojoba oil is unique among vegetable oils (Ayanoğlu, 2001). The fatty acid composition of jojoba oil is contain 65–80% Gadoleic acid, 10-20% Erustic acid, 3% Nervonic acid, 3% Palmitic acid, 5-12% Oleic acid, 1% Palmitoleic acid, 1% Behenic acid and 1% Lingoceric acid. Its specific gravity is 25 oC and 0.86 density. Jojoba oil does not change in its structure when exposed to 285 oC continuously, and for 4 days when exposed to 370 oC heat. Whale oil, which does not lose its stability and viscosity at high temperature and pressure and adheres well to the contact surface, is the indispensable oil of heavy construction machinery. Jojoba oil is also mono-fluid. This means that it shows the same adhesiveness at different temperatures (Naqvi and Ting 1990).

6. Industrial Use Case

A unique high-quality oil is produced from the jojoba seed, which can be used in a wide variety of applications such as medical and industrial products. In addition to being a plant used to combat erosion and flooding, the oil obtained from jojoba is used in cosmetics, medicine, pharmacy and industrial industry. The oil contents of jojoba seeds are used as raw materials in the production of many substances such as disinfectants, detergents, soaps, lubricants, emulsifiers, resins, adhesives and printing ink. Jojoba oil is also hydrogenated and used to make hard white waxes for use in furniture, floor, shoe and automobile polishes. It is also preferred for the lubrication of very sensitive machines such as watches. In some countries, jojoba oil is used as a material and engine oil additive for vehicles used in the defense industry (tank, armored vehicle, etc.). Most of the jojoba oil produced today is used in shampoos, creams and lotions in the cosmetic industry. Jojoba was used by the Indians for skin diseases. Likewise, in a trial conducted at the Ben-Gurion University Hospital in Israel, it was observed that the acne was completely eliminated after the use of creams containing jojoba oil in patients with advanced acne (Arya and Khan, 2017). Jojoba oil is preferred in the cosmetic industry for the production of anti-aging and cell-renewing creams due to its purity, odorless and non-deteriorating, anti-aging (cell activity enhancing, rejuvenating) structure. Today, the jojoba industry is a raw material for 400 types of cosmetic products (Kohorn, 1994). In addition, with the production of many new chemicals in the chemical industry, it is very promising in the industrial field. In conclusion, it can be said to have superior oil properties, used as a key ingredient in various pharmaceutical products.

Jojoba oil has a long shelf life as it does not oxidize, harden or deteriorate under high temperature and pressure. Because of this feature, it serves as a basic ingredient in various skin and hair care products such as moisturizers, facial cleansers, stretch marks reducers, conditioners, hair masks. Apart from that, jojoba oil is also rich in various vitamins and minerals

such as vitamin E and B complex, silicon, chromium, copper, iodine and zinc. Due to its richness in vitamin E, it is used in medicine as a regenerative agent for scratches, cuts, acne, hair and skin disorders, and as a lubricant in artificial hearts due to its 100% purity. It has an extraordinary skin softening feature. It is also used against sunburns (Al-Obaidi et al., 2017). Since jojoba oil contains myristic acid, it is used for the treatment of inflammation and swelling caused by joint rheumatism and injuries.

Oils are converted into semi-solids (like margarine oils) by hydrogenation, but hydrogenation turns jojoba oil into a white crystalline wax-like structure. This solid oil is used in candle making; in car, floor, furniture and shoe polishes; to wax and brighten fruits, to insulate batteries and cables, to make dustless chalk and colored pencils (Ayanoğlu, 2001). Since waxy substances cannot be used in the human digestive system, it is not considered appropriate to use jojoba oil directly as food. Indirectly, the use of jojoba oil in meals is highly appreciated. The oil absorption rate of the substance fried in jojoba oil is half compared to other oils. Such a conclusion was reached in the trial conducted in Nestle's laboratories in Switzerland, but it could not be included in the products because there was not enough Jojoba oil (Arya and Khan, 2017).

Some researches showed that antibiotic biosynthesis is increased with jojoba oil, and penicillin can be obtained as high as whale oil. The same is true for tetracycline production Penicillins coated with jojoba oil or hydrogenated jojoba oil are protected from inactivity in gastric juice and are assimilated into the system. Therefore, penicillin absorption becomes easier. In pharmacology, it can also be used as a high nitrogen fertilizer with animal feed and 20-30% protein, in penicillin production and tetracyline production (Yılmaz, 2012).

After extracting the jojoba oil, the remaining pulp is used as feed in the animal production cycle. Otherwise, their accumulation in nature will cause great environmental pollution. Oil-free jojoba pulp is a rich source of dietary fiber and contains high crude protein. Although this amount of protein is less than cottonseed and soybean meal, it contains many important nutrients and has good potential as an animal feed supplement (Arya and Khan, 2016). After taking the appetite suppressant Simmondsia in the pulp, it is used in the animal husbandry sector (Ayanoğlu, 2001). Jojoba pulp contains 25-35% protein, 9-11% fiber and 5-15% oil (Weiss, 1983). After oil extraction, Jojoba proteins contain 65% albumin, 21% globulin, 8% prolamine, and 6% glutelin. The first limiter in this protein as an essential amino acid is methionine.

7. Result

Jojoba is a very valuable plant with its use in many fields (cosmetics, pharmaceutical industry, lubrication industry, food industry, electrical insulation, oil heating) in industry. On the other

hand, the fact that it can grow in arid and weak soils constitutes a very important alternative for such areas. It is possible to grow jojoba, which has such great importance and superior characteristics, in barren areas in the south of our country. It is important criteria for our country that it can be used especially in the fields of medicine, cosmetics and machinery, and also that it does not deteriorate for many years. Considering that Turkey loses 500-600 million tons of soil with erosion every year, arid and barren lands will be protected by integrating jojoba into agriculture. In addition, jojoba is emerging as a new option for arid regions, and it is seen as a new source of income for the local farmer for arid and unproductive lands. Today, where alternatives that will provide the highest yield per unit area are sought in plant production, the most effective method that can be done in this regard will be to bring jojoba into agriculture.

Conflict of Interest

The authors have no conflict of interest.

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FULL TEXT–ORAL PRESENTATION

EFFECT OF PLANT AGE ON ESSENTIAL OIL CONTENT AND COMPOSITION OF *SALVIA FRUTICOSA* (MILL.)

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Abstract

Salvia fruticosa species known as Anatolian sage is widely used in many sectors like food, cosmetic, medicine etc. The essential oil content and composition are main guiding properties for determining usage area for this plant. The essential oil content and composition can vary according to many factors like plant cultivar and age. This study was carried out to determine the effect of plant age on essential oil ratio and component ratios for the sage oil. Clonally developed UYSAL and TURGUT candidate cultivars were used in the study. The essential oil content of the cultivars for 5 years was determined by using clevenger apparatus. Essential oil composition was analyzed by GC-MS/FID device using a capillary column. There were significant differences in essential oil composition with respect to age of plant for each cultivar. While, 1,8-cineol varied between 51.63-64.74% over the years for Uysal cultivar, it changed between 47.16-65.55% for Turgut cultivar. β-pinene reached the maximum level in last year for Uysal (14.16%) and Turgut (14.95%) variety. Camphor, one of the main components of sage, was determined as the highest for 2 years old plants for both cultivars, and the lowest value was obtained in 5 years old plants. As a result, the age of the plant for the sage was quite effective on essential oil content and composition.
Keywords: Sage, *Salvia fruticosa*, cultivar, plant age, essential oil.

1. Introduction

The genus *Salvia* belonging to the Lamiaceae family includes medicinal aromatic plant species that have been used for a long time and have never lost their importance until today. The most important of these species is *S. fruticosa* Miller. While this species is often known as Anatolian sage, it is also known as sage, grizzly or apple sage. Known to be good for respiratory tract infections, nervous diseases and diarrhea, Anatolian sage also has pain-relieving properties. Its essential oil is used in the perfumery and cosmetics industry, and in the manufacture of candy and cakes (Baytop, 1999). Its leaves are consumed as tea and the essential oil obtained from its leaves is called 'apple oil'. A significant part of the essential oil obtained is exported.
Yield is one of the main characteristics that determine the commercial value of *Salvia* species.

Since the side effects of synthetic origin materials have emerged in recent years, quality factors are gaining importance in addition to efficiency. The most important factor that determines the quality of Salvia species is the essential oil rate and the components in the essential oil. The components and ratios in the essential oil give information about the quality of the oil and the areas where it can be used. The medicinal quality of the plant is primarily determinedby genetics. However, the plant does not behave the same waythroughout its life and undergoes changes that may cause vari-ation in the concentration of its active components. (Chagas et al., 2011). Variations in plant age and nutritional availability are the mostsignificant factors influencing the chemical composition and con-tent of essential oils (Martins et al., 2006). The plant's age anddevelopmental stage may influence the total amount of secondarymetabolites as well as the relative proportion of these compoundsthroughout the life of the plant. Younger plant tissue usually hasincreased biosynthetic activity because of the increased productionof secondary metabolites, such as essential oils (Moraes, 2009).

Studies have shown that the aging process of plants may causea reduction in the content of essential oils and major compounds (Bayram et al. 1999, Baydar et al. 1999, Skoula et al. 2000, Aydın et al. 2019, Dinçer et al. 2012, Karık and Sağlam 2017, Karık and Sağlam 2018, Leontaritou et al., 2020), whereas other studies have detected variations in the chem-ical behavior of plants because of the age and development stageand shown that age can promote increased levels of essential oilsand major constituents (Santos and Innecco, 2004). Therefore, suchstudies are indispensable for determining the optimal conditionsfor cultivating and harvesting aromatic species because such infor-mation ensures that essential oils have the required quality andsafety because the market requires quality essential oils of a pre-determined composition (Carvalho Filho et al., 2006).

The effect of plant age on biochemical properties has been studied in many species, but such studies have been found to be insufficient in *S. fruticosa* species. Studies are generally limited to two years. No study on long-term changes was found. Depending on which sector the raw material to be used will be used, the essential components of essential oils differ. In this context, the right variety supplied at the right time is needed in order to obtain the highest component with the least input. In this study, it was aimed to determine the proportional change of essential oil and essential oil components according to plant age of cultivars developed in *S. fruticosa*.

2. Material and Methods

'UYSAL' and 'TURGUT' cultivars belonging to *S. fruticosa* species obtained from previous breeding studies were used as material. Study; It was carried out for 5 years between 2017-2021 in the Aksu campus of the Batı Akdeniz Agricultural Research Institute. In the region where the experiment was conducted, summers are hot and winters are mild. The climatic data of the years of the experiment are given in Table 1.

Table 1. Climatic data of Antalya-Aksu during the experiment

Years	2017							2018				
Months	June	July	August	September	October	Nov.	Dec.	Jan.	Feb.	March	April	May
Precipitation (mm)	0.0	0.0	0.0	0.0	29.0	48.0	74.0	93.0	91.0	94.0	2.0	19.0
Mean Rel. Hum. (%)	66.4	62.0	72.3	72.4	64.9	74.0	81.8	72.2	83.0	78.9	68.7	66.2
Mean Temp. (°C)	25.8	29.4	27.9	25.2	19.7	14.4	12.0	10.8	12.8	15.0	18.5	23.2
Max. Temp. (°C)	44.5	44.8	40.3	36.9	19.7	32.2	25.9	20.9	21.2	25.8	35.2	35.6
Min. Temp. (°C)	15.5	18.3	19.0	14.7	19.7	3.1	0.8	1.7	3.4	6.8	6.7	11.9
Years	2018							2019				
Months	June	July	August	September	October	Nov.	Dec.	Jan.	Feb.	March	April	May
Precipitation (mm)	65.0	18.0	0.0	13.0	24.0	57.0	156.0	300.0	127.0	72.0	149.0	7.0
Mean Rel. Hum. (%)	72.8	65.8	71.2	65.1	67.3	72.5	78.0	85.1	80.1	76.7	75.6	71.9
Mean Temp. (°C)	25.5	28.5	28.0	25.9	20.4	15.7	11.5	9.6	11.4	13.4	15.8	21.3
Max. Temp. (°C)	38.0	43.3	40.8	40.7	35.5	31.5	21.6	17.6	20.6	27.4	27.6	36.3
Min. Temp. (°C)	16.3	18.2	17.2	15.2	7.2	7.2	0.0	0.8	3.6	2.5	5.6	9.9
Years	2019							2020				
Months	June	July	August	September	October	Nov.	Dec.	Jan.	Feb.	March	April	May
Precipitation (mm)	13,0	0,0	0,0	77,0	19,0	71,0	262,0	142,0	97,0	22,0	27,0	53,0
Mean Rel. Hum. (%)	69,4	62,5	63,5	67,3	72,4	80,0	81,0	62,1	78,4	74,8	77,4	68,7
Mean Temp. (°C)	25,8	28,6	28,7	25,2	22,5	16,1	11,8	10,1	11,1	13,6	16,6	21,5
Max. Temp. (°C)	39,7	40,9	42,8	36,4	37,5	30,0	23,1	18,7	22,2	24,4	27,8	43,2
Min. Temp. (°C)	12,2	16,0	16,1	12,1	11,1	5,1	1,2	0,9	0,6	2,8	6,6	11,0
Years	2020							2021				
Months	June	July	August	September	October	Nov.	Dec.	Jan.	Feb.	March	April	May
Precipitation (mm)	1,0	0,0	1,0	0,0	26,0	33,0	440,0	317,0	26,0	35,0	4,0	5,0
Mean Rel. Hum. (%)	70,8	70,4	66,3	70,9	75,1	62,3	-	80,6	72,2	67,4	69,9	69,2
Mean Temp. (°C)	23,8	28,6	28,4	27,0	22,0	15,9	13,3	11,2	12,3	12,6	16,8	22,3
Max. Temp. (°C)	40,4	41,3	41,0	41,2	38,1	28,2	22,5	22,3	26,8	23,3	31,5	38,8
Min. Temp. (°C)	12,7	18,5	17,8	17,2	12,2	4,2	5,2	1,3	6,8	1,7	6,0	9,9

Experimental lay out was randomized blocks with 3 replications. A drip irrigation system was installed and mulch was laid on the soil prepared area. The genotypes were planted in the experimental area in March 2017 with a distance of 0.4*0.7 m between rows and rows. The experiment was planned with 4 rows and 10 plants in each row. All agricultural activities (fertilization, irrigation, removal of weeds, spraying, harvesting, etc.) were carried out during the study. As of May 2017, two months after planting the plants, the essential oil content and essential oil components were determined in the samples taken at the same time each year, with 3 replications for five years. The essential oil ratio (%) was obtained by hydrodistillation method in the Klavenger apparatus. Distillation was carried out for 3 hours by adding 300 ml of distilled water on 20 g sample and the essential oil yield was calculated (Anonymous 2011).

The essential oil component ratios were determined using a GC-MS (Gas chromatography (Agilent 7890A)-mass detector (Agilent 5975C) device and a capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm). The samples were diluted 1:100 with hexane for analysis. In the analysis, helium was used as the carrier gas at a flow rate of 0.8 ml/min, and the samples were injected into the device as 1 µl with a 40:1 split ratio. Injector temperature was set at 250°C, column temperature program at 60°C (10 minutes), from 60°C to 220°C at 4°C/minute and 220°C (10 minutes). In line with this temperature program, the total analysis time was 60 minutes. Scanning range (m/z) of 35-450 atomic mass units and electron bombardment ionization of 70 eV were used for the mass detector. The data of WILEY, NIST and OIL ADAMS libraries were used as basis for the identification of essential oil components. The component percentages of the results were made using the FID detector, and the components were identified using the MS detector.

3. Results and Discussion

The results showed that the content of *S. fruticosa* essential oils varied significantly among genotypes within each age group. In genotype UYSAL, the highest essential oil content was observed in the three and four-year-old plants (4,00%), whereas the lowest value was observed in the five-year-old plants (2,50%). In genotype TURGUT, the highest essential oil content was observed in the three-year-old plants (3.89%), whereas the lowest value was observed in the five-year-old plants (2,83%) (Table 2). When the ages of the genotypes were compared in terms of essential content, it was observed that it was higher in three- and four-year-old plants. When the ages of the genotypes were compared in terms of essential content, it was observed that it was lower in one- and two-year-old plants, and higher in three- and four-year-old plants. In five-year-old plants, this rate was lower than in one-year-old plants (Table 2). According to Matos and Innecco (2002), proportional reduction occurs in the activity of metabolic ways as the plant ages that reduces the synthesis of secondary metabolites. Essential oil ration less in old plants, which was also observed in this study.

Determining the essential oil ratio for medicinal plants is not enough, it is important to reveal the changes of essential oil according to plant age on the basis of components. Therefore, in the present study, the effect of plant age on essential oil components was determined and significant differences were found in some component ratios according to plant age.

The distinct presence of three components was observed among genotypes within each studied age: 1.8 cineol, camphor, α-pinene. However, for both genotypes, cineole was the major compound.

The cineole content of the UYSAL genotype varied between 51,63% and 64,74%, while the TURGUT genotype ranged from 47,16% to 65,55%. In both genotypes, the highest cineol ratio was detected in three-year-old plants, while the lowest ratio was detected in two-year-old plants (Table 2). In both genotypes, camphor was determined at the maximum rate in two-year-old plants, and at a minimum rate in five-year-old plants. While camphor varied between 0,763-15,53% in the UYSAL genotype over the years, it varied between 0,45-19,14% in the TURGUT genotype. One of the important components, α-pinene was determined the highest in old plants in both genotypes. While α-pinene varied between 3,21-4,24% in the UYSAL genotype over the years, it varied between 2,95-4,30% in the Turgut genotype (Table 2).

For the secondary compounds α-pinene, β-myrcene and β-caryophyllene, there was a significant variation genotype and between ages. The highest secondary compound content recorded among plant ages between UYSAL and TURGUT, respectively, was as follows: α-pinene in one year-old plants (4,24%) and one year-old plants (4,30%); β-myrcene in five-year-old plants (5,75%) and five-year-old plants (5,86%); β-caryophyllene in five-year-old plants (6,74%) and five-year-old plants (8,92%) (Table 2). Between one- and five-year-old plants, the genotype UYSAL presented an increase of β-caryophyllene content from 3,50% to 6,74% and viridiflorol content from 0,45% to 2,23%; genotype TURGUT presented an increase of α-terpineol content from 2,18% to 3,50%. Between one- and five-year-old plants, the genotype UYSAL presented a decrease of camphene content from 2,12% to 0,26% and limonene content from 1,81% to 0,84% and β-thujone content from 1,16% to 0,31%; genotype TURGUT presented an decrease of camphene content from 2,84% to 0,20% and limonene content from 1,78% to 0,74% and β-thujone content from 1,63% to 0,00%.

Table 2. Values for essential oil and components in essential oil

Genotypes	UYSAL					TURGUT				
Components/Age	1 year old	2 year old	3 year old	4 year old	5 year old	1 year old	2 year old	3 year old	4 year old	5 year old
α-Pinene	4,24	3,21	3,99	3,925	3,881	4,30	2,95	3,57	4,23	3,95
α-Thujene	0,45	0,55	0,41	0,509	0,581	0,28	0,82	0,26	0,57	0,72
Camphene	2,12	1,80	0,62	0,974	0,263	2,84	2,68	0,57	0,96	0,20
β-Pinene	9,33	7,28	10,49	10,019	14,157	7,84	5,32	9,81	10,92	14,95
β-Myrcene	3,07	2,47	3,42	3,752	5,746	4,94	1,82	3,34	3,70	5,86
Limonene	1,81	1,09	1,05	1,297	0,838	1,78	0,81	1,01	1,29	0,74
1.8-Cineole	54,30	51,63	64,74	60,19	55,61	52,54	47,16	65,55	59,67	54,07
γ-Terpinene	0,76	0,33	0,73	0,76	0,667	0,73	0,54	0,53	0,90	0,75
1-Octen-3-ol	0,45	0,33	0,41	0,369	0,274	0,55	1,05	0,28	0,32	0,26
α-Thujone	0,50	0,40	0,00	1,078	0,76	0,58	0,63	0,13	0,72	0,45
β-Thujone	1,16	1,13	1,09	0,36	0,307	1,63	1,62	1,32	-	-
Sabinene Hydrate	0,27	0,41	0,24	0,319	0,41	0,17	0,57	0,68	0,35	0,33
Camphor	11,82	15,53	3,45	5,524	0,763	11,96	19,14	3,61	5,09	0,45
Bornyl Acetate	1,37	1,56	0,00	0,656	-	1,01	2,15	0,14	0,55	0,69
β-Caryophyllene	3,50	4,34	3,48	3,539	6,737	4,75	7,40	3,74	3,69	8,92
Aromadendrene	0,22	0,64	0,10	-	0,474	0,25	0,27	0,10	0,42	0,72
Δ-Terpineol	1,15	0,97	1,21	1,171	0,997	0,89	1,27	1,18	1,17	1,01
α-Terpineol	2,93	3,66	4,10	4,776	3,579	2,18	2,95	3,92	4,89	3,50
Viridiflorol	0,45	0,73	0,48	0,478	2,234	0,78	0,85	0,26	0,57	2,46
Unidentified	0,09	1,92	0,00	0,30	1,72	0,00	0,00	0,00	0,00	0,00
Essential Oil	3,20	3,67	4,00	4,00	2,50	3,30	3,33	3,89	3,67	2,83

Table 3. Genotypes and years average of essential oil and main components in essential oil

Main Components and Essential Oil	Genotypes		Age				
	Uysal	**Turgut**	**1 year old**	**2 year old**	**3 year old**	**4 year old**	**5 year old**

1.8 Cineol	57,30[a]±1,263	55,80[a]±1,916	53,42[c]±1,067	49,40[d]±2,283	65,14[a]±0,990	59,93[b]±0,291	54,84[c]±0,405
Camphor	8,05[a]±1,506	7,42[a]±1,914	11,89[b]±0,605	17,34[a]±2,039	3,53[c] ±0,433	5,30[c]±0,280	0,61[d]±0,215
***α*-Pinen**	10,26[a]±0,618	9,77[a]±0,887	8,59[c]±0,519	6,30[d]±0,605	10,15[b]±0,249	10,47[b]±0,310	14,55[a]±0,335
Essential Oil	3,47[a]±0,163	3,40[a]±0,110	3,25[b]±0,050	3,50[b] ±0,143	3,95[a]±0,055	3,83[a]±0,098	2,67[c]±0,167

The averages of 1.8 cineole, camphor, α-pinene, which are the most abundant in essential oil and essential oil rate are given in table 3. There was no statistical difference between UYSAL and TURGUT genotypes in terms of 1.8 cineole, camphor, α-pinene and essential oil ratios. Significant differences were observed between different plant ages in terms of 1.8 cineole, camphor, α-pinene and essential oil ratios. While the highest 1.8 cineole ratio (65,14%) was detected in three-year-old plants, it is seen that this ratio decreases as the plant ages. Similarly, while the highest camphor ratio (11,89%) was detected in two-year-old plants, this ratio decreases as the plant ages. On the contrary, the lowest rate of α-pinene (6,30%) is detected in two-year-old plants, while this rate increases as the plant ages. While the essential oil ratio varied between 2,67-3,95% according to the age of the plant, the highest ratio was obtained from three-year-old plants. While the essential oil ratio is low in young plants, it increases with age, and this ratio decreases at later ages.

Many researchers have carried out studies on the effects of plant age on essential oil. For species *Hyptis suaveolens* (Martins et al., 2006) and *Alpiniazerumbet* (Murakami et al., 2009) and *Lippia sidoides* (Santos et al., 2015) plant age also influenced the proportion of volatile constituents in essential oils. According to Moraes(2009), the age and developmental stage of the plant can redirectthe metabolic pathway and lead to the biosynthesis of different compounds that might cause an increase of certain compounds.

In this study, essential oil and components of UYSAL and TURGUT genotypes were determined. In addition, the change in essential oil and its components according to the age of the plant was revealed. As a result; It has been revealed that the component ratios change according to the plant age, the genotypes have different component amounts according to the plant age, and there is a significant interaction between the components. Considering that the change between the components in terms of plant age has been ignored in many breeding studies to date; It is thought that the research offers the producer and the consumer the opportunity to evaluate different options. It is expected that the correct use of these options by the producer will contribute to the production and consumption of sage components in a more sustainable way.

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CORRELATION AND PCA ANALYSIS RESULTS IN 6 MORPHOLOGICAL PROPERTIES OF DIFFERENT FENUGREEK GENOTYPES

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Abstract

Fenugreek is one of the important plants among the fabaceae family as chickpea, common bean and soybean. It has anticarcinogenic, antiviral, and antioxidant effects as pharmacologically. It is used for many purposes by using different part of the fenugreek. In this study, six morphological properties were examined, and relationship among these properties were determined by using correlation and PCA analysis in 75 different fenugreek genotypes and two cultivars. Morphological properties as 50% seedling days (7.68-38.68 days), first pod height (6.67-41.24 cm), pod thickness (1.13 2.83 mm), stem diameter (2.58-6.63), leaf length (1.33-4.09 mm) and leaf width (0.66-1.94) were measured. Correlation analysis was performed to determine the relationship among these properties and two highly significant positive correlations were found between leaf width and stem diameter (r=0.413) and leaf length (r=0.455). One positive correlation was found between stem diameter and leaf length (r=0.226). PCA analysis was carried to classify and characterize different origin fenugreek genotypes and cultivars depending on the six morphological properties. PC1 and PC2 explained 51.53% in total variations and it was divided three groups. First group occurred with stem diameter, leaf length and width. Second group consisted of 50% seedling days and pod thickness. The last group included only first pod height. It can be said that first pod height is effective on many of the fenugreek genotypes, and stem diameter and 50% seedling days are effective for the Çiftçi and Gürarslan cultivars, respectively.

Keywords: *Trigonella foenum graecum* L., principal component analysis, seedling days, first pod height.

1. Introduction

Fenugreek is an annual dicotyledonous plant belonging to fabaceae family. The native of this plant is Southern Europe, Asia, and the Mediterranean region (Snehlata and Payal, 2012). Seeds and leaves of the fenugreek are used in traditional medicine and food industry (Syeda et al., 2008).

It was reported that fenugreek seeds include several important biochemical components as steroidal sapogenins (diosgenin), polysaccharide fiber (galactomannan), amino acid (4-hydroxyisoleucine) or etc, with important medicinal and pharmacological characteristics impacting human and animal health. There are noticeable differences in the quality of several phytochemicals found in fenugreek seed possibly due to variations in plant genotypes and agroclimatic conditions (Zandi et al., 2015). These different climatic conditions can be identified from warm temperatures to tropical regions (Malhotra and Vashishtha, 2008).

Different reports were noted by previous researchers for the species of the fenugreek. Because, morphology, growth habit, color of flower, leaves, stem and chemical compositions have a great variability among the fenugreek species (Svecova and Neugebauerova, 2010).

Fenugreek varieties have low production potential and new varieties should be developed including high yield and yield components. Also, genetic diversity reveals the variability of fenugreek and magnitude of variability provides to develop desired new varieties. With this study, 6 morphological properties, some of them yield component, were determined, and correlation and PCA analysis were conducted to determine the relationship among the these properties.

2. Material and Methods

The seeds of fenugreek genotypes were obtained from United States of America (USDA) and fenugreek cultivars were obtained from Ankara University, Faculty of Agriculture (Gürarslan) and Transitional Zone Agricultural Research Institute (Çiftçi). The materials consist of 75 genotypes and two cultivars (Gürarslan and Çiftçi) (Table 1).

The seeds of genotypes and cultivars were sown (15 April 2018) with augmented block design having 11 blocks with 11 entries in each block and having plot size of 3.3 m × 5.0 m with spacing of 30 cm.

Field experiments were carried out according to augmented trial desing during 2018 vegetation period at Field Crops Experimental Area (40°44’44’’ N, 31° 37’45’’ E), located at an altitude of approximately 881 m above sea level. Average temperature, rainfall and humidity were recorded as 17.10°C, 53.68 kg/m^2, 71.18% during the vegetation period, respectively (Yaldiz and Camlica, 2019). The experimental area soil properties were found as 23.74 kg/da phosphorus, 38 kg/da potassium and 1.6% organic matter, clay-loam and slightly alkaline (pH: 7.5) (Yaldiz and Camlica, 2021).

Table 1. The used materials in experiment

No	ID	Country	No	ID	Country	No	ID	Country
1	PI 302448	India	27	PI 568215	Turkey	53	PI 661008	Pakistan
2	PI 572540	Syria	28	PI 532862	Pakistan	54	PI 617078	Bulgaria
3	PI 469264	Egypt	29	PI 381062	Iran	55	PI 268434	Afghanistan
4	PI 194020	Ethiopia	30	PI 557489	Turkey	56	PI 143505	Iran
5	PI 173820	Turkey	31	PI 567879	Turkey	57	PI 613632	Australia
6	PI 286532	India	32	PI 229793	Iran	58	PI 170834	Turkey
7	PI 250235	Pakistan	33	PI 628787	Nepal	59	PI 628790	Jordan
8	PI 613633	Australia	34	PI 577712	Morocco	60	PI 426974	Pakistan
9	PI 617076	Bulgaria	35	PI 197471	Ethiopia	61	PI 269994	Pakistan
10	PI 226572	Ethiopia	36	PI 338679	Morocco	62	PI 661011	Palestinian
11	PI 175933	Turkey	37	PI 171872	Turkey	63	PI 577713	Spain
12	PI 572538	Egypt	38	PI 244291	Spain	64	PI 661009	Palestinian
13	PI 532867	India	39	PI 543072	Pakistan	65	PI 286533	India
14	PI 381061	Iran	40	PI 661007	Palestinian	66	PI 269996	Pakistan
15	PI 296394	Iran	41	PI 532863	Pakistan	67	PI 141725	Iran
16	PI 343170	Egypt	42	PI 173819	Turkey	68	PI 577711	Morocco
17	PI 639185	Armenia	43	PI 426972	Pakistan	69	PI 212124	Afghanistan
18	PI 613631	Pakistan	44	PI 572539	Egypt	70	PI 628790	Jordan
19	PI 214351	India	45	PI 660994	Bulgaria	71	PI 613630	Morocco
20	PI 269993	Pakistan	46	PI 199264	Greece	72	PI 462264	Egypt
21	PI 660995	Armenia	47	PI 257604	Ethiopia	73	PI 613629	Morocco
22	PI 251640	Ethiopia	48	PI 302449	India	74	PI 174393	Turkey
23	PI 613629	Morocco	49	PI 426973	Pakistan	75	PI 628785	Bulgaria
24	PI 215615	India	50	PI 250627	Egypt	76	Çiftçi	Turkey
25	PI 426971	Pakistan	51	PI 572540	Syria	77	Gürarslan	Turkey
26	PI 195854	Egypt	52	PI 661014	Pakistan			

DAP (diamonium phosphate) and ammonium sulphate (AS) were used as the base fertilizer at the rate of 6 kg/da and 4 kg/da, respecitvely. Half of the AS was applied as top dressed at 30 days after sowing (Çamlıca and Yaldız, 2019). These genotypes were harvested at physiological maturity from last of july to early August. The seeds were separated by hand threshing.

2.1. Statistical analysis

The analysis of variance (ANOVA) analysis was conducted to determine the differences among the 6 morphological properties at $p<0.05$ level by using JMP statistic program. Correlation and PCA analysis were carried out to determine the relationship among the examined properties.

3. Results and Discussion

3.1. Morphological properties

The results of the statistical analysis showed that significant differences were found among the fenugreek cultivars and genotypes in the examined properties ($p<0.05$).

Under Bolu conditions, these genotypes attained 50% seedling days between 7.68 and 38.68 days after sowing, while cultivars (Gürarslan and Çiftçi) began seedling after 31 days (Table 2). Ethiopia (PI 251640) genotype was the fastest seedling, but the slowest were Nepal (PI 628787) and Pakistan (PI 543072). Seven genotypes were later seedling than Çiftçi and Gürarslan cultivars. Çamlica and Yaldiz (2019) reported that 50% seedling ranged from 7.09-29.09, which was in partly agreement with this study.

First pod height of fenugreek cultivars and genotypes changed between 6.67-41.24 cm with mean 22.58 cm (Table 2). The highest first pod height was found in PI 286533 genotype and followed by PI 171872 genotype with 39.37 cm. The lowest first pod height was found from PI 269994 with 6.67 cm and followed by PI 613632 genotype with 6.86 cm. The first pod height of Çiftçi and Gürarslan cultivars were observed as 24.66 and 24.28 cm, respectively. The obtained results for first pod height were found partly similar with Çamlica and Yaldiz (2019) and Güzel and Özyazıcı (2021) who reported the first pod height of fenugreek between 17.00-35.78 cm and 25.26 to 41.76 cm, respectively. The differences can be explained depending on the genotypes, ecological and growing conditions.

Pod thickness of fenugreek cultivars and genotypes changed between 1.13-2.83 mm with mean 2.15 mm (Table 2). The highest pod thickness was found in PI 613629 genotype and followed by PI 286532 (2.79 mm) and PI 613630 (2.70 mm) genotypes, while the lowest pod thickness was seen PI 426973 genotype and followed by PI 469264 genotype with 1.56 mm. The cultivars were found close and the data was found as 2.11 mm for Çiftçi and 2.09 mm for Gürarslan. These cultivars were higher than 28 genotypes and lower than 42 genotypes.

There was a significant difference in terms of stem diameter. It changed between 2.58-6.63 mm among the fenugreek cultivars and genotypes (Table 2). The highest value was found in PI 661011genotype and followed by PI 577711 (6.32 mm) and PI 661009 (6.25 mm) genotypes. The lowest stem dimeter was found from PI 661008 and PI 617078 genotypes with 2.58 mm. The previous studies reported that stem dimeter in fenugreek changed between 3.68-4.12 mm (Zandi et al., 2011) and between 1.15-2.08 mm (Danesh-Talab et al., (2014). Our results were found similar with Zandi et al. (2011) and they were found higher than Danesh-Talab et al. (2014).

Leaf size (leaf length and width) and shape effect a range of important physiological processes as photosynthesis, transpiration and thermoregulation, and change with a series of environmental factors (Yates *et al.* (2010).

There was a significant difference among the fenugreek cultivars and genotypes for leaf length, which ranged from 1.33 to 4.09 cm with overall mean of 2.99 cm (Table 2). The tallest leaf lengths were noted in Pakistan (PI 613631) and India (PI 214351) genotypes with 4.09 cm and followed by Palestinian Territory (PI 661011) genotype with 3.80 cm. The smallest leaf length was seen in Pakistan (PI 532863) genotype and followed by Turkey (PI 174393) genotype with 1.95 cm, Syria (PI 302448) and Egypt (PI 469264) genotypes with 2.03 cm. The leaf lengths of cultivars (Çiftçi and Gürarslan) were found as 3.11 and 3.04 cm, respectively, and 38 genotypes had lower leaf length than cultivars. These results are partly in aggrement with the results of Aminkar et al. (2018), which was reported 1.5-2.08 cm leaf length of 21 fenugreek genotypes.

The leaf width trait ranged from 0.66 to 1.94 cm with overall mean value of 1.13 cm. The highest leaf width was seen in PI 661011 genotype and the lowest value was observed from PI 617078 genotype. The obtained results were found similar with Aminkar et al. (2018), who reported that leaf width ranged from 0.68 to 1.80 cm.

Table 2. Six morphological properties of fenugreek cultivars and genotypes.

Cultivars/ genotypes	50% SED	FPH	PdT (mm)	SD (mm)	LL (cm)	LW (cm)
PI 302448	27.68a-h	32.67a-f	1.71h-l	4.11g-q	2.03pqr	0.94h-r
PI 572540	27.68a-h	20.66g-v	2.03d-k	4.24e-q	2.51k-q	1.37b-h
PI 469264	24.68a-j	20.96g-v	1.56kl	4.21f-q	2.03pqr	1.10e-q
PI 194020	24.68a-j	22.76d-u	1.69h-l	3.53l-q	2.78f-o	0.97f-r
PI 173820	25.68a-ı	20.96g-v	1.97d-k	4.36d-q	2.38n-q	1.00e-r
PI 286532	26.68a-ı	21.16g-u	2.79ab	3.83j-q	2.84e-o	1.00e-r
PI 250235	23.68b-j	31.16a-ı	2.05c-k	4.16f-q	2.74f-p	0.70qr
PI 613633	36.68ab	21.62f-u	2.33a-h	5.72a-i	3.14b-m	1.26d-m
PI 617076	29.68a-f	18.02m-z	2.30a-i	5.77a-i	2.91d-o	1.29d-l
PI 226572	20.68c-k	19.22l-y	1.65i-l	5.82a-h	3.14b-m	1.39b-g
PI 175933	33.68a-d	24.22c-t	2.20a-k	5.67a-j	3.14b-m	1.23e-n
PI 572538	19.68d-k	20.92g-v	2.08c-k	5.50a-k	3.41a-g	1.43b-e
PI 532867	28.68a-h	24.33c-t	1.78g-l	5.45a-k	3.77abc	1.73abc
PI 381061	20.68c-k	27.13c-o	2.2a-k	4.62b-p	2.87e-o	1.39b-g
PI 296394	15.18f-k	30.47a-k	2.23a-j	5.82a-h	3.09b-n	0.92j-r
PI 343170	15.18f-k	25.27c-r	2.20a-k	4.94a-o	3.63a-d	1.26d-m
PI 639185	23.18b-j	30.27a-l	2.44a-g	5.03a-n	3.53a-e	1.26d-m
PI 613631	24.18a-j	33.97a-d	2.29a-i	4.22f-q	4.09a	1.32b-k
PI 214351	24.18a-j	30.87a-j	2.45a-f	5.46a-k	4.09a	1.36b-i
PI 269993	34.18a-d	22.57e-u	2.12c-k	4.07g-q	3.29b-i	0.96g-r
PI 660995	15.68e-k	29.67b-l	2.29a-i	4.81a-o	2.49l-q	1.24e-m

PI 251640	7.68k	26.67c-p	2.16b-k	4.57b-p	2.96d-o	1.01e-r
PI 613629	30.68a-d	16.87n-A	2.83a	4.57b-p	2.73f-p	1.07e-r
PI 215615	21.68c-k	27.87c-n	2.42a-g	3.72k-q	3.33b-i	1.34b-j
PI 426971	15.68e-k	19.47k-y	2.43a-g	4.89a-o	2.99d-o	1.11e-q
PI 195854	15.68e-k	29.07b-m	2.11c-k	3.11opq	3.16b-m	1.37b-h
PI 568215	33.68a-d	29.90b-l	2.11c-k	6.15a-d	3.31b-i	1.67a-d
PI 532862	25.68a-ı	16.10o-A	2.46a-f	5.02a-n	2.97d-o	0.90k-r
PI 381062	33.68a-d	19.50k-y	1.89f-k	4.07g-q	2.81e-o	1.17e-o
PI 557489	25.68a-ı	22.30e-u	2.37a-g	5.05a-n	2.87e-o	1.20e-o
PI 567879	33.68a-d	21.50f-u	2.22a-j	5.20a-n	3.01d-o	1.30c-k
PI 229793	33.68a-d	22.70e-u	2.39a-g	4.33d-q	2.97d-o	1.40b-f
PI 628787	38.68a	20.70g-v	2.59a-e	5.06a-n	2.47m-q	1.27d-l
PI 577712	22.68b-j	16.97n-A	1.93e-k	4.00h-q	3.18b-m	0.84m-r
PI 197471	33.68a-d	19.97ı-y	1.9f-k	4.84a-o	2.84e-o	1.34b-j
PI 338679	26.68a-ı	21.97e-u	1.97d-k	5.15a-n	2.74f-p	1.11e-q
PI 171872	10.68jk	39.37ab	2.4a-g	4.68b-o	2.64h-q	0.98f-r
PI 244291	29.68a-f	14.97r-A	2.13b-k	3.37n-q	2.84e-o	1.01e-r
PI 543072	38.68a	14.77r-A	2.10c-k	5.82a-h	3.11b-n	1.04e-r
PI 661007	30.68a-d	26.57c-p	2.4a-g	5.78a-h	3.28b-i	1.28d-l
PI 532863	27.18a-ı	21.47f-u	2.27a-j	5.86a-g	1.33r	0.91j-r
PI 173819	24.18a-j	24.27c-t	2.24a-j	6.01a-f	3.30b-i	1.15e-p
PI 426972	27.18a-ı	19.67j-y	2.11c-k	5.18a-n	3.23b-k	1.08e-r
PI 572539	37.18ab	13.67t-A	1.81f-k	5.74a-i	2.76f-p	0.98f-r
PI 660994	25.18a-j	21.07g-u	2.4a-g	4.77b-o	3.20b-m	0.71q-r
PI 199264	24.18a-j	34.47abc	1.68h-l	5.53a-k	3.20b-m	1.01e-r
PI 257604	23.18b-j	19.87j-y	1.90f-k	5.04a-n	3.10b-n	1.01e-r
PI 302449	27.68a-h	20.26h-y	1.92f-k	3.92i-q	2.54j-q	0.8n-r
PI 426973	27.68a-h	28.47b-m	1.13l	4.09g-q	3.31b-i	0.83m-r
PI 250627	23.68b-j	31.27a-h	2.05c-k	4.08g-q	2.84e-o	0.90k-r
PI 572540	26.68a-ı	14.46r-A	1.83f-k	3.11opq	2.78f-o	1.16e-p
PI 661014	26.68a-ı	20.86g-v	2.14b-k	2.81pq	2.71g-p	0.80n-r
PI 661008	25.68a-ı	26.46c-q	2.08c-k	2.58q	3.28b-i	1.20e-o
PI 617078	27.68a-h	15.86p-A	2.26a-j	2.58q	2.31o-q	0.66r-r
PI 268434	22.68b-j	21.66f-u	2.41a-g	5.40a-k	3.30b-i	1.03e-r
PI 143505	19.68d-k	11.66u-A	2.16b-k	4.40c-q	3.20b-m	1.10e-q
PI 613632	22.68b-j	6.86zA	2.38a-g	4.28e-q	3.34b-h	1.20e-o
PI 170834	20.68c-k	12.16u-A	1.96d-k	4.45c-p	2.87e-o	0.93i-r
PI 628790	20.68c-k	15.26q-A	2.12c-k	4.73b-o	3.10b-n	0.86l-r
PI 426974	22.68b-j	9.26yzA	2.25a-j	5.25a-m	2.94d-o	1.23e-n
PI 269994	14.68g-k	6.67A	2.03d-k	4.44c-p	2.94d-o	1.00e-r
PI 661011	29.68a-f	33.04a-e	1.61jkl	6.63a	3.80ab	1.94a
PI 577713	30.68a-d	25.04c-s	2.22a-k	5.40a-k	3.23b-k	1.74ab
PI 661009	29.68a-f	31.64a-g	2.60a-d	6.25a-c	3.03d-o	1.21e-o
PI 286533	12.68ıjk	41.24a	2.05c-k	4.57b-p	3.26b-j	1.11e-q
PI 269996	20.68c-k	22.04e-u	1.94e-k	4.82a-o	3.16b-m	1.08e-r
PI 141725	34.68abc	34.04abc	1.88f-k	6.09a-e	3.06c-n	1.18e-o
PI 577711	29.68a-f	20.84g-v	2.02d-k	6.32a-b	3.46a-f	1.28d-l
PI 212124	14.18h-k	18.37m-y	2.45a-f	5.30a-l	2.55j-q	0.93i-r
PI 628790	30.18a-e	9.77v-A	2.29a-i	3.39m-q	3.21b-l	1.16e-p

PI 613630	31.18a-d	13.97s-A	2.70a-c	5.23a-m	2.61i-q	0.93i-r
PI 462264	29.18a-g	22.02e-u	2.27a-j	3.73k-q	2.71g-p	0.73pr
PI 613629	30.18a-e	18.37m-y	2.59a-e	4.07g-q	3.15b-m	0.79opr
PI 174393	24.18a-j	23.77c-t	2.25a-j	4.47c-p	1.95q-r	0.99f-r
PI 628785	25.18a-j	23.57c-t	2.38a-g	4.51b-p	3.18b-m	1.29d-l
Çiftçi	30.91a-d	24.66c-t	2.11c-k	5.17a-n	3.11b-m	1.09e-q
Gürarslan	31.45a-d	24.28c-t	2.09c-k	4.54b-p	3.04d-n	1.22e-n
Mean	25.76	22.58	2.15	4.75	2.99	1.13
LSD (5%)	15.00	11.23	0.66	1.85	0.73	0.43
CV (%)	20.74	19.80	13.65	16.47	10.23	16.10

SED: Seedling days, PdT: Pod thickness, SD: Stem diameter, LL: Leaf length, LW: Leaf width, LSD: Least Significant Difference, CV: Coefficient of variation.

3.2. Correlation and PCA analysis results

Correlation and PCA analysis were conducted to determine the relationship among the 6 morphological properties of fenugreek cultivars and genotypes. Correlation analysis results showed that the least correlations were found among the 50% seedling days, first pod height, stem diameter, pod thickness, leaf length and width (Table 3). Totally, three correlations were found in fenugreek cultivars and genotypes. There were two highly significant positive correlations and one significant correlation. The highest significant positive correlations were obtained between stem diameter and leaf with r=0.413 and between leaf length and leaf width with r=0.455. The positive correlation was found between stem diameter and leaf length with r=0.226.

Table 3. Correlation analysis of 6 morphological properties

	FPH	**PdT (mm)**	**SD (mm)**	**LL (cm)**	**LW (cm)**
50% SED	-0,175	-0,012	0,16	-0,042	0,154
FPH		-0,116	0,172	0,187	0,217
PdT (mm)			0,044	-0,001	-0,051
SD (mm)				0,226*	0,413**
LL (cm)					0,455**

**: Significant at 5% level, **: Significant at 1% level, SED: Seedling days, PdT: Pod thickness, SD: Stem diameter, LL: Leaf length, LW: Leaf width.*

PCA analysis revealed that a total of 51.53% depending on PC1 and PC2 in examined properties and it divided the 3 groups (Figure 1). First group included the stem diameter, leaf length and leaf width. Second group occurred the 50% seedling days and pod thickness. The third group consisted of only a property as first pod height. First pod height affected the many of the fenugreek genotypes, and stem diameter and 50% seedling days effected the Çiftçi and Gürarslan cultivars, respectively. Principal Component 1 (PC1) accounted for 31.31 % of total variation and it was related to leaf length and leaf width consisted of PC1 as major factors. PC2 accounted for 20.22% of total variation with 50% seedling days and first pod height.

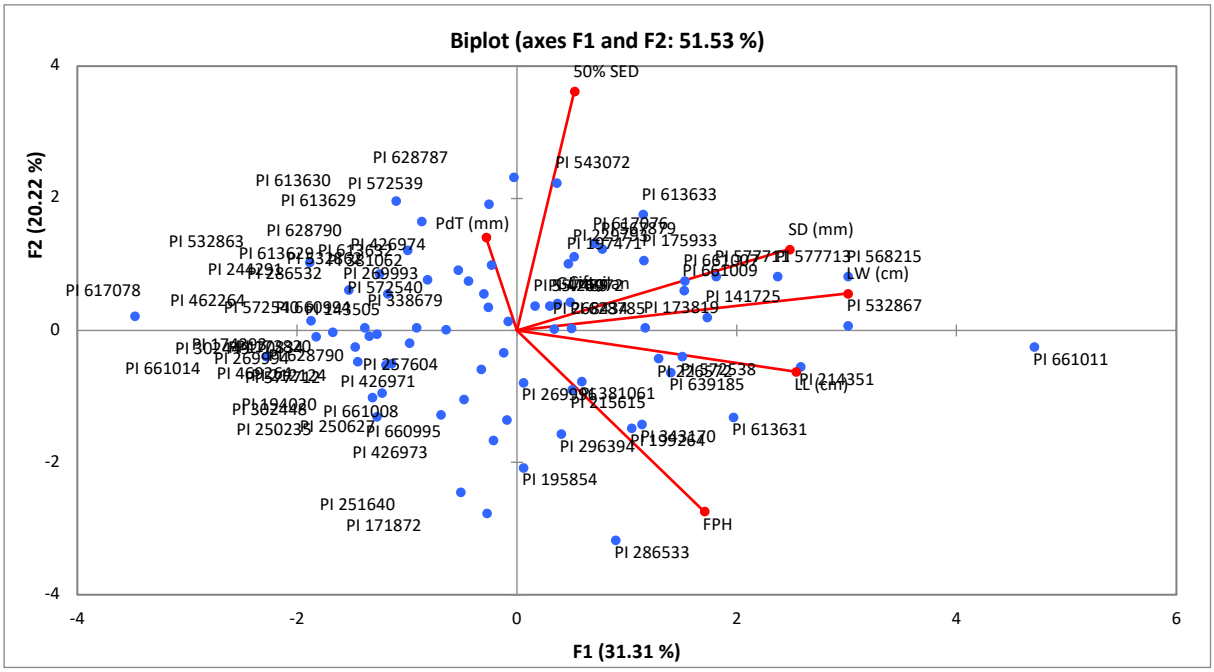

Figure 1. PCA analysis result of the examined properties of fenugreek cultivars and genotypes

4. Conclusion

Six morphological properties showed variability among the fenugreek cultivars and genotypes. So, the results of the study revealed that a wide genetic diversity were found in different origin fenugreek genotypes evaluated under field conditions. In addition, the examined properties of fenugreek genotypes used this study can be utilized to help in further crop improvement programme.

PI 251640 genotype had the earliest 50% seedling days and it can be recommended for the fastly seedling, and PI 286533 genotype had the highest first pod height and it can be suggested for mechanized farming. Generally, it can be said that correlation analysis showed the least correlations among the examined properties. It occurred only 3 correlations in 6 morphological porperties. Totally, PCA analysis showed 51.53% variation and leaf length and width the major factors of this variation.

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FULL TEXT–ORAL PRESENTATION

COMPARATIVE KARYOTYPE ANALYSIS OF POPULATIONS IN THE *MUSCARI MASSAYANUM* (ASPARAGACEAE) IN TURKEY

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Abstract
Muscari is a genus of bulbous plants belonging to the Asparagaceae family. The genus has a wide distribution in the Mediterranean basin, Central and Southwest Europe, the Caucasus, Southwest Asia and Central Asia. The species belonging to this genus have various medicinal and biological activities such as antioxidant, anti-inflammatory, emetic, diuretic, hypoglycemic and stimulating effects. In this study, the chromosome number and morphologies of four populations belonging to the endemic *Muscari massayanum* C. Grunert species were investigated. The detailed karyomorphologic features of the populations belonging to *M. massayanum* was reported and examined here for the first time. This work was carried out using the squashing method at the root tips and it was determined that all populations were diploid ($2n=2x=18$) and the basic chromosome numbers were $x=9$. Also, comparative karyotype analyzes showed that all populations have the common karyotype formula. According to asymmetry indices used in here Erzincan and Erzurum populations had the most asymmetric karyotype among all populations. These results indicate that the populations have the common chromosome number and a karyotype formula but display different karyo morphological features.
Key Words: Asparagaceae, endemic, karyomorphology, *Muscari*, Turkey.

1. Introduction

Our country is one of the most important and rich centers of the world in terms of plant resources, due to reasons such as climate and soil diversity, geomorphological difference and being a region where three floristic regions intersect (Avcı 2005). Anatolia, which is a bridge between Western Asia and Europe, contains many different species (Avcı 2005; Ekim 2014; Mammadov et al. 2016). These species have been known and used since ancient times and are still used as medicinal and aromatic plants today (Yeşilada 2002; Karaköse and Karaköse 2017; Akalın et al. 2020). Among geophytes, it is a decorative bulbous plant of the genus *Muscari*, known as grape hyacinths (Mammadov et al. 2012, 2016). It is represented by 77 species in the world (Eker 2021). These species are common in Central Asia, Europe and the Mediterranean Region. The genus known as müşkürüm in Turkey is represented by 51 species (Eker 2012;

Doğu and Uysal 2019, Eroğlu et al. 2019, Eroğlu and Pınar 2019; Yıldırımlı and Kılıç 2019; Eker 2019a,b; Eker et al. 2019; Eker et al. 2020; Eker and Armağan 2020; Eker and Kandemir 2020; Eker and Yıldırım 2021; Eker 2021). Of these 51 species, 34 are endemic, with an endemism rate of approximately 67%.

Some *Muscari* species are used in traditional medicine as diuretic, antirheumatic, expectorant, stomachic and anti-wart. In addition, some species are used as food, paint, ornamental plants in gardens and parks for humans and animals. Numerous floristic and karyological studies have been carried out on the genus (Çelik et al. 2004; Yılmaz 2004; Doğu and Uysal 2019, Eroğlu et al. 2019; Eroğlu and Pınar 2019; Yıldırımlı and Kılıç 2019; Eker et al. 2020; Eker and Armağan 2020; Eker and Kandemir 2020; Eker and Yıldırım 2021; Eker 2021; Stuart 1970; Karlén 1984a,b; Dalgıç 1991; Johnson 1994; Özhatay and Johnson 1996; Johnson and Brandham 1997; Demirci Kayıran and Özhatay 2017; Kıran et al. 2020a,b; Bozkurt 2020; Uysal et al. in press), which has pharmaceutical and economic importance (Casoria et al. 1999; Pehlivan et al. 2003; Baba et al. 2014).

Plant karyotype studies are important for understanding the origin and evolution of plant species, molecular phylogeny and floristic geography (Sun et al. 2019). Karyomorphological studies provide information about the potential evolutionary characteristics of karyotypes as well as the cytological mechanisms that drive the evolution of plant diversity on a phylogenetic step. In addition, karyomorphological studies are a fast and inexpensive approach to classify plant species by identifying key cytological parameters of a species, such as chromosome number, ploidy level, karyotype asymmetry, and karyotype coefficient of variation (Guerra 2008). The chromosome number and karyotype of a species are stable traits that can reflect its basic genetic information. The majority of Muscari species are diploid ($2n=2x=18$), while a few populations are polyploid (Stuart 1970; Karlén 1984; Dalgıç 1991; Johnson 1994; Özhatay and Johnson 1996; Johnson and Brandham 1997; Demirci Kayıran and Özhatay 2017; Kiran et al. 2020a,b; Bozkurt 2020; Uysal et al. in press). As conclusion, this study informs to us about the detailed karyotypes of four populations of *Muscari massayanum* endemic to Turkey.

2. Material and Methods

Four populations of *Muscari massayanum* were collected from different regions of Turkey (Table 1). Some of the collected samples were prepared for karyological studies and planted in green houses. Bulbs were germinated by placing them in foam floating in water, and chromosome counts were made in somatic metaphases using the crushing technique (Goldblatt 1996). Germinated root tips were fixed in Carnoy fixative for 24 hours at low temperatures after 8 hours of pretreatment with 0.002 M 8-hydroxyquinoline at 4°C. Root tips taken from Carnoy fixative for dyeing were hydrolyzed with 5 M hydrochloric acid (HCl) for 1 hour. Then, it was

stained with 1% aceto-orsein to which 45% acetic acid was added. For all counts, at least five metaphase plates from different individuals were examined and pictures were taken with an Olympus DP72 digital camera mounted on an Olympus BX53 microscope after the best metaphase image was obtained. Karyotype measurements were performed from the photographed metaphase images with the KAMERAM program and their karyomorphology was determined using various symmetry indices. The nomenclature of chromosomes, according to their morphology was carried out according to Levan (1964), and karyotype measurements and symmetry index calculations were carried out according to Romero-Zarco (1986) and Pazsko (2006).

Table 1. Taxa studied and localities

Populations of *Muscari massayanum*	**Localities**
Population Niğde	Niğde: Ulukışla, Ali hoca köyü, *Pinus nigra* açıklıkları, meyilli yamaçlar, 1060 m, 27 iv 2018, *T. Uysal* 3578.
Population Erzincan	Erzincan: Refahiye, Refahiye-İliç arası, 23 km, kumlu yamaçlar, 1630 m, 30 iv 2018, *T. Uysal* 3685a & *M. Armağan*.
Population Bayburt	Bayburt: Karşıgeçit koyu ile İspir güzergahı 2. km kumul tepeler, 1464 m, 28 v 2018, *T. Uysal* 3727 & *A. Sefalı*.
Population Erzurum	Erzurum: Erzurum-Bayburt Yolu, Serpantin döküntülü eğimli yamaçlar, 07 vi 2020, *T. Uysal* 4073.

3. Results and Discussion

Most species belonging to the genus *Muscari* are diploid (2*n*=18), although a few polyploid populations have been reported. It has been reported that the basic chromosome number of the genus is *x*=9 (Stuart 1970; Karlén 1984b; Dalgıç 1991; Özhatay and Johnson 1996; Johnson and Brandham 1997; Demirci Kayıran and Özhatay 2017; Bozkurt 2020; Kıran et al. 2020a, b; Uysal et al. in press). In this study, chromosome number and morphology of four populations of *Muscari massayanum* were performed (Figure 1). Details of the karyotypes of each population of the species are presented in Tables 2 and 3.

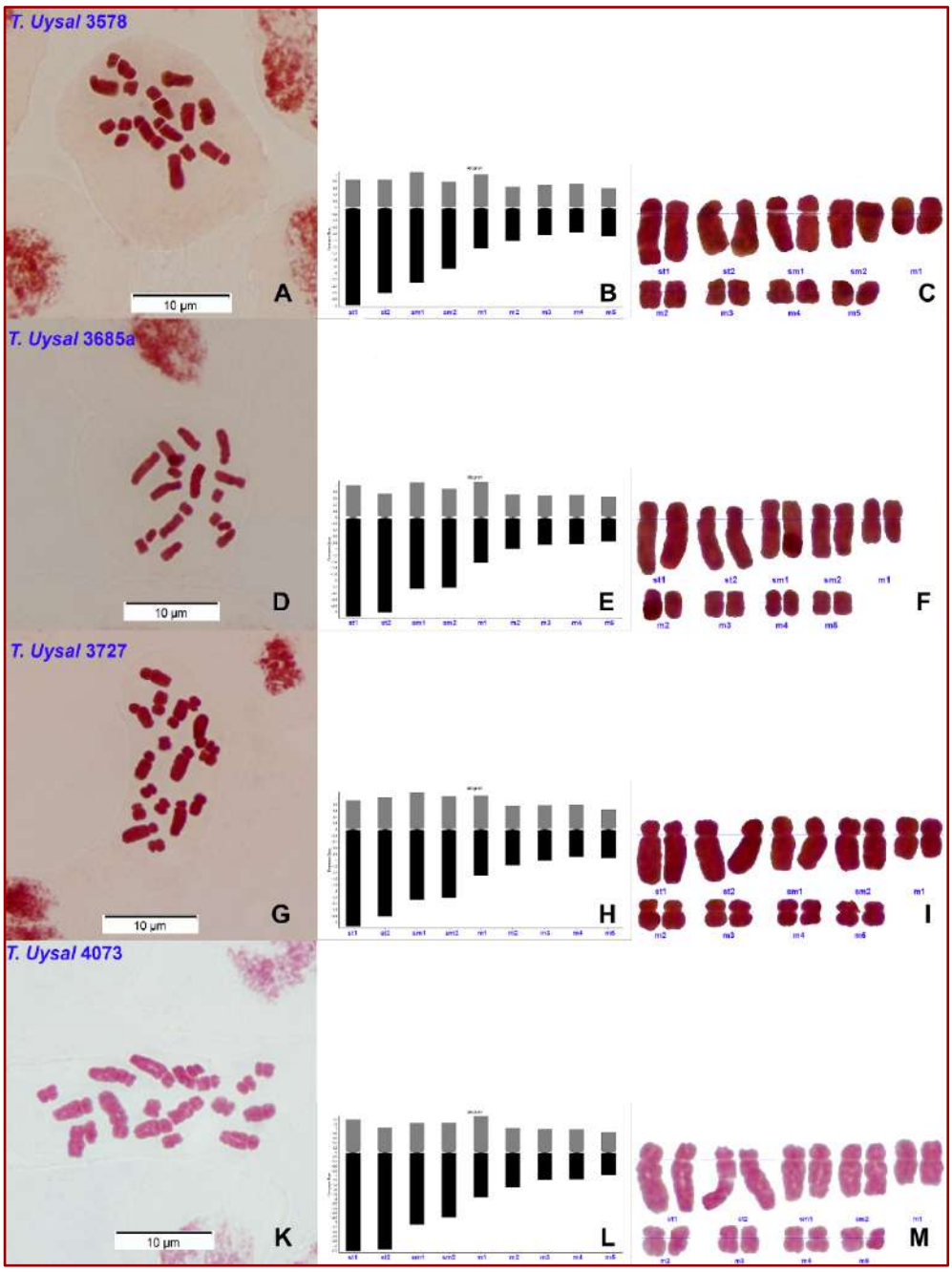

Figure 1. Mitotic metaphase chromosomes, idiograms and karyograms of populations belonging to *Muscari massayanum*. A-C: Niğde, D-F: Erzincan, G-I: Bayburt, K-M: Erzurum

Table 2. The chromosome features populations belonging to *Muscari massayanum*

Collection numbers	2n	SC-LC (µm)	LC / SC	p (µm) (±SD)	q (µm) (±SD)	CL(µm) (±SD)	TCL (µm)	CI (±SD)	KF
T. Uysal 3578	18	1.46 – 3.83	2.632	0.81 (±0.16)	1.61 (±0.80)	2.42 (±0.90)	21.752	36 (±0.09)	4st + 4sm + 10m
T. Uysal 3685a	18	1.40 – 4.15	2.964	0.85 (±0.18)	1.73 (±0.90)	2.58 (±1.01)	23.198	36 (±0.09)	4st + 4sm + 10m
T. Uysal 3727	18	1.58 – 4.07	2.582	0.92 (±0.18)	1.77 (±0.81)	2.70 (±0.94)	24.279	37 (±0.08)	4st + 4sm + 10m
T. Uysal 4073	18	1.83 – 5.61	3.063	1.15 (±0.21)	2.34 (±1.21)	3.49 (±1.32)	31.426	36 (±0.10)	4st + 4sm + 10m

Abbreviations: *R: Range, SC: shortest chromosome length, LC: longest chromosome length, p: mean length of the long short arm, q: mean length of the long arm, CL: mean chromosome length, TCL: total haploid complement length, CI: mean centromeric index, SD: standart deviation, KF: karyotype formula, m: metacentric, sm: submetacentric, st: subtelocentric.*

Table 3. The karyotype indices populations belonging to *Muscari massayanum*

Collection numbers	A_1	A_2	CV_{CL}	CV_{CI}	AI	M_{CA}
T. Uysal 3578 (Niğde)	0.4	0.371	37.08	24.683	9.153	33.05
T. Uysal 3685a (Erzincan)	0.393	0.391	39.059	26.008	10.158	34.1
T. Uysal 3727 (Bayburt)	0.398	0.347	34.743	20.826	7.236	31.59
T. Uysal 4073 (Erzurum)	0.392	0.378	37.752	26.795	10.116	34.09

Abbreviations: *A_1-Intrachromosomal asymmetry index, A_2-Interchromosomal asymmetry index, CV_{CL}-Coefficient of variation of chromosome length, CV_{CI}-Coefficient of variation of centromeric index, AI-Karyotype asymmetry index, M_{CA}-Mean centromeric asymmetry.*

In previous studies, the chromosome number of the species was reported as 2*n*=18 (diploid). The present study confirms previous reports (Speta 1989; Özhatay and Johnson1996). Chromosomes are named according to the position of the centromeres (Levan et al. 1964). The karyotype formula is the same in all populations of the species. Populations have four pairs of long, one pair of medium and four pairs of short chromosomes and they are without satellites. The chromosome number and morphology of *M. massayanum* were previously reported by Kiran et al. (2020a). The karyotype formula of this species is moderately different from the present study (Table 4).

Table 4. Comparison of the results with the previously reported karyotype formulas for *Muscari* taxa

Taxa	Karyotype formula	References
M. mirum	2n=18 (10m+2m^{SAT}+4sm+2st)	Kiran et al. 2020a
M. erdalii	2n=18 (8m+4sm+2st+2st^{sat}+2t)	Demirci kayıran and Özhatay 2017; Kiran et al. 2020a
M. tenuiflorum	2n=18 (8m+6sm^{SAT}+2st+2t)	Demirci kayıran and Özhatay 2017; Kiran et al. 2020a
	2n=18 (8m+2m^{SAT}+6sm+2st)	
M. longipes	2n=18 (8m+2m^{SAT}+6sm+2st)	Kiran et al. 2020a
M. babachii	2n=18 (8m+6sm+2st^{sat}+2t^{sat})	Demirci kayıran and Özhatay 2017; Kiran et al. 2020a
	2n=18 (10m+8sm)	
M. caucasicum	2n=18 (14m+4st)	Kiran et al. 2020a
M. comosum	2n=18 (14m+2sm+2t)	Kiran et al. 2020a
M. weissi	2n=18 (12m+2sm+4st)	Kiran et al. 2020a
M. massayanum	2n=18 (10m+8sm)	Kiran et al. 2020a
	2n=18 (4st+4sm+10m)	In this study

Demirci Kayıran and Özhatay (2017) reported different karyotype formulas in different populations of *Muscari neglectum* and they reported that such variations within the species may be due to altitude, weather conditions and geographic differences. *Muscari* is a taxonomically and karyologically complex genus. Species of the genus have adapted to different habitats. It has been reported that different habitats may have contributed to morphological differences and even genetic differences (Demirci Kayıran and Özhatay 2017).

Hybridizations and chromosome evolutions are very important to the genus. The reason for this variation and instability is the number and size of chromosomes, which leads to taxonomic problems in species identification (Valdez and Diaz Lifante 1992; Arslan and Uysal 2009). Additionally, a chromosomal list including chromosome number and morphology for the taxa of *Leopoldia* are submitted as based on the present literatures, thereby this chromosomal information would be supplied a comparative approach for them. Also, it is making it possible to see how much the species is different or not in terms of chromosome features (Table 4).

Karyotype asymmetry indices are widely used to make assumptions about the mechanisms of chromosomal evolution in plants (Paszko 2006). According to CV_{CL}, AI and M_{CA} indices of Erzurum and Erzincan populations have more asymmetric and evolved karyotypes than Bayburt and Niğde populations (Table 3). From the morphological observations in their natures, it is seen that there is a moderately morphological variations and differentiations between them, particularly in raceme length, flower shape and color. As connected with this observation, the

determined of the chromosomal variation among *M. massayanum* populations can be assessed as a positive correlation between two parameters (Chromosome and morphology).

4. Conclusion

In conclusion, the findings of this study reveal that the karyotype formula of the samples collected from different localities is the same. The results of this study may serve to clarify the taxonomic position of *Muscari massayanum* and related taxa.

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DETERMINATION OF SOME MORPHOLOGICAL, PHYSIOLOGICAL AND QUALITY PROPERTIES OF BAY LAUREL (*Laurus nobilis* L.) POPULATIONS

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Abstract

This study was carried out on single plants belonging to the laurel (*Laurus nobilis* L.) population in the experimental area of Ege University Faculty of Agriculture Department of Field Crops in Bornova. In the study, 40 laurel plants (12 females and 28 males) selected from the population in the experimental area were used as plant material. Leaf samples were taken from the lower, middle and upper parts of the plants for five months (May, June, July, August and September) in 2019 and some morphological, physiological and quality characteristics were determined in these samples. Parameters determined in the research were; plant height, leaf width and height, leaf fresh and dry weight, moisture content, leaf chlorophyll value (SPAD), leaf area, specific leaf area (SLA) and essential oil ratio. As a result of the study, the average width of the leaf was 28,16 mm, while the average leaf length was 70.23 mm. The average leaf fresh weight was determined as 0.33 g, the average leaf dry weight was 0.19 g, and the average fresh and dry weight ratio was 1.72. Moisture content of the leaves were between 34.55%-49.48% and 41.16% on average, and SPAD value was between 40.99-46.76% with an average of 43.32. As the months progressed from May to September, it was observed that there was a decrease in parameters such as fresh weight, moisture content and SPAD value from upper parts to lower parts of the plants. The average leaf area was 13.9 cm2, and the specific leaf area average was 0.73 dm2. The average essential oil ratio was 1.53% while, the values varied between 0.94% and 1.96%.

Keywords: Bay laurel, *Laurus nobilis* L., essential oil, leaf chlorophyll content, SPAD value.

1. Introduction

In agricultural activities, it is possible to produce the products necessary to meet the needs such as nutrition, clothing and shelter, which are vital for human beings, as well as the production of therapeutic plants that are beneficial for human health (Acıbuca, 2018). These plants, which we classify as medicinal aromatic plants, are used as a complement in traditional and modern medicine for the purpose of maintaining health, curing and preventing diseases.

Every year, the consumption of these plants increases with the increase in their use and awareness (Bayram et al., 2010). Especially using in spices, parfumery and cosmetics, in food additives, in aromateraphy, in natural paint production, in different industrial branches such as natural plant protection products and biofuels.

Turkey has different climate and soil characteristics because it is located in three different flora regions: Mediterranean, Europe-Siberian and Iran-Turan. This situation has brought with it a rich floristic diversity and various vegetation types. While approximately 3800 of Turkey's 12000 plant taxa are endemic species, approximately 2600 of 11000 taxa in Europe are endemic plant species (Yıldıztekin et al., 2019). Laurel (*Laurus nobilis* L.) is one of the important plants that spread naturally in the rich soil cover of Turkey, like many medicinal and aromatic plants. *Laurus nobilis* L. (Lauraceae), commonly known as bay laurel, sweet bay, true laurel or roman laurel. *Laurus nobilis* L. (bay tree), Laurel is known by different names such as defne, nehtel, tahnal, tefrin, tehnel, tenel, tenhel and teynel among the people in Turkey (Baytop, 1991). Mediterranean region is one of the main species of vegetation. It is evergreen and it can grow 2-20 m tall. It is dioecious tree. Laurel is generally found in Portugal, Spain, Italy, Yugoslavia, Turkey and the southern coast of Greece. It is distributed in 600-800 m altitudes in Turkey. It can be seen on all coasts from Hatay to the northeastern Black Sea, and on other coasts (Acar, 1987).

Bay leaves are divided into 5 classes in terms of quality (UNDP, 2018).

Selected by hand for packet: Leaves 4-7 cm apart, unbroken, uniform in color, without holes (Kilogram price is 20 TL),

• **Manual selection:** The color is smooth, without holes/broken (Kilogram price is 18 TL),

•**Semi-selection:** Cleaned from garbage and diseased leaves in the belt conveyor machine (Kilogram price 12 – 13 TL)

• **Serried**: Whipped, baled or pressed (Kilogram price 8 – 9 TL),

• **Under the sieve**: It is powdered and pressed (Kilogram price is less than 8 TL).

Today, the laurel plant is widely used in medicine and health, in cosmetics, in the chemical industry, in the field of food and biofuel (Adıyaman et al., 2008). If we want to give some examples of these; herbal tea (finely chopped branches and especially leaves), as a spice to make more delicious to meal, in pickle making, in liqueur making, perfume, (especially) soap, lotions, massage oil, shampoos, aromatherapy, cologne, natural dyeing, candle making, fence, landscaping, wreath making. To clean the room air by burning leaf. Finely chopped twigs are put in small cloth bag or diffrent methods and using insect repellent instead of naphthalene. The fact that it has many uses makes laurel a very important plant. Another reason why the laurel plant is an important plant for Turkey, the fact that approximately 90% of the world's need is met by Turkey.

Turkey's Total Bay Laurel Export Amount and Price by Years

YEARS	AMOUNT (TON)	PRICE (1000 $)
2000	4.423	7.964
2005	5.558	11.839
2010	8.891	25.618
2015	12.274	35.831
2019	13.513	38.235
Resource: UN Comtrade, 2021 and TUIK, 2021		

This study was carried out to determine the changes in some morphological, physiological and quality characteristics of Laurel (*Laurus nobilis* L.) in Bornova conditions in 2019. In the study, it was aimed to examine the change in yield and quality characteristics of the laurel plant in leaf samples collected from the lower, middle and upper parts of the plant once a month for five months (May, June, July, August and September).

2. Material and Methods

The research was carried out on 40 single plants selected from the laurel (*Laurus nobilis* L.) population in the experimental fields of Ege University Faculty of Agriculture, Department of Field Crops in Bornova. Selected plants consist of 12 female plants and 28 male plants. In 40 laurel plants, on the 15th day of each month for 5 months as of May in 2019; observation and measurement processes were carried out by taking samples from the lower, middle and upper parts of the Laurel tree. The lower, middle and upper parts were determined by dividing the length of the tree from the beginning of branching and leafing into 3 equal parts. The drying process of the harvested leaf samples was carried out at 30°C in the drying cabinet located in the Ege University Field Crops Department Warehouse. The analysis of the harvested leaf samples was carried out in the Medicinal Plants Laboratory of the Field Crops Department of Ege University.

The trial material consists of 40 plants selected from the existing laurel population. A selection was made from plants that reached harvest maturity. The basic statistical parameters (mean, minimum and maximum values, standard deviation of the mean, variance and coefficient of variation) were determined in the selected population plants separately.

Harvest processes in the experiment were carried out on the 15th day of each month between May and September 2019. Measurements were made for a total of five months. As a cultural process were used a hoeing machine for between rows area. Harvesting process was carried out with vineyard shears and hand-picking method. Samples were taken from three different plant parts, namely the lower, middle and upper parts of each plant. Since 40 single plants were selected from the population, a total of 120 samples were analysed and measured each month.

The soil properties of the trial area in Bornova are mil-clay textured at a depth of 0-20 cm, while it has a clay-loamy texture at a depth of 20-40 cm. The pH of the surface of the research area is 8.2, it is moderately alkaline, and at a depth of 20-40 cm, it is slightly alkaline with pH=7.8 (Sönmez, 2015). These parameters, which are measured and analysed every month;

Plant Height (cm)

In order to investigate the effect of plant height on other characteristics to be examined, the distance between the soil surface of the laurel trees selected to be harvested and the top of the plant was measured in cm.

Leaf Width (mm)

In order to calculate the leaf area; the widest part of the leaf width (mm) was measured. While obtaining the leaf width data; 10 leaves were taken from each plant part (lower, middle and upper) and their averages were calculated.

Leaf Length (mm)

In order to calculate the leaf area; the length (mm) from the end of the petiole to the tip of the leaf was measured. Data on leaf length; 10 leaves were taken from each plant part (lower, middle, upper) as well as the length of the leaf and their averages were calculated.

Fresh Leaf Weight (g/leaf)

After the harvesting process, foreign materials were removed and fresh weight measurement was carried out in each sample. Since it is not possible to keep the fresh weight of the leaves harvested as a standard in the samples taken every month, it is more meaningful to examine the fresh weight per leaf. In this way, it is important to be able to compare the number of leaves in 1 kg of fresh bay leaf and compare it with the dry leaf measurement.

Dry Leaf Weight (g/leaf)

Leaf samples were dried at 30°C for 24 hours in the drying cabinet in the Ege University Field Crops Department Warehouse. After drying, each sample was weighed again and dry weight was measured. As a result of the dry weight measurement, it is important in terms of estimating how much the weight difference will be proportional to the fresh leaf weight. Fresh and dry weight per leaf; it was calculated by dividing the number of leaves in the sample taken by the total weight.

Moisture Ratio (%)

It was calculated by making a ratio between fresh and dry weight. It is important to see what percentage of fresh leaves have moisture content.

SPAD (chlorophyll) Value

Measurements were made with the SPAD meter device, which allows estimation of the amount of chlorophyll in the leaf. SPAD values of three randomly selected leaves were taken from each sample. Each leaf was measured from three different parts, and the average of the three values was determined as the SPAD value for that leaf sample. The average of the SPAD value of the three different leaf samples obtained was evaluated as the average SPAD value of that sample.

As a result of the measurement of SPAD values, mean, lowest and highest values were determined.

Leaf Area (cm^2)

Leaf area was determined by pixel-area calculation over pixel values using Photoshop CS6 program. Starting from the leaf area, a coefficient was determined with the help of leaf width and length lengths of the same sample. The determined coefficient was calculated with the leaf width and length of the other samples.

Specific Leaf Area (dm^2)

It is based on the result of the calculation of the leaf area. It was found using the following equation. SLA = Total Leaf Area (dm^2) / Total Dry Leaf Weight (g) was calculated using the equation.

Essential Oil Rate (%)

Each sample was subjected to essential oil analysis in the Laboratory of the Department of Agronomy, Faculty of Agriculture, Ege University. Essential oil ratios were determined in 10 g dry leaves (Folia Lauri) using the Neo-Clevenger appliance by steam distillation method. The rate of essential oil in the leaf was calculated as % (ml/gr) over the dry air (Wichtl, 1971).

3. Results and Discussion

According to the data obtained; each parameter was been summarized graphically. Each cell or column in the graphs represents the mean of 40 plants.

3.1. Plant Height (cm)

Since the shortening in the plant height during the harvesting process will affect the healthy measurement of the plant height values of the other months, the plant height feature was carried out only before the harvesting in May.

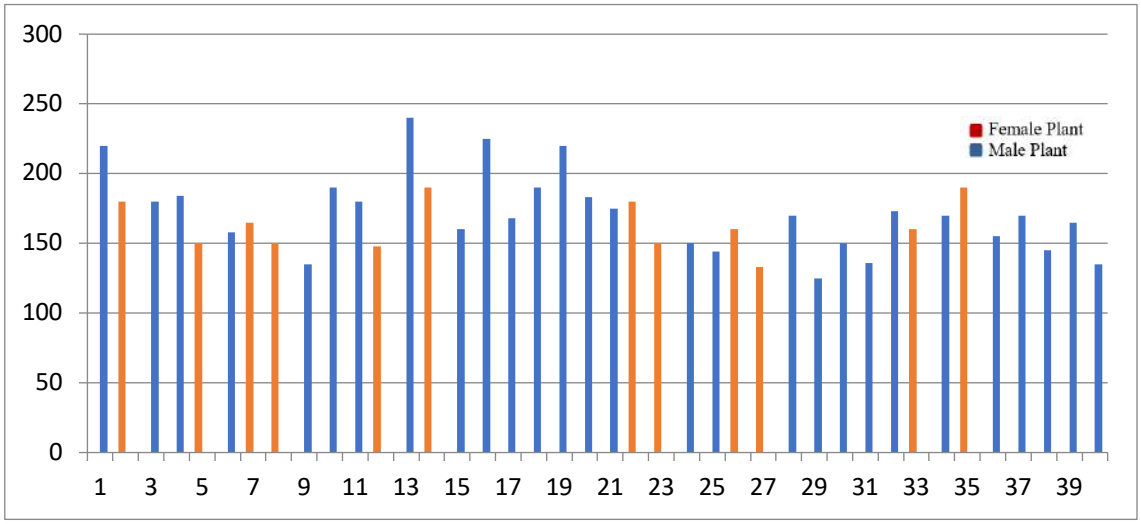


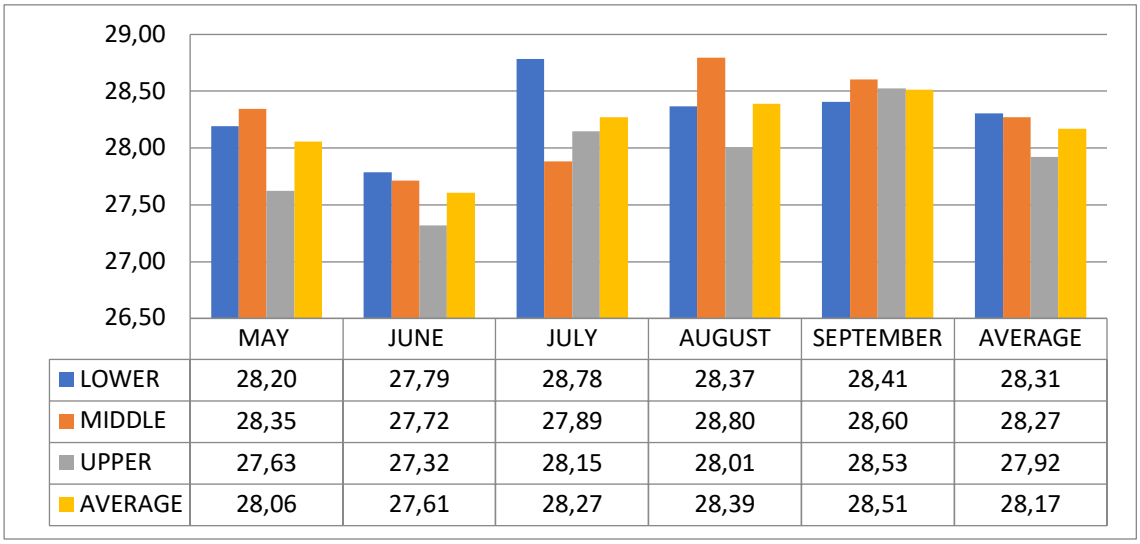
Figure 3.1. Plant Height Values Distribution Graph in Single Plants Belonging to the Laurel

When we examine generally the plant height according to the data obtained; average is 168.8 cm, highest height 240 cm belongs to the male plant and the lowest plant height is 125 cm belongs to the female plant. The average plant height of the female plants was 163 cm and the average plant height of the male plants was 171.3 cm (Figure 3.1). In the study conducted by Erat (2016), the average is 5.9 m, in the study by Baytöre (2016) it is 232, 235, 275 cm at 0-200-400 m heights, respectively. In the study of Pala (2010), it was found to be 144.34, 154.77 cm and 151.31 cm at different planting densities of 1x2 m, 2x2 m and 3x3 m, respectively.

3.2. Leaf Width (mm)

When we evaluate the leaf width values overall average is 28.17 mm. The average of September is the maximum month average and it is 28.51 mm. The average for June is the minimum and is 27.61 mm. In the other months except June, it is seen that the average leaf width increases as it progresses towards September (Figure 3.2).

When we evaluate the leaf width according to the plant part; The highest result was obtained form the lower part average of 28.31 mm, the lowest result is the upper part at 27.92 mm. It is seen that the leaf width increases in older leaves as one goes towards the lower parts of the plant. In this case, we can say that the development time of the plant and the length of the leaf are directly proportional. Accordingly, it can be suggested that a sample can be taken from the lower plant part in July or from the middle plant part in August in order to obtain a wider plant material in terms of leaf width. Researh of Yazıcı (2002) found 3.47 cm. Boza (2011) found between 2.5 and 3.5 cm, Erat (2016) found an average of 3.28 cm. Koçer (2019) calculated the average leaf width values as 3.76, 3.81, 3.82, 3.60 cm in the first year and 3.46, 3.50, 3.49, 3 49 cm in the second year in September, October, November, and December, respectively.

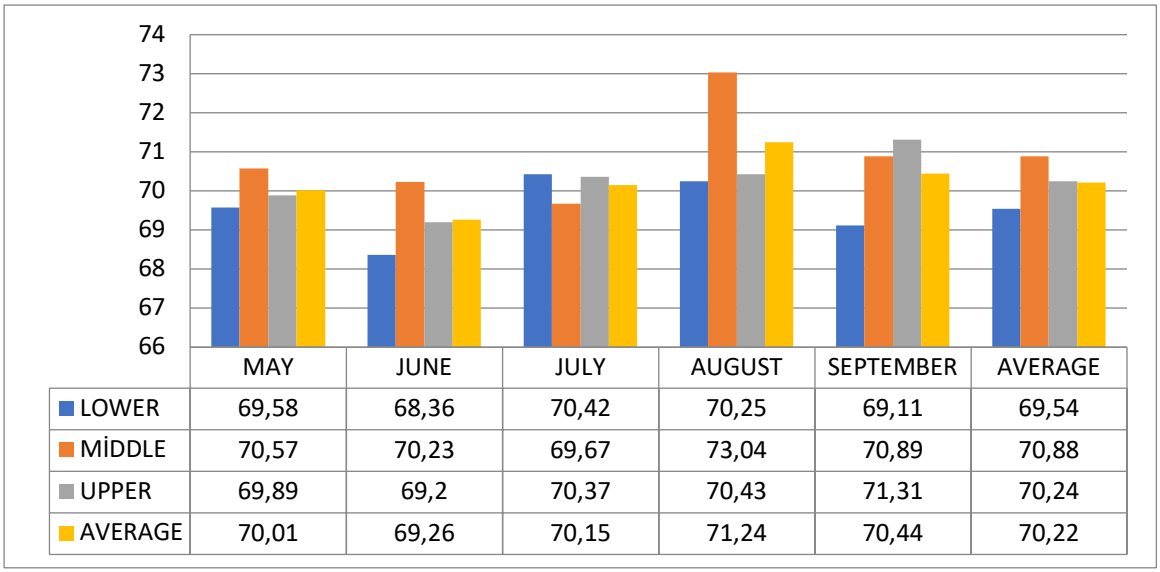


	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	28,20	27,79	28,78	28,37	28,41	28,31
MIDDLE	28,35	27,72	27,89	28,80	28,60	28,27
UPPER	27,63	27,32	28,15	28,01	28,53	27,92
AVERAGE	28,06	27,61	28,27	28,39	28,51	28,17

Figure 3.2. Mean Leaf Width in Plants Belonging to the Laurel Population (mm)

3.3. Leaf Length (mm)

When we examine the leaf length results; the average was calculated as 70.22 mm. When we evaluate it according to months, the highest average belongs to August and is 71.24 mm. The lowest month average belongs to June and is 69.26 mm. When we evaluate it according to the plant part; the middle part average is 70.88 mm and the lower part average is 69.54 mm (Figure 3.3).



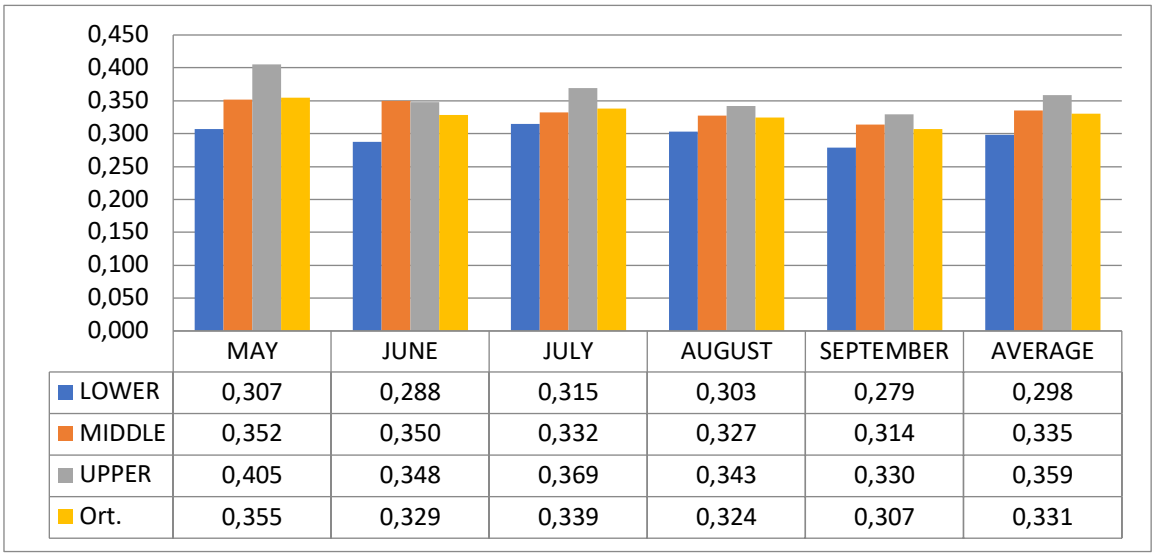
	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	69,58	68,36	70,42	70,25	69,11	69,54
MİDDLE	70,57	70,23	69,67	73,04	70,89	70,88
UPPER	69,89	69,2	70,37	70,43	71,31	70,24
AVERAGE	70,01	69,26	70,15	71,24	70,44	70,22

Figure 3.3. Mean Leaf Length in Plants Belonging to the Laurel Population (mm)

In this case, we can say that leaf length values do not show a homogeneous distribution according to months and plant parts. Accordingly, it can be suggested that a sample can be taken from the middle plant part of August in order to obtain a long leaf sample in terms of leaf length. Yazıcı (2002), found the average leaf length as 9.02 cm, and Erat (2016) found 7.99 cm. Boza (2011) measured the range of 7-8 cm on Dilek Peninsula types and in the range of 6-7 cm on Urla and Karaburun types. In the study of Koçer (2019), in September, October, November and December, respectively average values were detected 8.13, 8.18, 7.99, 8.02 cm in the first year and 7.90, 7.87, 7.90, 7.60 cm in the second year.

3.4. Fresh Leaf Weight (g/leaf)

When we evaluate the fresh leaf weight results in general, it is seen as 0.331 g/leaf on average. Average values of the months; May average is the highest and 0.355 g/leaf, and September average is the lowest, 0.307 g/leaf (Figure 3.4). As it progresses from May to September and from the lower part to the upper part, a regular decrease is observed depending on the increase in air temperature. When we evaluate it according to the plant part; the highest average of the upper part is 0.359 g/leaf, and the average of the lower part is the lowest result, 0.298 g/leaf.



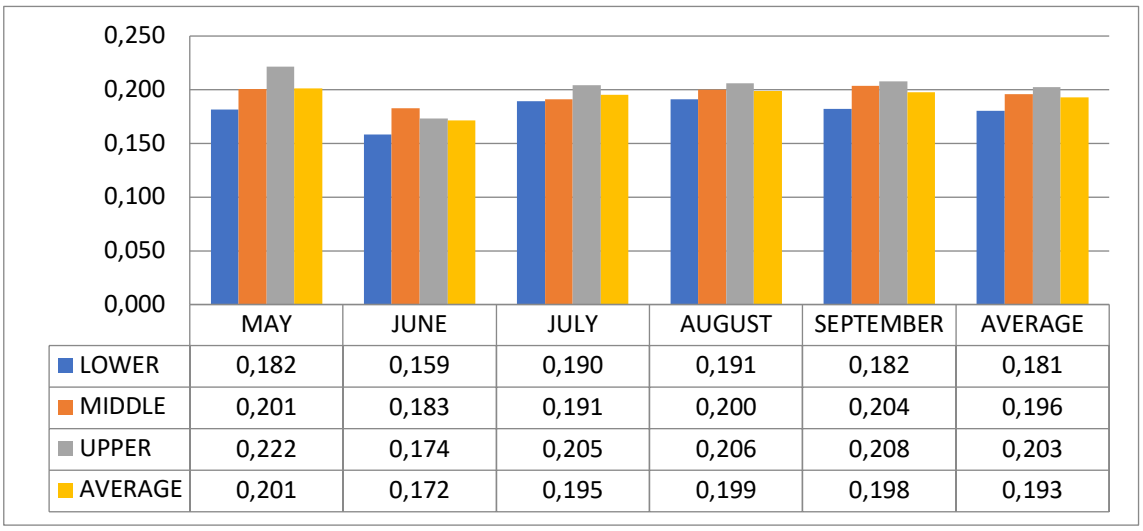
	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	0,307	0,288	0,315	0,303	0,279	0,298
MIDDLE	0,352	0,350	0,332	0,327	0,314	0,335
UPPER	0,405	0,348	0,369	0,343	0,330	0,359
Ort.	0,355	0,329	0,339	0,324	0,307	0,331

Figure 3.4. Fresh Leaf Unit Weight Average Values in Plants Belonging to the Laurel Population (g/leaf).

In this case, if we need to take a sample with a high fresh weight; it is advisable to take samples from the upper part of the plant in May. In the study conducted by Erat (2016); the average fresh leaf weight was 0.40 g, the average of the maximum data was 0.83 g, and the average of the minimum data was 0.18 g.

3.5. Dry Leaf Weight (g/leaf)

When we examine the average dry leaf weight values; the average is 0.193 g/leaf. The maximum month average value belongs to May and is 0.201 g/leaf. The minimum month average value belongs to the month of June and is 0.172 g/leaf (Figure 3.5). When we examine the average values of plant parts; the maximum value is 0.203 g/leaf, and it belongs to the upper



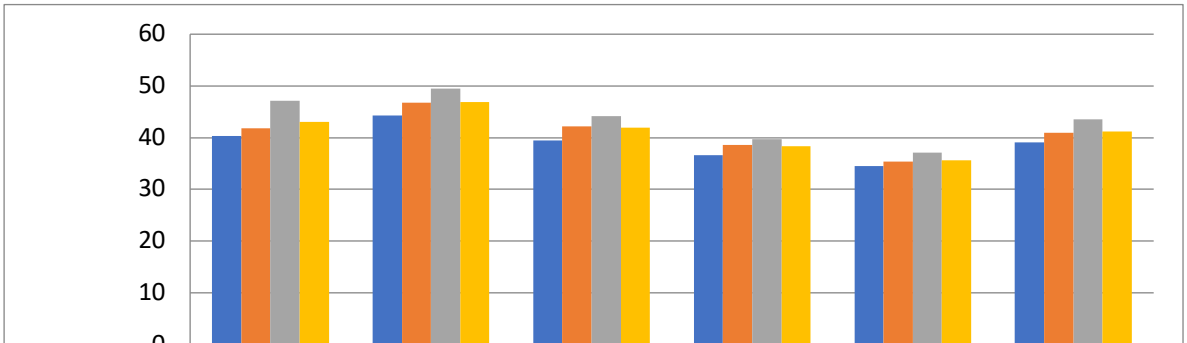
	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	0,182	0,159	0,190	0,191	0,182	0,181
MIDDLE	0,201	0,183	0,191	0,200	0,204	0,196
UPPER	0,222	0,174	0,205	0,206	0,208	0,203
AVERAGE	0,201	0,172	0,195	0,199	0,198	0,193

Figure 3.5. Average Dry Leaf Unit Weight Values in Plants Belonging to the Laurel Population (g/leaf).

part. The minimum value is 0.181 g/leaf and belongs to the lower part. According to our results, if we need to take a sample with a high dry leaf weight in Bornova conditions; It may be advisable to take samples from the upper part in May. The change in dry leaf weight is not consistent with the fresh leaf weight values. It is thought that this result is due to the rapid temperature change in the summer months. In the study conducted by Erat (2016), the average dry leaf weight was found 0.29 g, the average of the minimum value was 0.10 g, and the average of the maximum value was 0.69 g.

3.6. Moisture Ratio (%)

When we examine the average moisture values; average moisture is 41.16%. When we look at the average moisture ratio by months; the highest moisture average was 43% in May and the lowest moisture ratio average was 35.65% in September. When we look at the average moisture ratio according to the plant part; the highest part is the upper part with 43.53% and the



	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	40,28	44,27	39,45	36,64	34,55	39,04
MIDDLE	41,8	46,7	42,17	38,58	35,33	40,92
UPPER	47,18	49,48	44,19	39,74	37,06	43,53
AVERAGE	43,09	46,82	41,94	38,32	35,65	41,16

Figure 3.6. Mean Values of Moisture Content of samples in Single Plants Belonging to the Laurel

lowest part is the lower part with 39.04% (Figure 3.6).

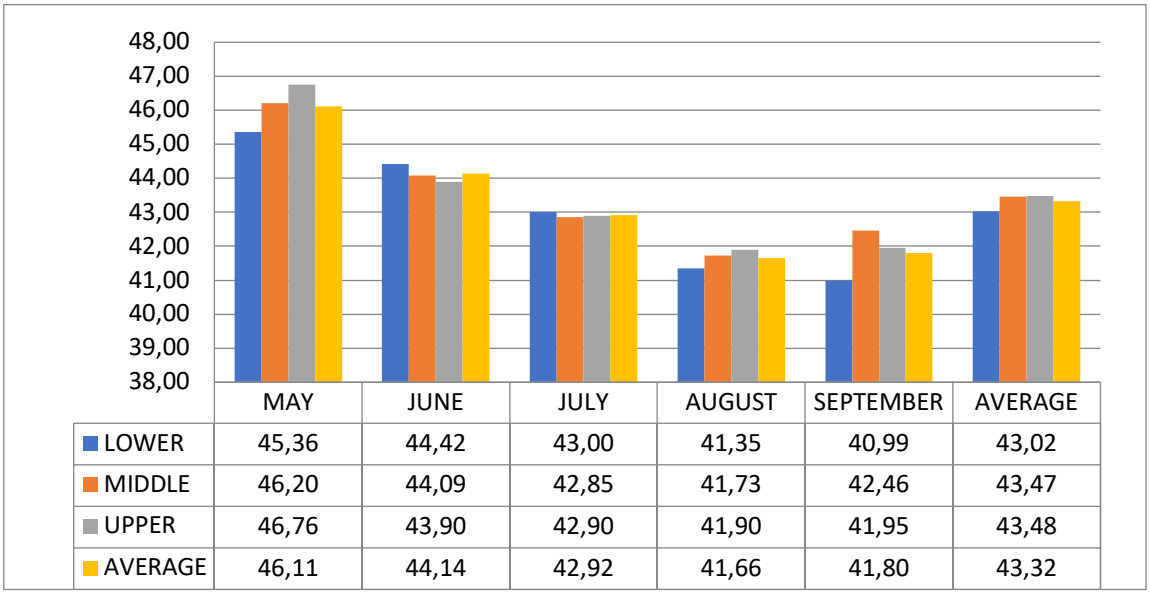
Accordingly, if it is desired to take leaf samples with low moisture, the leaves in the lower plant part should be preferred in September. In the study conducted by Erat (2016), the average was 28.57%, the average maximum value was 62.86%, and the average minimum value was 9.68%. In the study conducted by Çırpan (2017), the average results were 55.30% in Kurşunlu (0m) and 65.44% in Bayramdere (200m).

3.7. SPAD (chlorophyll) Value

According to the months; the maximum SPAD value average and 46.11 were obtained in May, and the minimum SPAD value average was 41.66 in August. When we examine it according to plant parts; the maximum SPAD value was found to be 43.48 from the upper part and the minimum SPAD value was found to be 43.02 from the lower part (Figure 3.7).

Accordingly, if we need to take a sample with a high SPAD value, samples taken in May and from the upper plant part should be preferred. When we examined the female and male plants separately according to the SPAD value, parallel values were obtained with the general average. While the average SPAD value of female plants was 43.48, the average SPAD value of male plants was 43.52. In the study conducted by Köse (2010), the lowest SPAD value was obtained as 43.30 and the highest SPAD value as 54.50. The average SPAD value was determined as

48.74. In the study conducted by Koçer (2019), the lowest SPAD value was found as 32.74 and the highest SPAD value as 54.25.

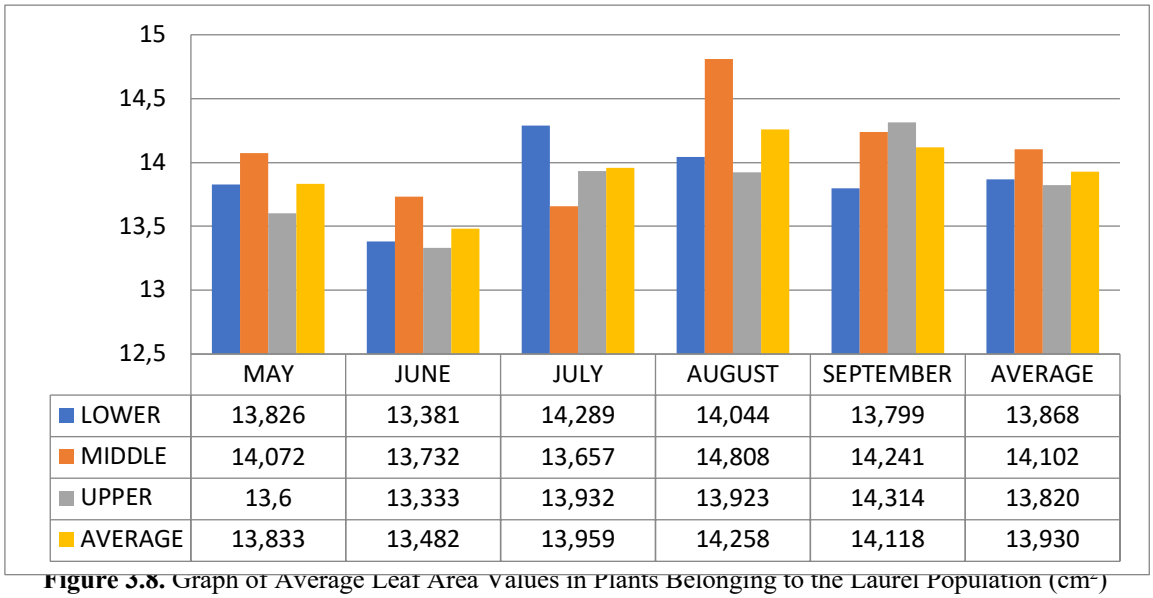


	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	45,36	44,42	43,00	41,35	40,99	43,02
MIDDLE	46,20	44,09	42,85	41,73	42,46	43,47
UPPER	46,76	43,90	42,90	41,90	41,95	43,48
AVERAGE	46,11	44,14	42,92	41,66	41,80	43,32

Figure 3.7. Average of SPAD Value in Plants Belonging to the Laurel Population

3.8. Leaf Area (cm^2)

When we evaluated the average leaf area results, the overall average was calculated as 13,930 cm^2. The maximum month result is august and 14,258 cm^2. The minimum month result is the June average and is 13,482 cm^2. When we evaluate the average of plant parts; the result



	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	13,826	13,381	14,289	14,044	13,799	13,868
MIDDLE	14,072	13,732	13,657	14,808	14,241	14,102
UPPER	13,6	13,333	13,932	13,923	14,314	13,820
AVERAGE	13,833	13,482	13,959	14,258	14,118	13,930

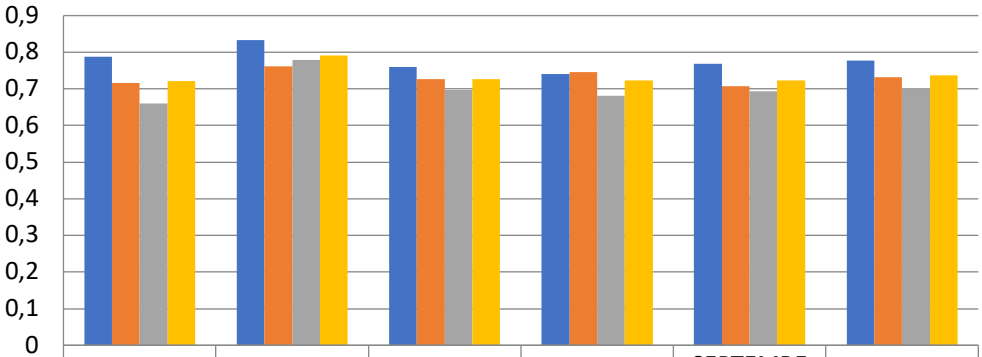
Figure 3.8. Graph of Average Leaf Area Values in Plants Belonging to the Laurel Population (cm^2)

of the maximum leaf area belongs to the middle part with 14,102 cm^2. As a result of the minimum leaf area, it was found to belong to the upper part with 13,820 cm^2 (Figure 3.8).

As a result of the evaluation, it is seen that it shows parallelism with the leaf length values. For this reason, there is no homogeneous distribution according to months and plant parts. In the study conducted by Köse (2010); average of leaf area was found 20 cm^2. In the study conducted by Boza (2011), results were calculated between 6.64-11.55 cm^2 and the average was found to be 9.48 cm^2.

3.9. Specific Leaf Area (SLA) (dm^2)

According to the results of the Specific Leaf Area; the average is 0.737 dm^2. When we evaluate according to months; the maximum SLA average is for the month of June and is 0.791 dm^2, while the minimum value is for the month of May and is 0.721 dm^2. When we evaluate it for plant parts; the maximum average value is the lower part and is 0.778 dm^2 and the minimum average is the upper part is 0.702 dm^2 (Figure 3.9). While there is no homogeneous order according to the months in terms of specific leaf area; According to the plant parts, it was determined that there was an increase from the upper plant part to the lower plant part.

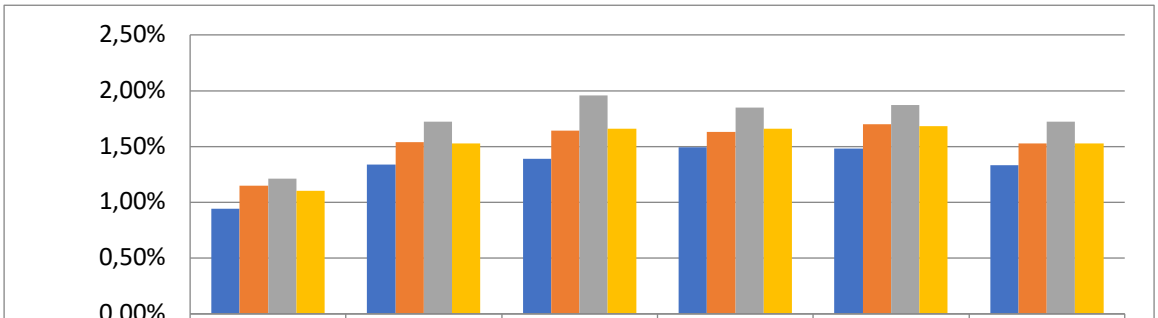


	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	0,788	0,833	0,759	0,741	0,769	0,778
MIDDLE	0,716	0,761	0,727	0,746	0,708	0,732
UPPER	0,66	0,779	0,696	0,681	0,694	0,702
AVERAGE	0,721	0,791	0,727	0,723	0,724	0,737

Figure 3.9. Mean Specific Leaf Area (SLA) in Plants Belonging to the Laurel Population (dm^2).

3.10. Essential Oil Ratio (%)

General average was 1.53%, the essential oil ratio of female plants was 1.51% and the essential oil ratio of male plants was 1.54%. When we evaluate according to months; The month with the maximum essential oil ratio is 1.68%, which is the average of September. The month with the minimum average value belongs to the average of May with 1.10%. When we examine according to plant parts; The maximum essential oil ratio was obtained from the upper part with 1.72%, and the minimum ratio was obtained from the lower part with 1.33% (Figure 3.10).



	MAY	JUNE	JULY	AUGUST	SEPTEMBER	AVERAGE
LOWER	0,94%	1,34%	1,39%	1,49%	1,48%	1,33%
MIDDLE	1,15%	1,54%	1,64%	1,63%	1,70%	1,53%
UPPER	1,21%	1,72%	1,96%	1,85%	1,87%	1,72%
AVERAGE	1,10%	1,53%	1,66%	1,66%	1,68%	1,53%

Figure 3.10. Average of Essential Oil Ratio in Plants Belonging to the Laurel Population (%).

In this case, when it is desired to obtain leaf samples with high essential oil; samples taken from the upper part in June or September may be preferred. In the study conducted by Pala (2010), the general essential oil average was obtained, respectively 1.68%, 1.82%, 1.65%, 1.60%, 1.49%, 1.57% in May, June, July, August, September and October. The average of female plants was 1.63%, and the average of male plants was 1.61%. In the study carried out by Çırpan (2017) in Kurşunlu Region, the highest essential oil ratio in dry leaves was found in September as 1.38%, the lowest essential oil ratio was found in May as 0,91% and the average value was calculated as 1,06%. As a result of the study conducted by Gölükçü (2017), essential oil values were found in the range of 1-1,75%.

4. Conclusion

Turkey is one of the important countries for world's laurel production. For this reason, studies on laurel are of great importance. Today, the need for laurel plants is mostly obtained by collecting from nature. Collecting from nature must be done consciously, producers must be supported and laurel production areas must be increased. In generaly, it is seen that there are not significant differences related to the data among the male and female plants. However, male plants showed higher results in terms of SPAD and essential oil. This may be due to the fact that female plants spend their energy on fruit formation rather than leaf formation. In this study,

which was carried out between May and September 2019, parameters such as the increasing essential oil ratio as September approaches can be examined in October and November in future studies. It was determined that June was an important month that changed the course of the chart in general. In addition to the essential oil ratio we calculated, it can be thought that component analysis will also be an important research topic.

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EVALUATION OF ANTI-TYROSINASE AND ANTIOXIDANT ACTIVITIES OF *LAMIUM* L. TAXA GROWING IN TURKEY

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Abstract
In this study, tyrosinase (TYR) inhibitory potential linked with skin hyperpigmentation and neuromelanin formation, which is associated with the pathophysiology of Parkinson's disease, and antioxidant activities of the ethanol extracts from the aerial parts and roots of thirty-one Lamium L. taxa were investigated. TYR inhibition was determined by using ELISA microtiter assay at 133.33 µg/mL as final concentration. Antioxidant activity of the extracts was assessed by microplate-modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric-reducing antioxidant power (FRAP) assays. The ethanol extract of the root of L. pisidicum R.R. Mill collected from Seydişehir to Manavgat was the most active one against TYR (35.46 ± 0.63%). Among the tested extracts, except 6 of them, 42 extracts showed varying levels of DPPH radical scavenging activity (11.06 ± 0.36 – 55.07 ± 1.82%), whereas all extracts showed antioxidant activity with FRAP method. The highest TYR inhibitory activity at 133.33 µg/mL was found in the root exract of L. (35.46 ± 0.63%), while the inhibitory activity of alpha-kojic acid at 133.33 µg/mL, used as the reference, was found 76.58 ± 0.85 (IC_{50}= 52.42 ± 2.67 µg/mL).
Key Words: *Lamium*, tyrosinase inhibition, DPPH, FRAP, antioxidant activity

1. Introduction

Tyrosinase (EC 1.14.18.1, TYR) is a copper-containing metalloprotein found in bacteria, fungi, plants and mammals, which plays a role in the biosynthesis of melanin by catalyzing the hydroxylation of monophenols to *o*-diphenols and the oxidation of *o*-diphenols to *o*-quinones(Gou et al., 2017). Melanin is a pigment produced by melanocyte cells provide pigmentation to the skin, iris, and hair. Melanin absorbs UV and protects the skin from the harmful effects of UV (Mapunya et al., 2012). Excess production of melanin causes freckles, hyperpigmentation, melasma, and skin cancer. For this reason, TYR inhibitors are often used to prevent hyperpigmentation. In recent years, with the increasing interest in skin-whitening products, the use of substances that cause TYR inhibition in cosmetics has increased a lot. These products are usually combined with antioxidants agents to strengthen the effect. The reason for

this is based on the hypothesis that reactive oxygen species increase in the skin, after UV exposure, where they thereby stimulate melanin synthesis (Farràs et al., 2019; Pillaiyar et al., 2017; Yasui & Sakurai, 2003).

On the other hand, TYR has become a target enzyme against Parkinson's disease (PD), which is defined as a progressive neurodegenerative disease characterized by degeneration of dopaminergic neurons in the substantia nigra and accumulation of α-synuclein proteins. TYR catalyzes the formation of neuromelanin by oxidizing dopamine to form melanin in brain cells. Neuromelanin can also be formed by auto-oxidation of dopamine. Neuromelanin-containing neurons are thought to be more susceptible to degeneration. For this reason, TYR also plays a role in the pathophysiology of PD (Carballo-Carbajal et al., 2019; Xu et al., 1997; Ye et al., 2020).

In the current study, the ethanolic extracts prepared from thirty-one *Lamium* taxa growing in Turkey were evaluated for their anti-TYR and antioxidant properties in order to find the most potential one.

2. Material and Methods

2.1. Plant materials and extraction procedure

Collection locations of the plant species screened in this study are tabulated in Table 1. Air-dried plant parts were finely powdered with a pestle and mortar, then weighed precisely on a digital balance (Radwag, Poland). Aerial parts and root of the plants were extracted with ethanol (EtOH, 96%) for 4 days with occasionally hand-shaking at room temperature. The ethanol phases were filtered and evaporated under vacuum using a rotary evaporator (Büchi, Switzerland). The percent yields (w/w) of the extracts are given in Table 1.

2.2 Microplate assay for TYR inhibition

TYR inhibition activity was determined by a spectrophotometric method (Lee et al., 2009) using L-DOPA (Sigma, USA) developed by (Masamoto & Kubo, 1980). 10 µl of the extracts and reference (kojic acid – Sigma, USA) for each were added to 96 well microplate. Followingly, adding phosphate buffer (pH:6.8) as well as L-DOPA as substrate, it was left to pre-incubate at 37°C for 10 minutes. After adding the enzyme solution (200 units/mL, Sigma, USA), it was incubated for 20 minutes at room temperature. Measurement was made at 492 nm. Formation of dopachrome by measuring optical density at 492 nm using ELISA microplate reader (Molecular devices spectramax i3x microplate reader, USA). All experiments were carried out in triplicate.

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 (Hatano et al., 1988) with minor modifications (Barros et al., 2007). 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). Analyzes were performed in triplicate.

2.3.2. Ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing antioxidant power (FRAP) of the extracts and reference (quercetin, Sigma, USA) was tested with minor modifications of (Oraiza, 1986). Reduction of Fe (III) to Fe (II) at acidic pH leads to evaluation of colored Fe (II)-tripyridyltriazine complex. The extracts dissolved in ethanol (96%) were added phosphate buffer (pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v, Sigma, USA). Following pre-incubation for 20 min at 50 ◦C, trichloroacetic acid (10%, Sigma, USA), distilled water, and $FeCl_3$ (17 µL, 0.1 %, w/v) was added respectively. After 30 min incubation at 25°C, absorbance was read at 700 nm using an ELISA microplate reader (Molecular devices spectramax i3x microplate reader, USA). Analyzes were performed in triplicate.

3. Results and Discussion

Among the tested extracts, except 6 of them, 42 extracts showed varying levels of DPPH radical scavenging activity (11.06 ± 0.36 – 55.07 ± 1.82%), whereas all extracts showed antioxidant activity with FRAP method. Our findings revealed that *L. cariense* R.R. Mill collected from Mount Ida showed the highest antioxidant activity in both methods with 55.07 ± 1.82% by DPPH radical scavenging and 1.143 ± 0.045 by FRAP methods. The highest TYR inhibitory activity at 133.33 µg/mL was found in the root exract of *L. pisidicum* R.R. Mill collected from Seydişehir to Manavgat (35.46 ± 0.63%). Inhibitory activity of alpha-kojic acid at 133.33 µg/mL, used as the reference, was found to be 76.58 ± 0.85 (IC_{50}= 52.42 ± 2.67 µg/mL under the same experimental conditions.

Table 1. Yield percentage, collection localities of plants, and TYR inhibitory effects of extracts

Species Name	Plant parts	Yield (w/ w%)	Collection localities	TYR Inhibition (Inhibiton % ± S.D.[a]) 133.33 µg/ml[b]
L. maculatum subsp. *villosifolium*	Aerial parts	22.89	Ksatamonu	10.78 ± 0.5
L. truncatum	Aerial parts	6.67	Hatay	10.50 ± 1.7
	Root	8.33		4.70 ± 1.3
L. moschatum	Aerial parts	5.67	Çanakkale	2.29 ± 0.3
L. sulphureum	Aerial parts	10.94	Gümüşhane	6.18 ± 0.2
L. galactophyllum	Aerial parts	4.76		12.69 ± 0.4
	Root	8.33	Erzurum	12.50 ± 0.33
L. ponticum	Aerial parts	3.42		5.90 ± 0.8
L. orientale	Aerial parts	5.06	Sivas	11.42 ± 0.4
	Root	5.56		7.88 ± 0.8
L. multifidum	Aerial parts	4.71		10.21 ± 3.1
	Root	4	Erzurum	NA[c]
L. galeobdolon	Aerial parts	3.7		32.15 ± 4.1
L. vremanii	Aerial parts	4.66	Erzurum	2.36 ± 0.2
	Root	2.22		4.76 ± 1.6
L. macularum susbsp. *maculatum*	Aerial parts	3.13	Trabzon	16.51 ± 1.85
	Root	4.48		5.40 ± 0.1
L. crinitum	Aerial parts	6.28	Gümüşhane	17.10 ± 0.53
	Root	5.26		22.64 ± 1.02
L. album	Aerial parts	3.74	Artvin	8.25 ± 1.47
	Root	3.24		NA
L. purpureum	Aerial parts	2.63	Tekirdağ	19.03 ± 0.74
	Root	7.69		NA
L. macrodon	Aerial parts	1.35		NA
	Root	7.65		NA
L. amplexicaule	Aerial parts	3.42	Antalya	10.41 ± 1.58
	Root	16.67		13.23 ± 2.42
L. amplexicaule	Aerial parts	4.26		20.07 ± 1.16
L. eriocephalum subsp. *glandulosidens*	Aerial parts	3.28		3.83 ± 2.21
L. eriocephalum subsp. *eriocephalum*	Aerial parts	4.62	Niğde	10.26 ± 0.53
L. armenum subsp. *armenum*	Aerial parts	19.56	Erzincan	14.55 ± 1.86
	Root	18.7		13.53 ± 0.32
L. cymbalarifolium	Aerial parts	7.27	Antalya	12.76 ± 1.14
L. microphyllum	Aerial parts	5.48	Denizli	10.04 ± 1.12
	Root	5.19		11.82 ± 2.31
L. veronicifolium	Aerial parts	4.14	Bursa	19.26 ± 1.26
	Root	6.67		3.57 ± 0.79

L. garganicum subsp. *reniforme*	Aerial parts	11.03	Niğde	19.55 ± 2.52
L. garganicum subsp. *rectum*	Aerial parts	6.45	Kahramanmaraş	32.12 ± 0.53
	Root	10		12.04 ± 0.74
L. garganicum subsp. *lasioclades*	Aerial parts	2.44	Adıyaman	NA
L. garganicum subsp. *laevigatum*	Aerial parts	4.83	Bursa	8.33 ± 0.74
L. tenuiflorum	Aerial parts	4.44	Karaman	6.62 ± 1.68
L. pisidum	Aerial parts	4.42	Konya: From Seydişehir to Manavgat	25.95 ± 0.21
	Root	7.89		35.46 ± 0.63
L. cariense	Aerial parts	5.77	Balıkesir: Ida	6.39 ± 0.95
	Root	10.53		9.29 ± 1.26
L. lycium	Aerial parts	5.26	Muğla	10.63 ± 0.21
Reference (Kojic acid 2000 µg/ml)[d]				76.58 ± 0.85 (IC_{50}= 52.42 ± 2.67 µg/ml)

[a] *Standard deviation (n=3),* [b] *Final concentration,* [c] *No activity,* [d] *Stock concentration*

Table 2. Antiradical activity and FRAP of the extracts

Species Name	**Plant parts**	**DPPH Radical Scavenging Activity (Inhibition% ± S.D.[a]) 2000 µg/mL[b]**	**FRAP (Absorbance at 700 nm ± S.D.[a]) 2000 µg/mL[b,c]**
L. maculatum subsp. *villosifolium*	Aerial parts	17.47 ± 2.33	0.444 ± 0.058
L. truncatum	Aerial parts	22.97 ± 1.22	0.707 ± 0.011
L. sulphureum	Aerial parts	35.05 ± 2.98	0.771 ± 0.005
L. galactophyllum	Aerial parts	28.47 ± 1.89	0.732 ± 0.043
L. ponticum	Aerial parts	4.21 ± 0.12	0.565 ± 0.018
L. orientale	Aerial parts	13.83 ± 3.28	0.676 ± 0.02
L. multifidum	Aerial parts	24.83 ± 1.82	0.711 ± 0.037
L. vremanii	Aerial parts	NA[d]	0.438 ± 0.018
L. macularum susbsp. *maculatum*	Aerial parts	11.6 ± 0.36	0.627 ± 0.007
L. crinitum	Aerial parts	NA	0.486 ± 0.022
L. purpureum	Aerial parts	14.35 ± 1.34	0.670 ± 0.032
L. amplexicaule	Aerial parts	NA	0.437 ± 0.013
L. amplexicaule	Aerial parts	NA	0.571 ± 0.009
L. eriocephalum subsp. *eriocephalum*	Aerial parts	NA	0.534 ± 0.004
L. armenum subsp. *armenum*	Aerial parts	17.61 ± 1.09	0.948 ± 0.018
L. cymbalarifolium	Aerial parts	NA	0.496 ± 0.028
L. microphyllum	Aerial parts	NA	0.512 ± 0.023
L. veronicifolium	Aerial parts	NA	0.710 ± 0.111
L. garganicum subsp. *reniforme*	Aerial parts	NA	0.701 ± 0.026
L. garganicum subsp. *rectum*	Aerial parts	NA	0.650 ± 0.056
L. garganicum subsp. *laevigatum*	Aerial parts	NA	0.902 ± 0.051
L. tenuiflorum	Aerial parts	8.85 ± 0.36	0.724 ± 0.032

L. pisidum	Aerial parts	9.19 ± 0.61	0.653 ± 0.013
L. cariense	Aerial parts	55.07 ± 1.82	1.143 ± 0.045
L. lycium	Aerial parts	NA	0.646 ± 0.012
Reference		83.08 ± 1.58[e]	1.126 ± 0.045[e]

[a] Standart deviation (n=3), [b] Stock concentration, [c] Higher absorbance indicates higher antioxidant activity in FRAP, [d] No activity, [e] Quercetin at 1 mg/ml

4. Discussion

Lamium genus belonging to the Lamiaceae family is used in the treatment of menorrhagia, uterine bleeding, hypertension, paralysis, trauma and fractures in folk medicine (Yalçin & Kaya, 2006). Previous studies on some *Lamium* species have indicated presence of mainly flavonoids, phenylpropanoids, and irioids. Despite its high flavonoid content, the *Lamium* species tested in previous reports did not show a notable TYR inhibition. DPPH radical scavenging and TYR inhibition activities of kaempferol glycoside, quercetin glycoside, and luteolin glycoside isolated from the methanolic extract of *L. amplexicaule* L. were examined and kaempferol and luteolin glycosides showed higher antioxidant and TYR inhibition activities than those of quercetin glycosides (Alipieva et al., 2006; Nugroho et al., 2009). In another study, 80% ethanolic extract of *L. purpureum* displayed high activity at 5 mg/mL concentration in FRAP and DPPH radical scavenging methods. In the same study, 80% ethanol extract of the aerial parts of *L. purpureum* at 666 µg/mL final concentration possessed 7.67 ± 1.15% (IC_{50} = 491.40 ± 5.60 µg/mL) inhibition against TYR. 1 mM kojic acid was used as reference, which showed 92.14 ± 2.49 (IC_{50} = 47.18 ± 0.51 µM) TYR inhibition (Deniz et al., 2021). The butanolic extract of *L. album* and *L. purpureum* has been revealed to have high antioxidant effect by DPPH radical scavenging method (Bubueanu et al., 2013). In another study, antioxidant capacity of the methanolic extracts of *L. maculatum* was higher than methanolic extract of *Lamium album* in DPPH assay (Danila et al., 2015).

5. Conclusion

As a result of our findings, although antioxidant activity of some *Lamium* species tested herein is variable, the *Lamium* taxa studied seem to be more active in FRAP method. However, their anti-TYR activity was observed to be low to moderate, in accordance with the reported data. Taking these outcomes into consideration, our further studies will focus on identification and isolation of compound(s) responsible for antioxidant and anti-TYR activities of the active *Lamium* species. In this regard, *L. pisidicum* seems to be the most promising species for further phytochemical studies, which is in progress in our laboratory.

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PHARMACOLOGY AND MOLECULAR BASED BIOINFORMATICS ANALYSIS ON CARVACROL

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Abstract

Pharmacology and molecular based bioinformatics approaches are an emerging discipline to elucidate potential molecular mechanisms and pharmacological properties of natural compounds. Therefore, target genes, proteins, and molecular pathways modulated by carvacrol in human genome and proteome were aimed to identify in this work. Network-based bioinformatics analyses was performed by using ChEBI database, DIGEP-Pred, GeneCards database, STRING and KEGG enrichment database in the current research. A total of 17 proteins including CASP8, CAT, CCL2, CD14, CD83, COL1A1, ESR2, FLT1, HMOX1, KLK3, MDM2, PPARA, RAC1, RARA, TIMP1, TNFRSF1A, and VDR effected by carvacrol were determined by gene set enrichment analysis. In addition, multiple molecular pathways such as cancer pathway, transcriptional misregulation in cancer, tuberculosis, human cytomegalovirus infection, Chagas disease, HIF-1 signaling pathway, p53 signaling pathway, and TNF signaling pathway were also detected to be regulated by carvacrol. This research demonstrated that carvacrol exhibits highly active pharmacological activity as an anti-inflammatory, antimicrobial, chemoprotective and neuroprotective agent. However, biological activities and pharmacological properties of carvacrol have already been determined, molecular signaling pathways, gene targets, and pharmacological properties based on bioinformatics analyses have not been fully revealed. Consequently, this work is network-based scientific research that will be very useful in understanding the biological, molecular and pharmacological properties of carvacrol for clinical applications.

Keywords: Carvacrol, network-based pharmacology, molecular pathway, bioinformatics, gene database, protein-protein interactions

1. Introduction

Carvacrol ($C_{10}H_{14}O$) is a volatile secondary metabolite that mainly found in oregano essential oils obtained from the genera Origanum, Thymus, Coridothymus, Thymbra, Satureja and Lippia. Commercial CV is synthesized by chemical and biotechnological methods Carvacrol, also named 5-isopropyl-2-methylphenol by the International Union of Pure and Applied Chemistry, is synthesized by chemical and biotechnological methods. It shows lipophilic properties at room temperature (25 °C). It is insoluble in water, whilst it is highly soluble in ethanol, acetone, and diethyl ether (Yadav and Kamble, 2009; Suntres et al., 2015; Marinelli et al., 2018). The chemical structure of carvacrol was given in the Figure 1.

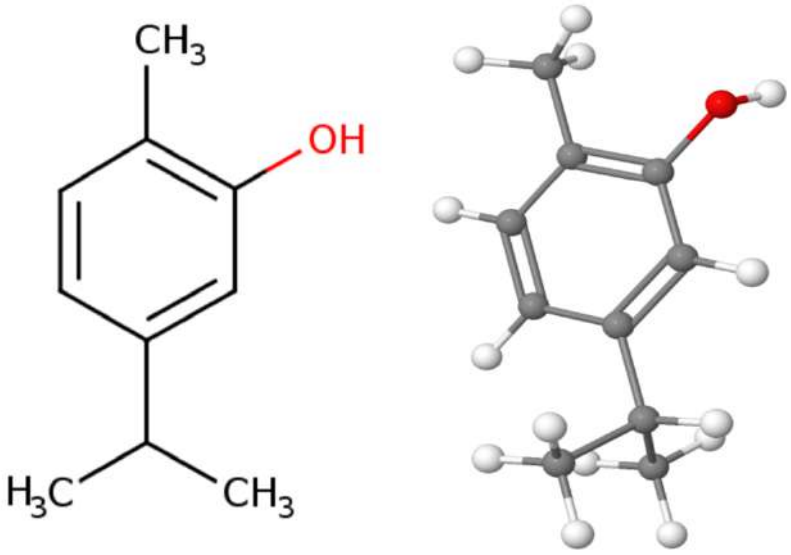

Figure 1. Chemical structure of carvacrol

Numerous reports have shown that carvacrol possesses many diverse of biological properties for instance anti-inflammatory, anti-cancer, anti-microbial, anti-cholinesterase, anti-genotoxic, anti-tumor, and anti-viral (De Vincenzi et al., 2004; Nosto and Papalia, 2012; Zotti et al., 2013; Suntres et al., 2015; Sharifi-Rad et al., 2018). The biological and pharmacological properties were summarized in the Figure 2. Although, studies have been conducted concerning the biological activity of carvacrol, network-based molecular and pharmacological activities of carvacrol have not been proposed yet. Thus, we aimed to evaluate the probable interactions of carvacrol by gene-set enrichment and network pharmacology analyses to provide a new approach to uncover the therapeutic mechanisms of carvacrol that will facilitate its future clinical applications in the treatment of diseases.

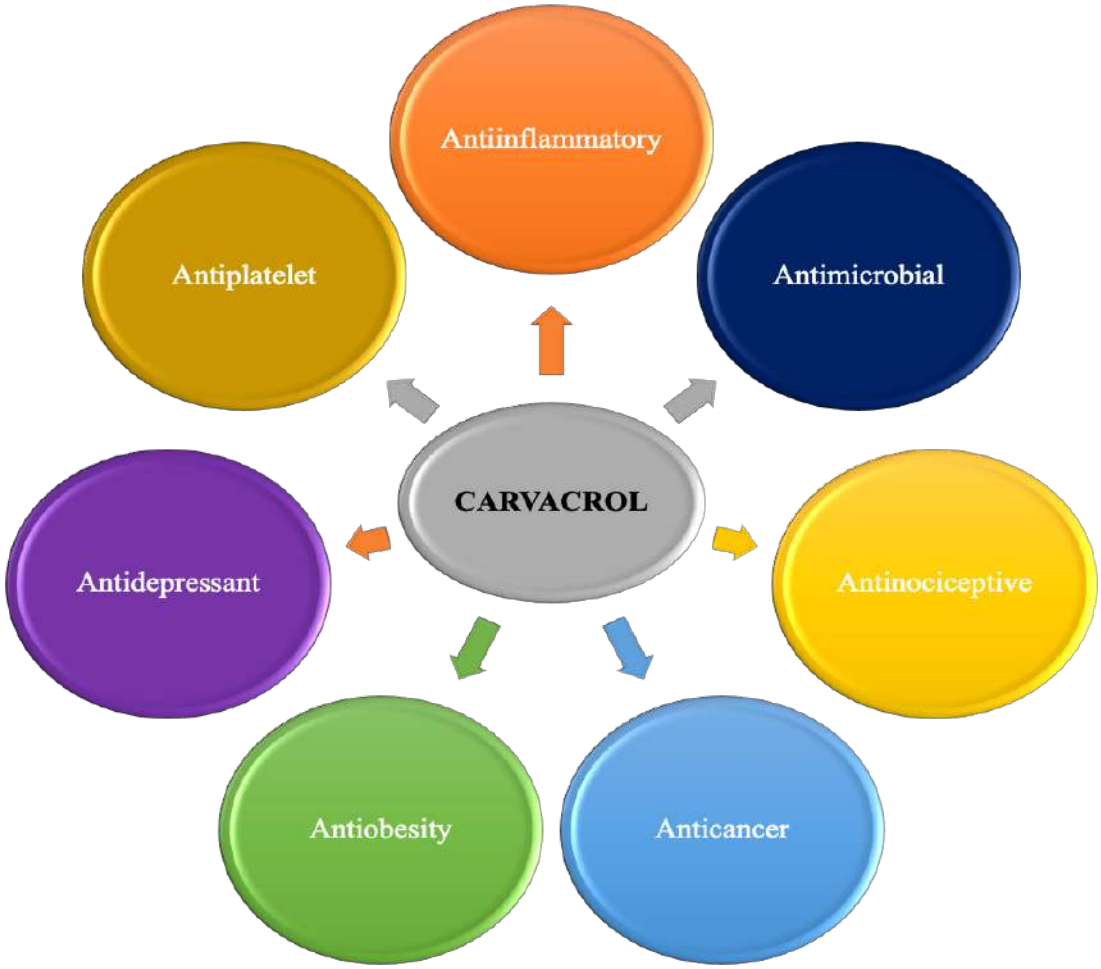

Figure 2. Multiple biological properties of carvacrol

2. Material and Methods

2.1.Chemical Properties and Targets

Chemical Entities of Biological Interest (ChEBI) database, a part of ELIXIR Core Data Resources, was used for dictionary of molecular entities and chemical properties of carvacrol (Figure 1) (Hastings et al., 2016). The targets of carvacrol were identified using DIGEP-Pred (Prediction of drug-induced changes of gene expression profile) based on structural formula of carvacrol (Lagunin et al., 2013).

2.2.Gene Set Enrichment Analysis

GeneCards, The Human Gene Database, was used to determine probable interacting genes of carvacrol. Based on this database, top interacting genes were analyzed using unique GeneCards identifiers (GC ids) and GeneCards Inferred Functionality Scores (GIFtS), provided by the GeneLoc Algorithm (Harel et al., 2009; Fishilevich et al., 2016).

2.3.Protein-Protein Interaction (PPI) Analysis

STRING database was used to annotate the role of probable interacting genes and proteins associated with carvacrol. PPI network mapping was conducted on carvacrol and protein targets using the Retrieval of Interacting Genes database with the species limited to "homo sapiens" and a confidence score > 0.4 (Wu et al., 2009; Athanasios et al., 2017).

2.4.KEGG Pathway Analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) is an integrated database of genes and genomes used for mapping pathways at molecular level. KEGG enrichment analysis was performed for construction the network regulated by carvacrol (Aoki-Kinoshita and Kanehisa, 2007; Kanehisa et al., 2017).

3. Results

3.1.Chemical and molecular information of carvacrol

Carvacrol ($C_{10}H_{14}O$) is a monoterpenoid phenol that the characteristic compound of oregano (*Origanum vulgare*) and thyme essential oils. Carvacrol, or cymophenol, $C_6H_3(CH_3)(OH)C_3H_7$ is a phenolic secondary metabolite with a molecular mass of 150.218 g/mol. Chemical structure and molecular properties of carvacrol are presented in the Table 1.

Table 1. Chemical and Molecular Properties of Carvacrol

ID	ChEBI: 3440
Name	Carvacrol
Synonyms	1-Hydroxy-2-methyl-5-isopropylbenzene, 1-Methyl-2-hydroxy-4-isopropylbenzene, 2-Hydroxy-p-cymene, 2-Methyl-5-(1-methylethyl)phenol, 2-Methyl-5-isopropylphenol, 2-p-Cymenol, 3-Isopropyl-6-methylphenol, 5-Isopropyl-2-methylphenol, 5-Isopropyl-o-cresol
Formula	$C_{10}H_{14}O$
Net Charge	0
Average Mass	150.218
Monoisotopic Mass	150.1044
InChI	1S/C10H14O/c1-7(2)9-5-4-8(3)10(11)6-9/h4-7,11H,1-3H3
SMILES	CC(C)c1ccc(C)c(O)c1
Top Chemical Roles	Radical scavenger, antioxidant, antineoplastic agent
Top Biological Roles	Votalite oil component, flavouring agent, the human ion channels transient receptor potential V3 (TRPV3) and A1 (TRPA1) agonist, antimicrobial and antifungal agent,

3.2.Prediction of carvacrol induced changes of gene expression profile

The targets of carvacrol were analyzed based on prediction of drug-induced changes of gene expression profile for proteins at the pharmacological activity (Pa) > 0.5. The findings were given in the Table 2. According to the data presented in the table, carvacrol exerts highly active pharmacological activity as an anti-inflammatory, antimicrobial, chemoprotective and neuroprotective agent.

Table 2. Prediction of carvacrol-induced changes of gene expression profile

Pa	Pi	Activity
0,931	0,004	Ubiquinol-cytochrome-c reductase inhibitor
0,908	0,001	SULT1A3 substrate
0,888	0,003	Alkane 1-monooxygenase inhibitor
0,884	0,002	Carminative
0,880	0,005	Mucomembranous protector
0,888	0,015	CYP2C12 substrate
0,876	0,003	Fibrinolytic
0,883	0,012	Aspulvinone dimethylallyltransferase inhibitor
0,864	0,008	HIF1A expression inhibitor
0,866	0,011	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,863	0,009	Alkenylglycerophosphocholine hydrolase inhibitor
0,834	0,002	SULT1A2 substrate
0,830	0,004	UGT1A6 substrate
0,832	0,006	Membrane permeability inhibitor
0,828	0,005	Linoleate diol synthase inhibitor
0,824	0,004	Antiseptic
0,819	0,007	Dehydro-L-gulonate decarboxylase inhibitor
0,816	0,008	Glutamyl endopeptidase II inhibitor
0,808	0,004	Sulfotransferase substrate
0,809	0,011	Feruloyl esterase inhibitor
0,811	0,016	Antieczematic
0,810	0,017	Antiseborrheic
0,811	0,018	CYP2J substrate
0,794	0,004	APOA1 expression enhancer
0,791	0,002	Aryl-alcohol dehydrogenase inhibitor
0,791	0,005	Fatty-acyl-CoA synthase inhibitor
0,784	0,005	Antiinfective
0,787	0,011	Alkylacetylglycerophosphatase inhibitor
0,783	0,009	Glutathione thiolesterase inhibitor
0,802	0,036	Membrane integrity agonist
0,784	0,023	Chlordecone reductase inhibitor
0,779	0,020	Sugar-phosphatase inhibitor
0,753	0,002	SULT1A1 substrate
0,761	0,012	Glucan endo-1,6-beta-glucosidase inhibitor

0,763	0,018	Acylcarnitine hydrolase inhibitor
0,755	0,010	NADPH-cytochrome-c2 reductase inhibitor
0,748	0,010	Oxidoreductase inhibitor
0,740	0,008	Phosphatidylserine decarboxylase inhibitor
0,742	0,014	Ribulose-phosphate 3-epimerase inhibitor
0,747	0,022	CYP2J2 substrate
0,737	0,014	JAK2 expression inhibitor
0,728	0,006	UGT1A9 substrate
0,728	0,008	Dextranase inhibitor
0,722	0,004	Antihelmintic (Nematodes)
0,734	0,017	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor
0,726	0,009	Glucan endo-1,3-beta-D-glucosidase inhibitor
0,729	0,012	Antidyskinetic
0,729	0,021	Mucositis treatment
0,708	0,003	Catechol 1,2-dioxygenase inhibitor
0,729	0,025	Taurine dehydrogenase inhibitor
0,716	0,013	Phosphatidylcholine-retinol O-acyltransferase inhibitor
0,704	0,002	3,4-Dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione 4,5-dioxygenase inhibitor
0,704	0,003	Plastoquinol-plastocyanin reductase inhibitor
0,714	0,015	UDP-N-acetylglucosamine 4-epimerase inhibitor
0,720	0,021	G-protein-coupled receptor kinase inhibitor
0,720	0,021	Beta-adrenergic receptor kinase inhibitor
0,702	0,003	Plasmanylethanolamine desaturase inhibitor
0,702	0,006	L-glucuronate reductase inhibitor
0,700	0,005	Antipyretic
0,699	0,008	Cholesterol antagonist
0,694	0,004	UGT2B1 substrate
0,692	0,004	Protein-Npi-phosphohistidine-sugar phosphotransferase inhibitor
0,691	0,007	Anesthetic general
0,698	0,014	Arylsulfate sulfotransferase inhibitor
0,692	0,018	Aldehyde oxidase inhibitor
0,679	0,007	Steroid N-acetylglucosaminyltransferase inhibitor
0,680	0,008	Aminobutyraldehyde dehydrogenase inhibitor
0,675	0,006	Beta-carotene 15,15'-monooxygenase inhibitor
0,683	0,016	Respiratory analeptic
0,680	0,016	UDP-glucuronosyltransferase substrate
0,677	0,015	CYP2B6 substrate
0,686	0,024	Omptin inhibitor
0,668	0,009	Alkenylglycerophosphoethanolamine hydrolase inhibitor
0,664	0,007	UGT1A4 substrate
0,663	0,006	H+-exporting ATPase inhibitor
0,666	0,011	Adenomatous polyposis treatment
0,674	0,019	Antiinflammatory
0,671	0,020	Venombin AB inhibitor
0,660	0,009	MAP kinase stimulant

0,661	0,014	Vasoprotector
0,662	0,015	Spasmolytic, urinary
0,713	0,070	Phobic disorders treatment
0,651	0,009	Cholestanetriol 26-monooxygenase inhibitor
0,667	0,025	Protein-disulfide reductase (glutathione) inhibitor
0,652	0,010	MMP9 expression inhibitor
0,645	0,003	3-Demethylubiquinone-9 3-O-methyltransferase inhibitor
0,652	0,013	Aspartate-phenylpyruvate transaminase inhibitor
0,651	0,012	Trimethylamine-oxide aldolase inhibitor
0,644	0,007	Lipid peroxidase inhibitor
0,656	0,019	Nitrate reductase (cytochrome) inhibitor
0,656	0,020	Kidney function stimulant
0,666	0,033	NADPH peroxidase inhibitor
0,647	0,014	HMOX1 expression enhancer
0,644	0,012	Gastrin inhibitor
0,660	0,032	Fusarinine-C ornithinesterase inhibitor
0,636	0,009	Tpr proteinase (Porphyromonas gingivalis) inhibitor
0,646	0,021	Electron-transferring-flavoprotein dehydrogenase inhibitor
0,642	0,018	Bisphosphoglycerate phosphatase inhibitor
0,674	0,051	Saccharopepsin inhibitor
0,674	0,051	Acrocylindropepsin inhibitor
0,674	0,051	Chymosin inhibitor
0,644	0,022	2-Hydroxyquinoline 8-monooxygenase inhibitor
0,652	0,032	Lysase inhibitor
0,636	0,016	NAD(P)+-arginine ADP-ribosyltransferase inhibitor
0,637	0,018	Cytoprotectant
0,630	0,011	Laccase inhibitor
0,622	0,003	NF-E2-related factor 2 stimulant
0,666	0,048	Polyporopepsin inhibitor
0,621	0,004	Urease inhibitor
0,634	0,018	Gluconate 5-dehydrogenase inhibitor
0,628	0,014	Acetylcholine neuromuscular blocking agent
0,631	0,022	Thioredoxin inhibitor
0,629	0,023	Lipoprotein lipase inhibitor
0,617	0,013	N-formylmethionyl-peptidase inhibitor
0,619	0,015	Threonine aldolase inhibitor
0,625	0,021	Peroxidase inhibitor
0,627	0,024	Limulus clotting factor B inhibitor
0,616	0,012	NADH kinase inhibitor
0,652	0,050	Nicotinic alpha6beta3beta4alpha5 receptor antagonist
0,633	0,032	Phosphatase inhibitor
0,607	0,009	Pyruvate decarboxylase inhibitor
0,616	0,019	Lipid metabolism regulator
0,614	0,017	2-Hydroxymuconate-semialdehyde hydrolase inhibitor
0,611	0,014	Gamma-guanidinobutyraldehyde dehydrogenase inhibitor

0,601	0,005	Muscular dystrophy treatment
0,604	0,012	Mannan endo-1,4-beta-mannosidase inhibitor
0,601	0,013	TNF expression inhibitor
0,611	0,023	Membrane integrity antagonist
0,592	0,006	Hydroxylamine reductase (NADH) inhibitor
0,615	0,029	Arginine 2-monooxygenase inhibitor
0,599	0,013	UGT1A substrate
0,594	0,009	Peptidoglycan glycosyltransferase inhibitor
0,589	0,004	Vanilloid 1 agonist
0,619	0,034	Glucose oxidase inhibitor
0,605	0,020	CYP2C8 inhibitor
0,589	0,004	CYP2B11 substrate
0,603	0,019	Analeptic
0,604	0,021	CYP2C9 substrate
0,587	0,005	Acaricide
0,590	0,009	Antinociceptive
0,605	0,024	Polyamine-transporting ATPase inhibitor
0,598	0,018	Antihypoxic
0,591	0,011	Taurocyamine kinase inhibitor
0,591	0,011	Opheline kinase inhibitor
0,592	0,013	Antipruritic, allergic
0,583	0,006	Indanol dehydrogenase inhibitor
0,592	0,016	Corticosteroid side-chain-isomerase inhibitor
0,580	0,004	Indoleacetaldoxime dehydratase inhibitor
0,611	0,036	Complement factor D inhibitor
0,590	0,015	Sulfite reductase inhibitor
0,602	0,026	Pterin deaminase inhibitor
0,588	0,016	Insulin promoter
0,596	0,024	Oxygen scavenger
0,583	0,012	CYP2E1 substrate
0,605	0,035	Carboxypeptidase Taq inhibitor
0,587	0,018	Chenodeoxycholoyltaurine hydrolase inhibitor
0,577	0,008	Acetylgalactosaminyl-O-glycosyl-glycoprotein beta-1,3-N-acetylglucosaminyltransferase inhibitor
0,582	0,014	Leukotriene-B4 20-monooxygenase inhibitor
0,574	0,007	Vitamin-K-epoxide reductase (warfarin-insensitive) inhibitor
0,589	0,022	CYP2C8 substrate
0,593	0,026	Calcium channel (voltage-sensitive) activator
0,579	0,012	CYP2E substrate
0,594	0,028	Dimethylargininase inhibitor
0,571	0,007	Trans-1,2-dihydrobenzene-1,2-diol dehydrogenase inhibitor
0,578	0,015	Antihypercholesterolemic
0,606	0,044	Pseudolysin inhibitor
0,568	0,007	Lactaldehyde reductase inhibitor
0,562	0,004	Phloroglucinol reductase inhibitor

0,573	0,017	Cardiovascular analeptic
0,567	0,012	N-hydroxyarylamine O-acetyltransferase inhibitor
0,566	0,011	Carbon-monoxide dehydrogenase inhibitor
0,583	0,029	27-Hydroxycholesterol 7alpha-monooxygenase inhibitor
0,564	0,013	Cyclohexyl-isocyanide hydratase inhibitor
0,569	0,019	Erythropoiesis stimulant
0,568	0,018	Malate dehydrogenase (acceptor) inhibitor
0,573	0,025	Methylamine-glutamate N-methyltransferase inhibitor
0,572	0,024	3-Hydroxybenzoate 6-monooxygenase inhibitor
0,565	0,017	AR expression inhibitor
0,562	0,015	Reductant
0,573	0,027	Alopecia treatment
0,556	0,011	UGT2B substrate
0,567	0,023	Platelet adhesion inhibitor
0,561	0,017	D-lactaldehyde dehydrogenase inhibitor
0,567	0,024	Phenol O-methyltransferase inhibitor
0,561	0,019	CYP2A4 substrate
0,559	0,016	Arylmalonate decarboxylase inhibitor
0,553	0,011	1-Acylglycerol-3-phosphate O-acyltransferase inhibitor
0,568	0,028	1,4-Lactonase inhibitor
0,547	0,008	3-Hydroxybenzoate 4-monooxygenase inhibitor
0,559	0,020	tRNA-pseudouridine synthase I inhibitor
0,567	0,028	CYP3A5 substrate
0,548	0,010	Mediator release inhibitor
0,545	0,007	Membrane permeability enhancer
0,560	0,022	Antisecretoric
0,560	0,022	CYP3A4 inducer
0,547	0,010	CYP2C18 substrate
0,558	0,021	Urethanase inhibitor
0,549	0,013	Crotonoyl-[acyl-carrier-protein] hydratase inhibitor
0,558	0,022	CYP2D16 substrate
0,544	0,009	Clavaminate synthase inhibitor
0,551	0,017	Cis-1,2-dihydro-1,2-dihydroxynaphthalene dehydrogenase inhibitor
0,554	0,021	Ferredoxin-NAD+ reductase inhibitor
0,554	0,021	Naphthalene 1,2-dioxygenase inhibitor
0,570	0,040	Macrophage colony stimulating factor agonist
0,570	0,040	CYP3A2 substrate
0,537	0,008	Peroxidase substrate
0,562	0,033	Preneoplastic conditions treatment
0,545	0,016	Long-chain-aldehyde dehydrogenase inhibitor
0,539	0,011	N-(long-chain-acyl)ethanolamine deacylase inhibitor
0,565	0,038	2-Dehydropantoate 2-reductase inhibitor
0,545	0,017	Eye irritation, inactive
0,552	0,026	Acetylesterase inhibitor
0,547	0,022	CYP2F1 substrate

0,549	0,024	Antipruritic
0,557	0,033	CYP2C substrate
0,537	0,013	Glyoxylate oxidase inhibitor
0,547	0,023	All-trans-retinyl-palmitate hydrolase inhibitor
0,534	0,010	2-Oxoaldehyde dehydrogenase (NADP+) inhibitor
0,547	0,025	CYP2B5 substrate
0,542	0,021	CYP2C19 substrate
0,553	0,031	Prostaglandin-E2 9-reductase inhibitor
0,544	0,023	CYP3A inducer
0,544	0,024	Glucan 1,4-alpha-maltotriohydrolase inhibitor
0,558	0,040	Platelet aggregation stimulant
0,600	0,083	CDP-glycerol glycerophosphotransferase inhibitor
0,522	0,005	Dihydroxy-acid dehydratase inhibitor
0,535	0,020	Prostaglandin-A1 DELTA-isomerase inhibitor
0,557	0,042	Phospholipid-translocating ATPase inhibitor
0,541	0,027	CYP2A substrate
0,528	0,013	N-Acyl-D-aspartate deacylase inhibitor
0,528	0,015	CYP2E1 inducer
0,525	0,013	Antiparasitic
0,524	0,012	Glycerol-3-phosphate dehydrogenase inhibitor
0,529	0,018	3-Phytase inhibitor
0,526	0,015	Hyponitrite reductase inhibitor
0,570	0,060	TP53 expression enhancer
0,523	0,013	Chitosanase inhibitor
0,548	0,040	Lysine 2,3-aminomutase inhibitor
0,545	0,036	Exoribonuclease II inhibitor
0,539	0,031	Hypolipemic
0,529	0,023	Radioprotector
0,518	0,012	Antiamyloidogenic
0,537	0,031	Endopeptidase So inhibitor
0,522	0,017	FMO1 substrate
0,524	0,020	Antiviral (Influenza)
0,527	0,023	5 Hydroxytryptamine uptake stimulant
0,520	0,017	Phosphopantothenoylcysteine decarboxylase inhibitor
0,512	0,008	UGT2B4 substrate
0,519	0,017	CYP4A11 substrate
0,527	0,025	Nucleoside oxidase (H2O2-forming) inhibitor
0,510	0,010	CYP2A5 substrate
0,579	0,079	Antineurotic
0,544	0,044	Glyceryl-ether monooxygenase inhibitor
0,520	0,020	Phosphoinositide 5-phosphatase inhibitor
0,513	0,014	Antimutagenic
0,509	0,011	3-Hydroxy-4-oxoquinoline 2,4-dioxygenase inhibitor
0,529	0,030	Neurotransmitter antagonist
0,512	0,013	DELTA14-sterol reductase inhibitor

0,531	0,034	Cl--transporting ATPase inhibitor
0,519	0,023	Hydroxylamine oxidase inhibitor
0,500	0,005	Antiuremic
0,503	0,010	CYP7 inhibitor
0,502	0,010	CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase inhibitor

3.3.Protein-based prediction results

A total of seventeen proteins were identified as top interacting genes that are regulated by carvacrol in human proteome. Among the predicted proteins, COL1A1, PPARA, FLT1, MDM2, ESR2, CASP8, and KLK3 were determined as downregulated proteins, whilst VDR, CAT, RAC1, TNFRSF1A, TIMP1, CCL2, RARA, CD14, HMOX1, and CD83 were found as upregulated proteins (Pa > 0.5). The list of top proteins regulated by carvacrol was summarized in the Table 3.

Table 3. List of the proteins regulated by carvacrol

Symbol	Description	Regulation
COL1A1	Collagen alpha-1(I) chain	↓
PPARA	Peroxisome proliferator-activated receptor alpha	↓
FLT1	Vascular endothelial growth factor receptor 1	↓
MDM2	E3 ubiquitin-protein ligase Mdm2	↓
ESR2	Estrogen receptor beta 2	↓
CASP8	Caspase-8	↓
KLK3	Prostate-specific antigen	↓
VDR	Vitamin D3 receptor	↑
CAT	Catalase	↑
RAC1	Ras-related C3 botulinum toxin substrate 1	↑
TNFRSF1A	Tumor necrosis factor receptor superfamily member 1A	↑
TIMP1	Metalloproteinase inhibitor 1	↑
CCL2	C-C motif chemokine 2	↑
RARA	Retinoic acid receptor alpha	↑
CD14	Monocyte differentiation antigen CD14	↑
HMOX1	Heme oxygenase 1	↑
CD83	CD83 antigen	↑

3.4. Protein-protein interaction (PPI) network

The relationship of a total of 17 proteins between each other were constructed from STRING database with FDR < 0.05. According to the interaction network diagram, MDM2, TIMP1, CCL2, CASP8, HMOX1, and TNFRSF1A are located in the center of the network (Fig. 3).

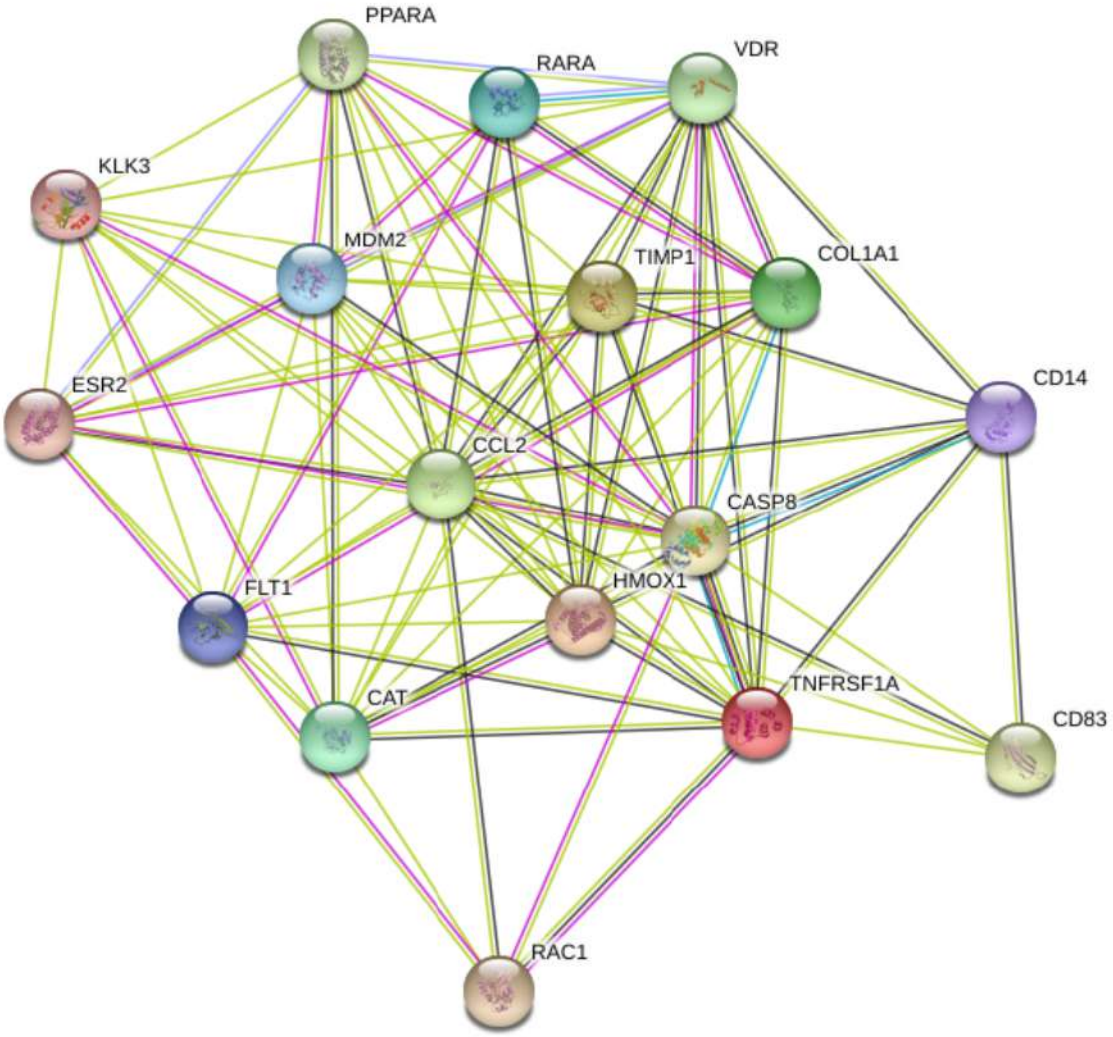

Figure 3. Protein-protein interaction of regulated proteins by carvacrol

3.5. KEGG Enrichment Pathway Analysis

The probably modulated pathways were determined regarding the KEGG pathway database. According to the KEGG analysis, different pathways were identified corresponding to 17 protein targets. The pathways modulated by carvacrol were presented in the Table 4.

Table 4. KEGG Enrichment results of modulated proteins

#term ID	term description	observed gene count	background gene count	false discovery rate	matching proteins in your network
hsa05200	Pathways in cancer	6	517	0.0015	HMOX1, RARA, MDM2, KLK3, ESR2, CASP8
hsa05152	Tuberculosis	4	168	0.0028	TNFRSF1A, CD14, CASP8, VDR
hsa05202	Transcriptional misregulation in cancer	4	171	0.0028	RARA, MDM2, FLT1, CD14
hsa05163	Human cytomegalovirus infection	4	218	0.0039	TNFRSF1A,CCL2,MDM2,CASP8
hsa05142	Chagas disease	3	99	0.0080	TNFRSF1A,CCL2,CASP8
hsa04066	HIF-1 signaling pathway	3	106	0.0082	HMOX1,TIMP1,FLT1
hsa04668	TNF signaling pathway	3	112	0.0082	TNFRSF1A,CCL2,CASP8
hsa05165	Human papillomavirus infection	4	325	0.0089	TNFRSF1A,COL1A1,MDM2,CASP8
hsa05418	Fluid shear stress and atherosclerosis	3	130	0.0098	TNFRSF1A,HMOX1,CCL2
hsa04932	Non-alcoholic fatty liver disease	3	148	0.0128	TNFRSF1A,CASP8,PPARA
hsa04215	Apoptosis - multiple species	2	30	0.0136	TNFRSF1A,CASP8
hsa05160	Hepatitis C	3	156	0.0136	TNFRSF1A,CASP8,PPARA
hsa05164	Influenza A	3	165	0.0136	TNFRSF1A,CCL2,CASP8
hsa05132	Salmonella infection	3	209	0.0247	TNFRSF1A,CD14,CASP8
hsa05131	Shigellosis	3	218	0.0260	TNFRSF1A,MDM2,CD14
hsa05134	Legionellosis	2	55	0.0312	CD14,CASP8
hsa04978	Mineral absorption	2	58	0.0326	HMOX1,VDR

hsa05221	Acute myeloid leukemia	2	66	0.0394	RARA,CD14
hsa01524	Platinum drug resistance	2	70	0.0407	MDM2,CASP8
hsa04010	MAPK signaling pathway	3	288	0.0407	TNFRSF1A,FLT1,CD14
hsa04115	p53 signaling pathway	2	72	0.0407	MDM2,CASP8
hsa04920	Adipocytokine signaling pathway	2	69	0.0407	TNFRSF1A,PPARA

As can be seen in the Table 4, several target proteins are simultaneously involved in one pathway, while one target protein is also present in many pathways. Pathways in cancer, transcriptional misregulation in cancer, tuberculosis, human cytomegalovirus infection, Chagas disease, pathways related to lipids and atherosclerosis, HIF-1 signaling pathway, TNF signaling pathway, MAPK signaling pathway, p53 signaling pathway, and so forth were the top carvacrol-regulated pathways with the lowest false discovery rate (FDR<0.05), and screened in Fig 4.

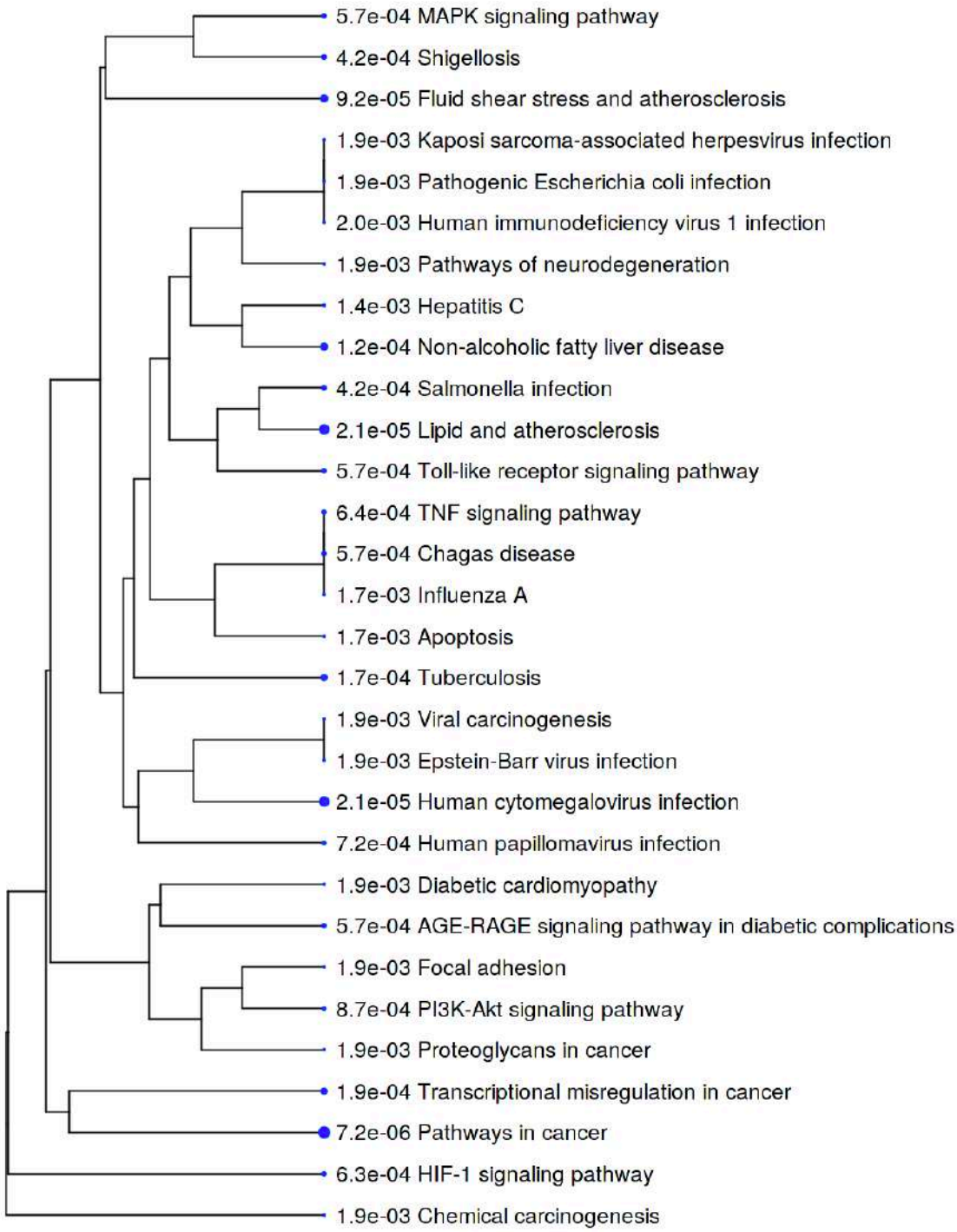

Figure 4. Top molecular pathways construction according to KEGG enrichment

4. Conclusion

Carvacrol is a volatile secondary metabolite that mainly found in oregano essential oils obtained from the genera Origanum, Thymus, Coridothymus, Thymbra, Satureja and Lippia. It has been showed that carvacrol possesses many diverse of biological properties for instance anti-inflammatory, anti-cancer, anti-microbial, anti-cholinesterase, anti-genotoxic, anti-tumor, and anti-viral. In this research, pathways in cancer, transcriptional misregulation in cancer, tuberculosis, human cytomegalovirus infection, Chagas disease, pathways related to lipids and atherosclerosis, HIF-1 signaling pathway, TNF signaling pathway, MAPK signaling pathway, p53 signaling pathway were defined as the top molecular pathways modulated by carvacrol. TNFRSF1A, CCL2, HMOX1, RARA MDM2, CASP8, and CD14 are main core proteins involved in top the signaling pathways. Consequently, these proteins and targets could be the key points of the therapeutic potentials of carvacrol. According to the results of network pharmacological analysis, carvacrol may exhibit a wide range of pharmacological properties involved in multiple targets and biological processes, thereby regulating the metabolism in human. Further studies are required to reveal the clinical efficacy of carvacrol and its mechanisms of action.

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EFFECT OF OSMOTIC STRESS ON GERMINATION, SEEDLING GROWTH AND PHYSIOLOGICAL PARAMETERS OF *BRASSICA OLERACEA* L.

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Abstract

This study objective was to evaluate the tolerance of *Brassica oleracea* var. *gongylodes* L. to osmotic stress. For this purpose, the osmotic stresses between 0 and -18.05 MPa osmotic potential were obtained using polyethylene glycol-6000 (PEG) solutions. The osmotic potential effect on germination and seedling growth of B. oleracea were determined. Results showed that enhanced osmotic stress led to substantial reduction in seed germination and germination index (%) as well as shoot length, root length and fresh and dry biomass. Seed germination was completely inhibited at -18.55 MPa osmotic potential. This plant supported only moderate osmotic stress up to -1.55 MP. Effects of stress levels (0 to 400 mg/ml of PEG 6000) were also tested in a greenhouse experiment. Increasing PEG concentration decreased strongly the stem diameter and the number, length and area of leaves. At 400 mg/ml of PEG, the shoot dry weight and the root dry weight were significantly reduced compared to control. For all the data, the impact of severe drought was more pronounced than that of moderate drought.

Keywords: *Brassica oleracea* var. *gongylodes* L., osmotic stress, PEG-6000, morphological and physiological parameters.

1. Introduction

Water is an important limitation for development, growth and yield of plants. During the life cycle, plants commonly experience a variable water amount due to continually shifting climatic factors (Passioura et al. 1993; Bohnert et al. 1995; Blum, 1998; Chaves et al. 2002; Tan et al. 2006). The plant responses to water stress rely on plant age, plant species, stage of development and growth, physical parameters and drought level and duration. Plants establish biochemical, physiological and morphological mechanisms which suppress the stresses noxious effects. Deficit of water generated by osmotic stress and drought modificated in water status,

morphology, chlorophyll content and gas exchange (Jackson et al. 1996). Polyethylene glycol (PEG) application provoked osmotic stress and caused variation in tissue water status and a reduction of biomass production and growth of plants (Kicheva et al. 1994; Grzesiak et al. 2003). Gas exchange and water content of leaves are physiological parameters extremely sensitive to drought stress. Photosynthesis limitations by non-stomatal and stomatal mechanisms rely on the intensity and period of drought stress, species of plant, plant development stage, and age of the leaf (Berkowitz et al., 1983; Passioura et al., 1993; Kicheva et al. 1994). In addition, the net photosynthetic rate diminution in water stress conditions is allied to troubles in a non-stomatal nature biochemical processes, produced by changes in the proteins and pigments structure and chloroplast lipid oxidation. Once water is not accessible in adequate amount to the leaves, higher water use efficiency (WUE) seems to be a substitute approach to advance crop yield (Araus et al. 2002). WUE may be changed through an enhance in a capacity of photosynthesis and a reduction in stomatal conductance. In plants exposed to water deficit, osmotic regulation is done by osmolytes accumulation such as proline and soluble carbohydrates (Zhang et al. 2009). Proline is stored in stressed plants in higher quantities than other amino acids (Ghaderi and Siosemardeh, 2011).

This work intended to studied the effects of polyethylene glycol produced osmotic stress on *Brassica oleracea* var. *gongylodes* L. synonym of *B. oleracea* L. to determine osmotic stress tolerance strategies. For that reason, the osmotic stresses between 0 and -18.05 MPa osmotic potential were obtained using polyethylene glycol-6000 (PEG-6000) solutions. The osmotic potential effect on germination, germination index, seedling growth and water content of *B. oleracea* were determined. Effects of stress levels (0 to 400 mg/ml of PEG-6000) were also tested in a greenhouse experiment and morphological parameters, water status, plant biomass were established in the studied plant exposed to PEG-6000.

2. Material and methods

2.1. Petri dish assay

The seeds of *B. oleracea* var. *gongylodes* (Early white Vienna kohlrabi seeds) were germinated for 7 days, in 90 mm diameter Petri dishes (20 seeds/Petri dish) on filter paper soaked with distilled water (control) or polyethylene glycol-6000 (PEG-6000) solution (50-400 mg/ml). Three replicates were prepared for each PEG concentration. Petri dishes were covered with Parafilm to water loss prevention and stored in the dark for 7 days at 25°C. Seeds that did not germinate were considered to have a radical length of 0 mm. The percentage of germination and germination index were calculated after 7 days. Then, the seedlings of *B. oleracea* var. *gongylodes* were collected, the shoots and roots lengths were determined, and the fresh and dry weights per Petri dish were measured to evaluate the effect of PEG. The stimulatory or inhibitory effects were calculated using the following equation, with slight modifications from Chung et al. (2001):

Stimulation(+)/Inhibition (−) % = ((Se − Ce)/Ce) × 100; where Se (PEG solution effect) is the parameter measured in the presence of PEG solution and Ce (control effect) the parameter measured in the presence of distilled water.

2.2. Greenhouse assay

The plastic pot experiment was done in the greenhouse of the Regional Center for Research in Horticulture and Organic Agriculture, Chott-Mariem, Sousse, Tunisia, using the seeds of *B. oleracea* var. *gongylodes* L. (Early white Vienna kohlrabi seeds), to assess the osmotic stress effects on the morphological and physiological parameters of this plant. Seedlings were grown with sand and potting soil (2:1) and they were fully irrigated before the PEG solution treatment (0 ; 100 ; 200 and 400 mg/ml). The relative water content of soil was established every day, at the same time. The treatments were operated after 75 days after sowing. The whole experiment included 24 pots.

2.2.1. Growth plant parameters

At the end of experiment and for each treatment, these measurements were taken: number and length (cm) of leaves, leaf area (cm^2) was measured with a planimeter, stem diameter (cm), root length (cm), dry and fresh weights (g) of root and aerial parts of plant.

2.2.2. Physiological parameters

2.2.2.1. Total water content

Biomass of fresh and dry plant was evaluated gravimetrically. Dry weight was assessed after drying plant material to constant weight at 70°C. Plant water content (%) was determined from the ratio of the difference between fresh and dry biomass to fresh biomass (Efimova et al., 2018). Also, water saturation deficit was evaluated by the gravimetric method (Farissi et al., 2013).

2.2.2.2. Relative water content

The relative water content (RWC) of leaf was evaluated one week after osmotic stress application. From each treatment six leaves were weighed (FW) and they were immersed immediately in distilled water until saturation for 24h in darkness at 5°C (TW). Then the dry weight (DW) was determined after drying for 48h at 80°C, and RWC (%) was evaluated as following (Beadle et al., 1993):

$$\text{RWC (\%)} = \left(\frac{\text{FW}-\text{DW}}{\text{TW}-\text{DW}}\right) * 100$$

2.3. Statistical analysis

The data statistical analysis was performed by SPSS 20.0 (Inc., Chicago, IL, USA). Statistical differences were evaluated using ANOVA variance test, and the differences significance

between means ± the standard deviation (SD) values was performed using Duncan's multiple range tests and was considered when $P < 0.05$.

3. Results and discussion

3.1. Osmotic potential of the growing medium

Germination tests were conducted at different levels of osmotic potential using a polyethylene glycol solution (PEG-6000). PEG-6000 solutions with increasing concentrations (0; 50; 100; 200 and 400 mg/ml), causing decreases in osmotic potential (0; -0.44; -1.55; -5.08 and -18.05 MPa), were used to induce the different levels of osmotic stress tested (Table 1).

Table 1. Osmotic potential values as a function of PEG-6000 concentration.

PEG-6000 (mg/ml)	Osmotic potential Ψ_O (MPa)
0	0
50	-0,44
100	-1,55
200	-5,08
400	-18,05

3.2. Effect on germination

To assess the capacity of *B. oleracea* var. *gongylodes* L. to tolerate osmotic stress, during the germination phase, seeds of this variety were germinated for seven days in the presence of a solution of PEG-6000 at different concentrations. The effect of osmotic treatment on the germination rate of *B. oleracea* var. *gongylodes* seeds for 7 days is shown in Figure 1. The results obtained show that the germinative faculties of plant seeds treated with PEG at doses of 50 and 100 mg/ml are not too affected. Indeed, on the 7th day, germination rates are 83.4% (50 mg/ml) and 82.5% (100 mg/ml), compared to 85.53% for control seeds (0 mg/ml of PEG). By contrast, treatment of seeds with PEG at 200 and 400 mg/ml affects the germination rate, which remains considerably lower (16.66 and 0%, at 200 and 400 mg/ml PEG doses, respectively) than that of control seeds.

The variation in the germination index of the control and treated seeds is represented by Figure 2. The germination index is inversely proportional to the concentration of PEG-6000. This parameter varies from 7.66 for control seeds (Ψ_O = 0 MPa) to 0 for seeds treated with 400 mg/ml of PEG (Ψ_O = -18.05 MPa).

A 400 mg/ml solution of PEG-6000 completely inhibits the germinative capacity of seeds. Thus, when the osmotic pressure increases in the growing medium by increasing the concentration of PEG-6000, the germination of the seeds decrease. Dirik (2000) has shown that generally the germination rate decreases considerably with the increase in osmotic water stress. This inhibition of germination would result in particular from a difficulty of the penetration of water molecules in the seeds, which does not allow osmotic adjustment and causes a difficulty

of hydration of the tissues. This has an impact on the process of root emergence out of seed coat and consequently delayed or even stopped seed germination (Gill et al. 2001; 2003). In addition, this reduction in germinative capacity may be due to alteration of seed enzymes and phytohormones (Botia et al. 1998). According to Prado et al. (2002), the decrease in germination is due to an osmotic dormant process developed under these stressful conditions, representing a strategy of adaptation to environmental constraints.

Our results also showed that the germination index decreases when the osmotic potential of the growing medium decreases. Similar results were described by Jaouadi et al. (2010), which demonstrated that the increasing osmotic pressure of the substrate decreases the germination rate of *Acacia tortilis* seeds and increases their average germination times. Our results show that in a concentration greater than or equal to 400 mg/ml of PEG-6000 (Ψ_O = - 18,05 MPa), the species are unable to extract water because of the increase in osmotic pressure of the culture medium and the germinative capacity is cancelled. *B. oleracea* var. *gongylodes* is found to be susceptible to severe osmotic stress during the germinative phase.

According to our bibliographic research, such work on *B. oleracea* var. *gongylodes* cultivated in Tunisia, has not been discussed previously. Nevertheless, Xiong et al. (2018) showed that germination of seeds of other species of the genus *Brassica* (*Brassica napus* L.) was affected by a 15% PEG dose and the germination decreased from 99.2% in control seeds to 60.4% in treated seeds (15% PEG).

Seed germination, such as growth, is a criterion for the selection of plant species for water stress tolerance. However, it does not necessarily correlate with other growth stages (Sy et al., 2001; Hamrouni et al. 2012), but it is still a step to consider in assessing species tolerance to water stress. Various environmental factors such as light, temperature, salinity and drought have a major effect on seed germination and seedling growth. Germination and Seed vigour can be significantly influenced by changes in several parameters (Zhang et al., 2005; Qu et al., 2005).

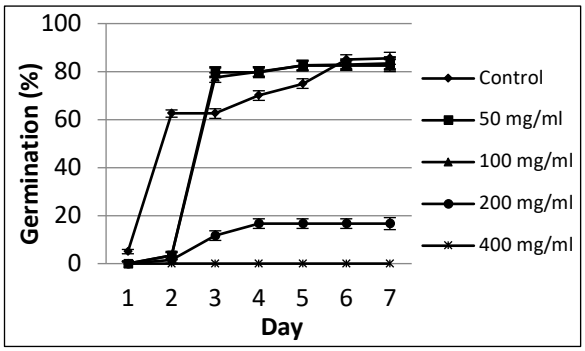

Figure 1. Germination kinetics of *B. oleracea* var. *gongylodes* in the presence of PEG-6000 solution at different concentrations.

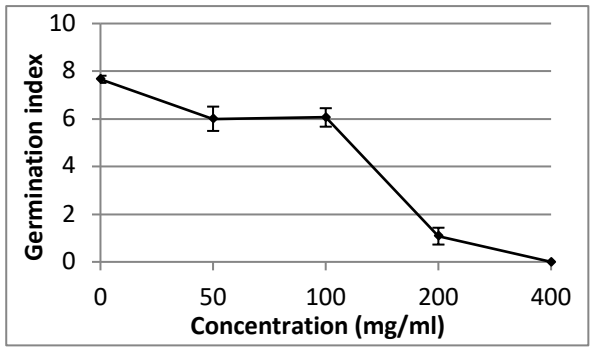

Figure 2. Variation in seed germination index of *B. oleracea* var. *gongylodes* as a function of PEG-6000 concentration.

3.3. Effect on seedling growth

After 7 days of incubation in the presence of the PEG solution at increasing concentrations (50-400 mg/ml), the parameters related to the seedlings growth of *B. oleracea* var. *gongylodes* were evaluated. The lengths of the aerial and root parts have been measured. The results are given as a percentage of inhibition or stimulation of growth of both plant parts and are shown in Table 2. The growth of the seedlings aerial part is stimulated by 31.29% at a dose of 50 mg/ml of PEG. However, at PEG concentrations of 100, 200 and 400 mg/ml, the aerial part growth is inhibited by 30.39, 99.55 and 100%, respectively. Root growth is stimulated by 113.99 and 61.28% at PEG doses of 50 and 100 mg/ml, respectively. However, at a PEG concentrations of 200 and 400 mg/ml, root growth is statistically inhibited by 92.37 and 100%, respectively ($p<0.05$).

Our results showed that a low PEG-6000 concentration (50 mg/ml) induced an increase in growth parameters such as, the length of the aerial and root parts compared to the control. A PEG concentration of 100 mg/ml (Ψ_O = - 1.55 MPa) stimulated the growth of the root part, but inhibited the growth of the aerial part. The decrease in the osmotic potential of the growing medium beyond - 1.55 MPa, negatively affected the growth of the plant vegetative apparatus. This decrease in the aerial part growth could be the result of a decrease in the cell divisions number. It is an adaptive ability that allows the survival of plants exposed to abiotic stress (Zhu, 2001). It is also noted that the root system is less sensitive to the reduction of osmotic potential than the aerial part. Indeed, under stress conditions, the assimilation of water by the plant is directly related to the degree of development of the root system. However, the characteristics of the root system vary according to the soil and climatic conditions. The relationship between the degree of root system development and water stress tolerance has been demonstrated in several species. Root length has increased as a result of water stress in millet and sorghum (Temagoult 2009). There is a positive relationship between root length and drought tolerance. An extensive and long root system can confer an advantage by increasing the water supply of the aerial part.

Table 2. Inhibition (-) or stimulation (+) of the growth of the aerial and root parts (in % of control) of *B. oleracea* var. *gongylodes* treated with the PEG-6000.

	Inhibition (-) or stimulation (+)	
PEG concentration (mg/ml)	**APL**	**RL**
50	31,29±1,64^{c}	113,99±2,6^{d}
100	-30,39±2,46^{b}	61,28±1,93^{c}
200	-99,55±1,38^{a}	-92,37±1,94^{b}
400	-100,0±0,0^{a}	-100,0±0,0^{a}

APL : aerial part length; RL: root length; the various letters indicate statistically different results p<0.05 (Duncan's Test)

3.4. Effect on water content

After 7 days, the fresh and dry weight of the seedlings of *B. oleracea* var. *gongylodes* treated with PEG solution at different concentrations, were determined. The water contents are thus calculated and the results are shown in Figure 3. The water content of the seedlings treated with the PEG solution at concentrations of 50 and 100 mg/ml is 224.58 and 130.23%, respectively. However, this content decreases considerably ($p<0.05$) for seedlings treated with high concentrations of PEG. It is 18.77 and 0% for doses of 200 and 400 mg/ml, respectively.

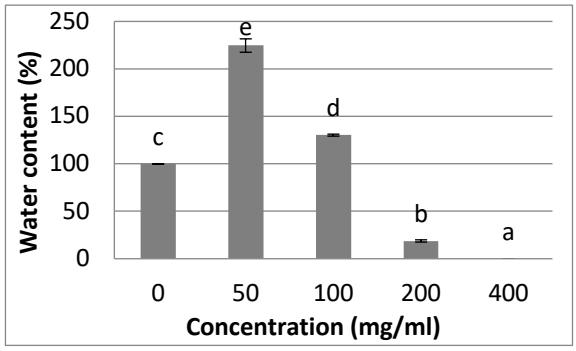

Figure 3. Variation in water content (% of control) of *B. oleracea* var. *gongylodes* seedlings as a function of PEG-6000 concentration. Bars followed by various letters indicate statistically different results $p<0.05$ (Duncan's Test).

Water content analysis provides a comprehensive description of the water status in response to drought stress, as well as an assessment of the ability to achieve good osmoregulation and maintain cellular turgescence (EL Jaafari et al., 2000). The water content of root seedlings increased significantly at doses of 50 and 100 mg/ml PEG. Thus, it turns out that this species tolerates a decrease in osmotic potential of the culture medium to a value of - 1,55 MPa. Above this value, the water content drops considerably until it cancels out for - 18,05 MPa. The same result was demonstrated by Sairam et al. (1998), who reported that the high osmotic pressure of the external environment reduces the water content of the entire plant. High water content under stress conditions was observed in soybeans (Zeghida et al. 2004). Plants that maintain a higher water content for longer periods in the presence of water stress are generally tolerant plants (Nouri, 2002).

3.5. Effect on morphological and physiological parameters (greenhouse test)

The leaves number, leaves length and the foliar surface of plants of *B. oleracea* var. *gongylodes* treated with increasing concentrations of PEG-6000 (0; 100; 200 and 400 mg/ml) are shown in Figure 4, 5 and 6, respectively. The results demonstrated that the mean number of leaves decreased statistically ($p<0.05$) under osmotic stress. It ranges from 24.5 in control plants to 13.5 leaves in plants irrigated with 400 mg/ml of PEG-6000 (Figure 4). The leaves length decreased statistically with the increase in PEG concentration, to stabilize ($p<0.05$) at a value between 11.9 cm (400 mg/ml of PEG) and 12.1 cm (200 mg/ml of PEG). The leaves length of

the control plants is 16.3 cm (Figure 5). The variation in leaf area is inversely proportional to the concentration of PEG-6000. The leaf area decreased significantly ($p<0.05$) from 59.5 cm^2 in control plants to 32.5 cm^2 in plants treated with 400 mg/ml of PEG (Figure 6).

The stem diameter was measured for the different plants, which are grown in the presence of PEG-6000 at increasing concentrations, for 17 days. The analysis of the results (Figure 7) shows that the stem diameter decreases as the concentration of PEG-6000 increases. At harvest (17^{th} day), the diameter is 8.97; 8.41 and 7.51 mm for concentrations of 100; 200 and 400 mg/ml of PEG, respectively, compared to 17.65 mm for the control plants.

The results (Figure 8) show that root growth was stimulated to 45.6 and 48.6 cm, at PEG concentrations of 100 and 200 mg/ml, respectively, compared to 39.2 cm for control plants. At a dose of 400 mg/ml, root length decreases to 34.7 cm.

The Variation in water content of *B. oleracea* var. *gongylodes* plants grown in the presence of increasing concentrations of PEG-6000 are given in Figure 9. The results show that osmotic stress caused a significant reduction in water content. This diminution becomes important with the increase in PEG-6000 concentration and this parameter ranges from 80.9 in control plants to 77.9% in PEG-treated plants at 400 mg/ml.

The relative water content (RWC) of the leaves of *B. oleracea* var. *gongylodes* grown in the presence of increasing concentrations of PEG-6000 is shown in Figure 10. The results demonstrate that the leaves of plants grown with 400 mg/ml of PEG have statistically the same relative water content (77%; $p<0.05$) as the control plants (76.5%). At doses of 100 and 200 mg/ml, the RWC decreases to 73.5 and 75.3%, respectively.

Water saturation deficiency (WSD) of *B. oleracea* var. *gongylodes* leaves grown in increasing concentrations of PEG-6000 is shown in Figure 11. The control plants as well as the stressed ones, mark a deficit of water saturation of their leaves. However, WSD is statistically more important in stressed plants compared to control plants. The values found were 26.5, 24.7 and 23%, respectively, at 100, 200 and 400 mg/ml, compared with 20.5% for control plants.

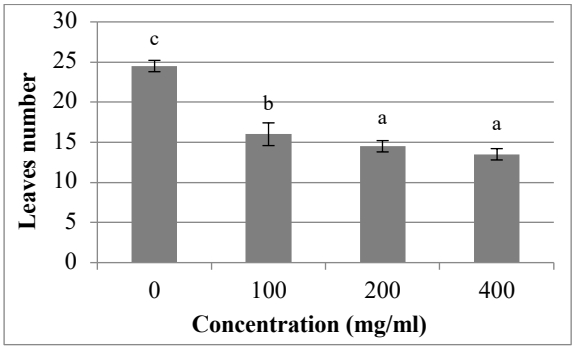

Figure 4. Variation in leaves number of *B. oleracea* var. *gongylodes* plants based on PEG-6000 concentration. The bars followed by different letters indicate statistically different results p<0.05 (Duncan Test).

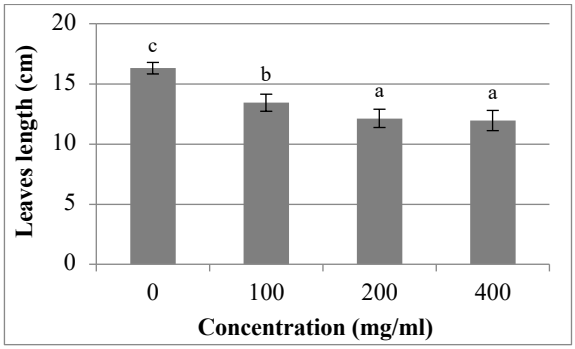

Figure 5. Variation in leaf length of *B. oleracea* var. *gongylodes* plants based on PEG-6000 concentration. The bars followed by different letters indicate statistically different results p<0.05 (Duncan Test).

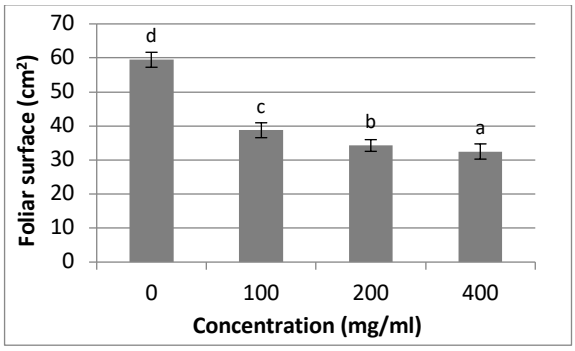

Figure 6. Variation in foliar surface of *B. oleracea* var. *gongylodes* plants based on PEG-6000 concentration. The bars followed by different letters indicate statistically different results p<0.05 (Duncan Test).

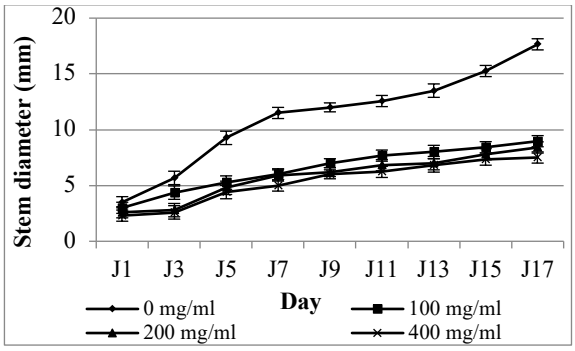

Figure 7. Variation in stem diameter of *B. oleracea* var. *gongylodes* plants cultivated in the presence of increasing concentrations of PEG-6000.

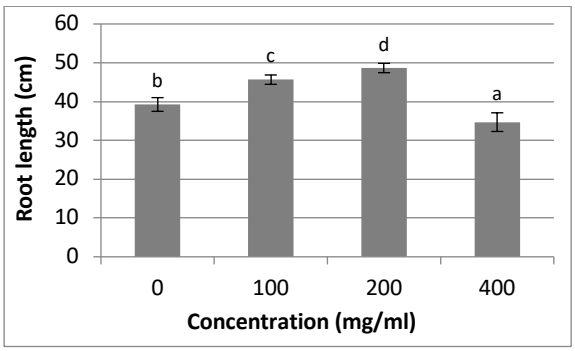

Figure 8. Variation in root length of *B. oleracea* var. *gongylodes* plants based on PEG-6000 concentration. The bars followed by different letters indicate statistically different results p<0.05 (Duncan Test).

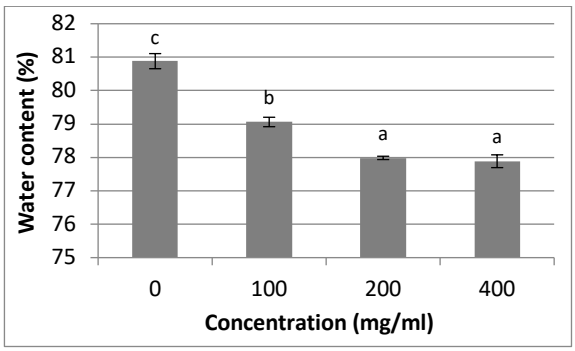

Figure 9. Variation in water content of *B. oleracea* var. *gongylodes* plants based on PEG-6000 concentration. The bars followed by different letters indicate statistically different results p<0.05 (Duncan Test).

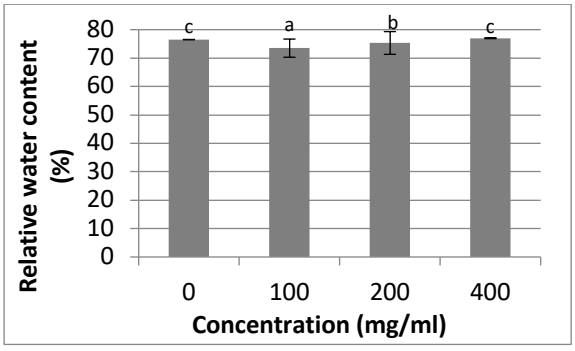

Figure 10. Variation in relative water content of *B. oleracea* var. *gongylodes* leaves based on PEG-6000 concentration. The bars followed by different letters indicate statistically different results p<0.05 (Duncan Test).

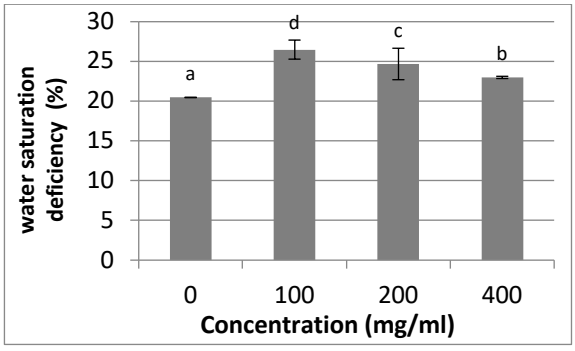

Figure 11. Variation in water saturation deficiency of *B. oleracea* var. *gongylodes* plants based on PEG-6000 concentration. The bars followed by different letters indicate statistically different results p<0.05 (Duncan Test).

At the end of the osmotic treatment, the fresh and dry weights of the aerial and root parts of *B. oleracea* var. *gongylodes* plants were determined to estimate dry matter production and assess the root dry matter (RDM) to aerial dry matter (ADM) ratio. The results are given in Table 3. The results show that in *B. oleracea* var. *gongylodes* plants cultivated with 100 and 200 mg/ml of a PEG solution, root dry matter (RDM) production increased compared to control plants (2.2 g; $p<0.05$). However, at a dose of 400 mg/ml, the RDM decreased significantly compared to the control (1.7 g; $p<0.05$). The production of aerial dry matter (ADM) decreased significantly as the concentration of PEG increased. The ADM decreased from 2.4 g in control plants to 1.7 g in plants treated with PEG at 400 mg/ml (Table 3). The distribution of biomass between the different plant parts is estimated by the RDM/ADM ratio, which allows us to evaluate the effect of osmotic stress on the allocation of dry matter from plants. Due to osmotic stress, this ratio is greater than or equal to 1, whereas it is equal to 0.8 in control plants (Table 3).

Table 3. Production of root dry matter (MSR) and aerial dry matter (ADM) in *B. oleracea* var. *gongylodes* plants grown in different concentrations of PEG-6000.

	Production of dry matter (g)		
PEG concentration (mg/ml)	**RDM (g)**	**ADM (g)**	**RDM/ADM**
0	2,17±0,02^{b}	2,70±0,12^{d}	0,80±0,02^{a}
100	2,26±0,01^{c}	2,15±0,05^{c}	1,05±0,04^{b}
200	2,43±0,10^{d}	1,80±0,01^{b}	1,35±0,01^{c}
400	1,70±0,03^{a}	1,70±0,02^{a}	1,00±0,06^{b}

RDM: root dry matter; ADM: aerial dry matter; different letters indicate statistically different results $p<0.05$ (Duncan Test).

Water stress is considered the main limiting factor for plant growth. To highlight the adaptive potentialities of *B. oleracea* var. *gongylodes* in response to osmotic stress, morpho-physiological parameters were followed. Seedlings were grown for 17 days under osmotic stress, applying different concentrations of PEG-6000. All the results showed that the different growth parameters studied (number, length and surface area of the leaves, stem diameter, root length and biomass) showed a reduction which is accentuated by the increase in the level of osmotic stress.

Our results corroborate those of Sritharan and Lenz (1990), who studied the effect of CO_2 concentration and irrigation on photosynthesis, growth and nitrate concentration in *B. oleracea* var. *gongylodes* grown in Germany. Indeed, these authors showed that the number and surface of the leaves and the diameter of the stem decrease under water regimes of 100, 50 and 25%, both in the presence of 300 and 900 µl/l of CO_2.

Xiong et al. (2018) showed that the length of the seedling, with its aerial and root parts, decreases under osmotic stress (15% PEG) applied to *Brassica napus*. Several authors have

reported a reduction in growth parameters for various species such as alfalfa (Siakhene 1984), soybean (Vidal et al. 1981) and fish (Ben Mbarek 2012).

In addition, Albouchi et al. (2003) found that a water deficit causes a reduction in the height of plants, which will show a certain dwarfism with a shortening of the stem. Vegetative development under water deficit conditions is severely disrupted (Ferryra et al. 2004). There is mainly a significant decrease in the size and surface of the leaves, a reduction in the height and diameter of the stem and a shortening of the internodes. This decrease is due to a reduction in cell elongation (Temagoult, 2009), which is one of the plant responses to dehydration and contributes to the conservation of water resources and allowing the plant to survive (Blum, 1996, Lebon et al., 2004). Depending on the adaptive strategy of each species, the effect of water stress can result in morphological changes to increase water uptake and decrease sweating and organ competition for assimilates. These changes affect aerial and root parts of the plant (Bajji, 1999).

Our results showed that under moderate osmotic stress (100 and 200 mg/ml PEG), root length increases. Conversely, when the stress level increases (400 mg/ml of PEG), the root length is inhibited. These findings are consistent with those of Ludlow and Muchow (1990), which showed that under limited water conditions, the plant develops a largely vigorous root system to properly exploit the soil and to continue the water absorption. In the same context, Fraser et al. (1990) reported that the water stress caused a reduction in root length in wheat and this reduction is likely due to a cessation of root cell division and elongation.

Our study showed a decrease in dry biomass production of *B. oleracea* var. *gongylodes* plants cultivated under severe levels of osmotic stress (400 mg/ml PEG). This is consistent with the results of Sritharan and Lenz (1990), which demonstrated that the dry matter of the whole plant, leaves, stems and roots of *B. oleracea* var. *gongylodes* decreased under 50 and 25% water regimes. The ability of the plant to produce more aerial biomass is related to its water state. Thus, in this work we have shown that the aerial part is more sensitive to osmotic stress than the root part of *B. oleracea* var. *gongylodes*. Indeed, we noted a reduction in aerial biomass and an increase in root biomass, especially at PEG concentrations of 100 and 200 mg/ml.

The increase in the RDM/ADM ratio reflects a reduction in the biomass of the aerial part and an increase in the biomass of the root part in order to limit the loss of water by the transpiration and to guarantee the cellular turgescence and the absorption of a maximum of water. Thus, it appears that these osmotic stress levels induced in *B. oleracea* var. *gongylodes* an increase in the allocation of assimilates to roots to the detriment of aerial organs. This result is consistent with those achieved by Timbal et al. (1995), Albouchi (1977), Yin et al. (2005) and Martin et al. (2006). These authors showed that the plant reduces the production of biomass from the aerial part and ensures the increase of biomass from the root part, followed by a significant increase in the ratio of the mass of the root part to that of the aerial part.

The water deficit modulates the root biomass to maintain a maximum aerial growth rate. This phenomenon is considered as a criterion of resistance to drought. It allows for better use of available water, which is becoming increasingly inaccessible (Albouchi et al., 2003). Under conditions of osmotic stress, the osmotic pressure of the culture medium increases, which prevents the absorption of water by the root system and therefore the size of the aerial vegetative apparatus will be reduced.

The water content (WC) is a useful indicator to highlight the state of a plant's water balance (Bajji et al., 2001). In stressed plants of *B. oleracea* var. *gongylodes*, WC decreased significantly compared to control plants. In fact, water stress causes the decreases of external osmotic pressure, which reduces the water content of the whole plant, resulting in a decrease in the mobility of nutrients (Sairam et al. 1998). There is also a decrease in the volume of the aqueous medium where the biochemical reactions that ensures the best development of the plant occur. Water molecules are directly involved in many biochemical hydrolysis or condensation reactions. Water is also a source of essential elements for plant metabolism. Its decomposition provides different constituents necessary for the biosynthesis of organic molecules. The decrease in WC is more rapid in susceptible plants than in resistant plants (Scofield et al., 1988). Nevertheless, plants that maintain a higher WC for longer periods in the presence of water stress are generally tolerant plants (Nouri 2002).

Osmotic stress caused the decrease in relative water content (RWC), this decrease is pronounced with the severity of stress. Indeed, the hydration of the different tissues of the plant decreases as the water stress increases. According to Albouchi et al. (2003), water deficit is a key determinant of plant growth, particularly in arid and semi-arid regions. It induces in stressed plants a decrease in relative water content. On the other hand, Matin et al. (1989) and Nouri (2002) show that plants that maintain high RWC in the presence of water stress are tolerant plants. Water saturation deficiency (WSD) indicates the level of water stress to which plants are subjected. WSD is more important in stressed plants compared to control plants.

Based on the present results, it is clear that the species *B. oleracea* var. *gongylodes* could tolerate osmotic stress caused by a PEG solution with a concentration lower than or equal to 200 mg/ml.

4. Conclusion

Brassica oleracea var. *gongylodes* L., a variety of cabbage with very important nutritional and economic values, has been the subject of our present work. Thus, in vitro germination of plant seeds were performed with increasing concentrations of PEG-6000 and under different osmotic potentials. The effect on germination, growth and water parameters of 7-day older seedlings was studied. Study of the effect of osmotic stress on *B. oleracea* var. *gongylodes* showed that the response of this plant is variable depending on the degree of stress severity. It was found

that at a dose of 400 mg/ml of PEG-6000, corresponding to an osmotic potential of -18.05 MPa, all germination and growth parameters and water content were totally inhibited. This suggests that osmotic potentials up to -5.08 MPa would be tolerable by seeds and seedlings. Culture test for *B. oleracea* var. *gongylodes* in the greenhouse has shown that osmotic stress has influenced the general appearance of plants as the intensity of stress increases. The effects of this osmotic stress result in a decrease in the average number of leaves, the length and foliar surface and the diameter of the stem. The water deficit response of plants results in a more vigorous root system in plants. The production of the biomass of the aerial part was significantly reduced, with the degree of severity of osmotic stress. Thus, under the effect of osmotic stress, the correlation between the root and aerial parts of plants is affected.

Changes in the water relations of plants, namely the water content (WC) and the relative water content (RWC) reflect their water status and their ability to incorporate water, especially in situations of water constraint. The dehydration of plants is increasingly important when the level of osmotic stress increases. In addition, water saturation deficiency (WSD) provides information on the degree of stress to which plants are subjected. The WSD was found to be significantly higher in stressed plants compared to controls.

It appears from this work that the variety *B. oleracea* var. *gongylodes* L. adopt several strategies to counter water stress, such as the avoidance strategy by limiting water loss by perspiration (decrease in the number and surface area of leaves), and improving water absorption, by establishing a long and vigorous root system.

Conflict of interest

All the authors declare no conflict of interest.

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EFFECT OF INORGANIC FERTILIZER AND MICROBIAL CONSORTIUM TO GROWTH AND PRODUCTION OF SWEET BASIL (*OCIMUM BASILICUM* L.)

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Abstract

Sweet basil, belonging to the Lamiacea family, is of economic importance culinary aromatic herbs and is cultivated throughout the world, and its aromatic leaves are used fresh or dried as a flavoring for foods, confectionery products and beverages. The study assessed possible effects of NP (100 kg ha^{-1} N and 100 kg ha^{-1} P) and P fertilizer (100 kg ha^{-1}) mineral fertilizer, two commercial liquid bio-fertilizer and inoculation with multi-traits bacteria based three consortium in three strains combinations (*Pseudomonas fluorescens* RC512 + *Bacillus licheniformis* RC601 + *Bacillus subtilis* RC210, *Bacillus megaterium* RC16 + RC512 + RC210, and RC512 + RC601 + RC16) on the growth, yield, and oil content in various sweet basil (Ispir, Yusufeli and Kayseri) populations in field conditions of Çanakkale. The experiment was arranged as a completely randomized block design with eight treatments and three replicates. The biofertilizers and bacterial consortia inoculation involved dipping the root system of the seedling into a suspension of each formulations for 60 min, prior to planting. Inoculations of basil with RC512+RC601+RC210, RC512+RC210+RC16, and RC512+RC601+RC16, BMusaGreen, and BMusaVita and gave increases over control respectively of by 16.9, 19.1, 22.9, 18.1, and 20.7 % in plant height, by 14.1, 20.4, 24.9, 25.3, and 25.9 % in branch number per plant, by 7.2, 13.3, 16.4, 13.5, and 14.0% in fresh herba yield per plants, by 9.6, 18.1, 24.7, 17.9, and 19.5 % in dried leave yield per plants and by 15.3, 18.5, 17.7, 20.2, and 18.1 and 2.6 % in essential oil ratio. NP and P applications, however, increased height of plant up to 22.9 and 13.1%, branch number per plant by 24.8 and 6.0 %, fresh herba yield of plants by 18.4 and 4.0 %, yield of dry leaves by 22.1 and 4.4 % and essential oil ratio by 18.1 and 2.5%, respectively.

Key Words: *Ocimum basilicum* L., fresh herb yield, dry leaf yield, Co-inoculation, bio-fertilizers, essential oil content

1. Introduction

Sweet basil (*Ocimum basilicum* L.), a member of the Lamiaceae family, is one of the most popular aromatic herbs widely used in important economic areas such as perfumery, pharmaceutical and medical industries, as well as being used as food, spice, and fresh vegetable plant (Telci et al., 2006; Castronuovo et al., 2019). Sweet basil is often referred to as 'the king of herbs' and is used in cooking and medicinal applications, as well as an ornamental plants due to its unique flavour and phenolic content (Makri and Kintzious, 2008; Dou et al., 2018). Sweet basil is one of the economically important aromatic and edible annual herbs, its leaves, flowers, and seeds are used in herbal medicine, perfumery, and for culinary purposes, food, aromatic, sacred, fragrances, cuisine, beverages, vegetable, antimicrobial and insecticides, and it is cultivated as a popular ornamental plant (Vlase et al., 2014; Yaldız et al., 2015; Kalisz et al., 2016; Çakmakçı and Milton, 2019).

The medicinal and aromatic properties of basil are associated with the presence of essential oil, which is used in the pharmaceutical industries due to its medicinal properties accumulated in its leaves and flowers (Nurzyńska-Wierdak et al., 2013). Basil is one of the popular culinary herbs used as a flavoring and commercial spice for foods, confectionery, and beverages due to its distinctive flavour and aroma. Basil is widely used as a flavouring in soups, soft drinks, liquor, tea, slivovitz, pesto, desserts, honey, pizza, spaghetti sauce, catsups, eggs, bakery products, cheese dishes, pickles, ragouts, sauces, sausages, tomato pastes, ice cream, tomato juice, vinegars, salads, salad dressings, condiment products, confectionery, and meat products, as well as perfumery, dental, and oral products. Because of their active ingredients, insect repellent properties, and antibacterial activities, this herb has been used industries such as food, pharmaceutical, and cosmetics, as well as in the treatment of cough, headache, kidney diseases and parasitic diseases. Although it has many uses, the most common use is to flavour foods and for culinary purposes.

Sweet basil, one of the most economically important aromatic culinary herbs, is widely used as seasoning in the form of fresh and dried herbs (Bączek et al., 2019). It has been reported by Singletary (2018) that basil has commercial importance for the production of phytochemicals used for medicinal purposes, as well as being a culinary and ornamental fresh market herb. On the other hand, since flowering basil herb contains much-appreciated fragrance components used in cosmetics such as linalool, citronellol, geraniol and limonene (Pitman, 2004; Sharmeen et al., 2021), the cosmetic industry uses basil oil in shampoos, soaps, lotions, and perfumes. Basil is grown as an ornamental, medicinal and spice plant, especially in small areas, and the cultivar, phenological stages, planting season, ontogeny and plant parts have important effects on the yield and quality of essential oil (Toncer et al., 2017).

Since PGPR directly or indirectly affects the physical, chemical, and biological properties of the soil, and promotes plant growth, yield and nutrient uptake by a number of mechanisms and their use is seen as an important approach in agriculture. Therefore, this study was conducted in order to investigate the effect of different bacteria-based biofertilizer formulations on the growth, leaf yield, oil content and oil yield in various sweet basil.

2. Material Methods

The objective of this study was to evaluate possible effects of NP (100 kg ha^{-1} N and 100 kg ha^{-1} P) and P fertilizer (100 kg P ha^{-1}) mineral fertilizer, two commercial liquid bio-fertilizer and inoculation with multi-traits bacteria based three consortium in three strains combinations (*Pseudomonas fluorescens* RC512 + *Bacillus licheniformis* RC601+ *Bacillus megaterium* RC16, *Bacillus subtilis* RC210+ RC512 + RC601, and RC512+RC210+RC16) on the growth, yield, and oil content in various sweet basil (İspir, Yusufeli and Kayseri) populations in field conditions of Çanakkale.

Seeds were sown at the mid-April into a mixture of peat, perlite and soil and watered regularly for germination. Seeds of the basil genotypes were germinated under greenhouse conditions and when the seedlings reached 8-10 cm in length, they were planted on 25 May in the trial field. Basil seedlings were surface-sterilized by soaking in 25% commercial-grade bleach for 5 min prior to inoculation, then thoroughly washed under running tap water, dried, inoculated, and planted.

Frozen bacterial culture was cultivated on nutrient agar-containing medium and incubated for 24 hours at 27°C for the triple bacteria formulation and commercial biological fertilizers. Pure colonies were taken and transferred to NB medium, developed in the 24-hour culture prepared previously by fermenters and sterilized by autoclaving at 121 °C for 20 min on a horizontal shaker (Çakmakçı et al., 2013). Bacteria were grown by providing optimum pH, oxygen and temperature for 24 hours and then inoculated at a ratio of 1:10 to the liquid carrier mixture, which was completely sterilized by steam. The bacteria-inoculated organic liquid carrier incubated in the bioreactor under optimum growth conditions. When the number of viable bacteria per millilitre exceeds 1 x 10^8 cells (cfu) at the end of 48 hours, the product was packaged under sterile conditions and stored in a cold room at 5 °C until use (Çakmakçı et al., 2013). Uniform height young seedlings were inoculated prior to planting by dipping on in a suspension of each of the bacteria-based biofertilizer formulations of the root system of the seedling for 60 minutes, and then planted (Çakmakçı et al., 2012). The remainder of the suspension was injected into the root zone, control plants received 5 ml of diluted SPB without bacteria. The inoculated seedlings were planted without waiting and water was applied after planting.

The experiment was conducted using a completely randomized factorial design with three replicates. Planting was done with hand in to 30 cm intra-row spacing with 40 cm between rows in 2 x 4 m plots on 25th May in 2020. All the phosphorus fertilizer and half of the nitrogen fertilizer were applied during planting, and the remaining half of the nitrogen fertilizer was applied to the before the first hoeing. Hoeing was done by hand and repeated as required.

The first harvest of whole plants was done on 15 July at the beginning of the flowering stage. Data on different growth parameters of sweet basil, namely, height and branch number of the plant, length and width of leaf, diameter of plant, fresh and dry herb yield, dry leave yield, essential oil ratio and oil yield were recorded at harvest time. Plants at flowering stage were collected by cutting the aboveground part of the stem from a height 10 cm and dried at 35°C, the essential oil content was extracted from air-dry material by distillation. The data for basil genotypes were subjected to analysis of variance using STATISTICA 12 and the means were separated according to Duncan's multiple range test.

3. Result and Discussion

As all selected treatments had promising positive effects on growth and yield parameters of Sweet basil under field conditions. As an average of genotype all treatments tested increased plant height of sweet basil significantly compared to the control; the maximum branch number, leaf length and width, plant diameter, fresh and dry herbage yield in basil were mineral fertilizer NP application, followed by BF3, commercial liquid bio-fertilizer, and BF2 formulation (Table, 1, 2). Differences in terms of growth and yield parameters were, however, not significant between triple inoculations with IAA-producing, ACC deaminase-containing, N_2-fixing, and P-solubilizing bacteria based BF3 formulation (*Pseudomonas fluorescens* RC512 + *Bacillus subtilis* RC210+ *Bacillus megaterium* RC16) and NP application.

As an average of the three genotypes, BF2 and commercial liquid bio-fertilizers were superior to BF1 formulations in terms of fresh herb yield per plant, but these treatments further increased yield over control (Table 1) although still behind the NP fertilization. Of the treatments, BF3 inoculation produced the highest dried leaf yield per plant and hectare while P fertilizer application alone and BF1 inoculation gave the lowest leaf yield. All the treatments, except for P fertilizer, enhanced essential oil ratio and oil yield in basil as compared to the control. As an average of the genotype, among the treatments, the best treatment was the BF3 inoculation, followed by NP, commercial liquid bio-fertilizers, and BF2 formulation in terms of oil yield per hectare. However, there were no significant change in oil yield among these applications. Inorganic fertilizer, biofertilizers, and bacterial formulations significantly affected the growth parameters investigated in basil compared with the control, depending on the genotypes, bacterial consortium and growth parameters assessed.

Table 1. Effect of bio- and inorganic fertilizer, and PGPR combinations on growth parameters of three population of sweet basil in the field experiment

Populations	Treatments*	Plant height (cm) **	Branch number (plant^{-1})	Leaf length (mm)	Leaf width (mm)	Plant diameter (cm)	Fresh herba yield (g plant^{-1})	Fresh herba yield (t ha^{-1})
İspir	Control	45.8 de	22.3 a-e	5.34 ef	3.27 cd	7.87 f	498 g	23.7 f
	NP	55.8 a-c	26.7 ab	6.22 b-d	3.64 c	12.40 a	583 ab	27.8 ab
	P	53.0 a-c	22.3 a-e	5.41 d-f	2.95 d	7.93 ef	499 g	23.8 f
	BF1	54.7 a-c	23.3 a-d	5.25 f	2.96 d	9.33 c-f	507 fg	24.2 ef
	BF2	54.2 a-c	25.0 a-c	6.18 b-d	3.51 cd	11.00 a-c	557 a-d	26.5 a-d
	BF3	56.7 ab	26.2 ab	6.08 c-e	3.65 c	12.13 a	580 ab	27.6 ab
	BMG	55.7 a-c	27.3 a	6.12 c-e	3.65 c	12.43 a	553 a-e	26.3 a-d
	BMV	52.2 a-c	24.3 a-c	6.20 b-d	3.40 cd	8.67 d-f	536 c-g	25.5 c-f
Average		53.5 a	24.7 a	5.85 b	3.38 b	10.22 b	539 b	25.7 b
Yusufeli	Control	45.9 de	18.5 de	5.25 f	3.27 cd	7.98 ef	499 g	23.8 f
	NP	57.3 a	24.1 a-d	6.06 c-f	3.65 c	10.68 a-d	594 a	28.4 a
	P	50.0 cd	20.3c-e	5.25 f	3.01 d	8.49 ef	513 e-g	26.0 b-e
	BF1	52.5 a-c	21.3 b-e	5.53 c-f	3.16 cd	9.19 c-f	526 d-g	27.2 a-c
	BF2	53.8 a-c	22.7 a-e	5.67 c-f	3.24 cd	9.43 c-f	553 a-e	27.9 ab
	BF3	55.0 a-c	23.4 a-d	5.84 c-f	3.33 cd	9.71 c-f	570 a-c	28.3 a
	BMG	54.0 a-c	25.0 a-c	5.57 c-f	3.10 cd	9.00 c-f	555 a-d	28.2 a
	BMV	55.3 a-c	22.7 a-e	5.98 c-f	3.51 cd	10.00 b-e	577 a-c	28.3 a
Average		53.0 a	22.3 b	5.64 b	3.28 b	9.31 b	572 a	27.2 a
Kayseri	Control	43.1 e	17.6 e	6.32 bc	4.95 b	9.95 b-e	500 g	23.8 f
	NP	52.6 a-c	23.8 a-d	8.06 a	6.31 a	12.42 a	596 a	28.3 a
	P	49.6 de	19.3 c-e	6.95 b	6.18 a	11.81 ab	545 b-f	24.2 ef
	BF1	50.6 b-d	22.1 a-e	7.85 a	6.58 a	11.82 ab	571 a-c	25.0 d-f
	BF2	52.6 a-c	22.8 a-e	7.97 a	6.50 a	12.07 a	586 ab	26.3 a-d
	BF3	54.0 a-c	23.4 a-d	8.19 a	6.25 a	12.44 a	594 a	27.1 a-c
	BMG	49.7 cd	21.0 b-e	8.22 a	6.35 a	12.47 a	592 a	26.4 a-d
	BMV	55.3 a-c	26.7 ab	6.94 b	6.41 a	10.67 a-d	594 a	27.5 a-c
Average		50.9 b	22.1 b	7.56 a	6.19 a	11.70 a	548 b	26.1 b
Average	Control	44.9 c	19.5 c	5.64 d	3.82 c	8.60 d	499 d	23.7 d
	NP	55.2 a	24.8 a	6.78 a	4.53 a	11.83 a	591 a	28.1 a
	P	50.9 b	20.7 bc	5.87 cd	4.05 bc	9.41 cd	519 cd	24.6 cd
	BF1	52.6 ab	22.3 a-c	6.21 bc	4.23 ab	10.11 bc	535 c	25.5 c
	BF2	53.5 ab	23.5 ab	6.61 ab	4.42 a	10.84 ab	565 b	26.9 b
	BF3	55.2 a	24.4 a	6.70 a	4.41 a	11.43 a	581 ab	27.7 ab
	BMG	53.1 ab	24.4 a	6.64 ab	4.37 a	11.30 a	567 b	27.0 ab
	BMV	54.3 ab	24.6 a	6.38 ab	4.44 a	9.78 bc	569 b	27.1 ab
		52.5	22.4	6.35	4.28	10.41	553	26.3

*Control: without bacteria inoculation or mineral fertilizers; NP (100 kg ha^{-1} N in the form urea form and 100 kg ha^{-1} P in the form of triple superphosphate) and P fertilizer (100 kg ha^{-1} in the form of triple superphosphate); BF1: *Pseudomonas fluorescens* RC512 + *Bacillus licheniformis* RC601+*Bacillus subtilis* RC210; BF2: *Bacillus megaterium* RC16+ *P. fluorescens* RC512 + *B. subtilis* RC210; BF3: *P. fluorescens* RC512 + *B. licheniformis* RC601+ *B. megaterium* RC16; BMG: BMusaGreen, BMV: BMusaVita; **Different letters within the same column indicate significant differences according to Duncan's Multiple Range Test ($P \leq 0.01$)

On an average of three populations, inoculations of basil with RC512+RC601+RC16, RC512+RC210+RC601, and RC512+RC210+RC16, BMusaGreen, and BMusaVita increased plant height by 16.9, 19.1, 22.9, 18.1, and 20.7% as compared to the control; branch number per plant by 14.1, 20.4, 24.9, 25.3, and 25.9%; length of leaf by 10.2, 17.2, 18.9, 17.7, and 13.1; width of leaf, by 10.8, 15.6, 15.6, 14.3 and 16.2%; diameter of plant by 17.6, 26.0, 32.9, 31.4, and 13.7%; fresh herba yield per plants by 7.2, 13.3, 16.4, 13.5, and 14.0%; dry herba yield per plants by 6.0, 10.6, 13.2, 11.1, and 11.4 %; dried leave yield per plants by 9.6, 18.1, 24.7, 17.9, and 19.5 %, and essential oil ratio by 15.3, 18.5, 17.7, 20.2, and 18.1 and 2.6 %, respectively. NP and P applications, however, increased height of plant up to 22.9 and 13.1%, branch number per plant by 24.8 and 6.0 %, length of leaf by 20.3 and 4.2%, width of leaf by 18.7 and 6.0%, diameter of plant by 37.6 and 9.4%, fresh herb yield of plants by 18.4 and 4.0 %, dry herb yield of plants by 14.6 and 2.5 %, dried leave yield of plants by 22.1 and 4.4 % and essential oil ratio by 18.1 and 2.5%, respectively.

On average of eight treatments, under Çanakkale conditions by conducting field experiment, İspir genotype caused the maximum enhancement in plant height and branch number in basil, while the Kayseri genotype was the most effective in terms of trunk diameter, and leaf length and width. On the other hand, as an average of treatments, Yusufeli genotype showed higher performance compared to the other two genotypes in terms of fresh herb, dry herb and dry leaf yield per plant and hectare. In the field as an average of treatments, Kayseri and Yusufeli genotype were superior to İspir genotype in terms of essential oil ratio and oil yield. Despite the stimulating effects of some *A. brasilense* strains (Mangmang et al., 2016) and the combination of *Azotobacter, Azospirillum*, and *Bacillus* (Roshanpour et al., 2014) on basil growth, fresh and dry yield, and essential oil yield, plant response to applications in terms of biomass production varies depending on plant species and genotype (Kolega et al., 2020).

On the average of all three genotypes, bacterial formulations, mineral (NP) fertilizer and commercial liquid bio-fertilizer stimulated overall plant growth, yield parameters including plant height, trunk diameter and branch number per plant, fresh herb yield, dry herb yield, and dried leaf yield per plants and per hectare, essential oil ratio and oil yield. Among the various treatments and bio-formulations tested, NP fertilization and co-inoculation of IAA-producing, N_2-fixing, P-solubilizing, and ACC deaminase containing *Pseudomonas fluorescens* RC512, *Bacillus subtilis* RC210, and *Bacillus megaterium* RC16 were found to be most effective in promoting the growth and yield parameters of sweet basil. These results were validated by the work of Çakmakçı and Milton (2019) who also showed a substantial elevation in root weight, propagation of lateral and incidental roots and affect nutrient uptake, as well as fresh and dry grass and root weight, chlorophyll content, number of lateral roots and branches, and trunk diameter in lemon basil (*Ocimum* x *citriodorum* Vis) following PGPR inoculations. In the studies conducted with microorganisms, inoculations of *Pseudomonades* sp., *Bacillus lentus*,

and *Azospirillum brasilense* have increased increase in growth, chlorophyll content and yield the essential oil content, weight of root and stem, and total biomass in *Ocimum basilicum* (Heidari et al., 2011), increased the growth of *Ocimum basilicum* with the inoculations of *G. fasciculatum*, *P. fluorescens*, and *B. megaterium* (Hemavathi et al., 2006), increased the dry weight of root and stem, N, P, K, and essential oil content in *Ocimum basilicum* with the inoculations of *P. putida* and *A. Chroococcum* similarly (Ordookhani et al., 2011). Inoculation with *Bacillus subtilis* GB03 increased the a-terpineol and eugenol content together with the yield of essential oil, as well as the shoot and root biomass increase in sweet basil (Banchio et al., 2009). Bacterial inoculation significantly affected and increased the glandular hair abundance, essential oil yield, and monoterpene biosynthesis in basil (Çakmakçı et al., 2020).

In basil as an average of three populations, the highest growth and yield parameters were obtained in NP plots. Studies have noted that nitrogen fertilizer rates significantly affected basil growth and essential oil yield (Alhasan et al., 2020), and the use of a moderate amount of approximately 50 to 60 kg N ha-1 N fertilizer may be appropriate to maximize basil oil yield (Zheljazkov et al, 2008). It has been found that nitrogen applications increase aerial and leaf yield, oil concentration and yield in sweet basil (Sifola and Barbieri (2006), while essential oil yield is promoted by rate of nitrogen and potassium (Nurzyńska-Wierdak et al., 2013), and increasing nitrogen rates (Zheljazkov et al. 2008).

It has been revealed that the inoculation of effective bacteria, which gives similar results to the application of mineral fertilizers in terms of yield and growth parameters, could significantly increase the nutrient use efficiency and reduce the use of fertilizers. Similarly, combination of inoculation of basil with *Azospirillum brasilense*, *Pseudomonas synxantha*, and *Bacillus subtilis* and 75% of recommended quantity of recommended chemical fertilizer showed improvements of plant growth, herb yield, essential oil yield as compared to 100% N fertilized plants (Kassem et al., 2014). Combined inoculation of *Trichoderma afroharzianum* T22, *Azotobacter chroococcum* 76A and 6-pentyl-α-pyrone bioformulation with and without added carboxymethyl cellulose-based biopolymer as carrier was found to modulate fresh basil cultivation by increasing photosynthetic efficiency, rosmarinic acid content, yield and quality (Comite et al., 2021). It has been pointed out that combined inoculation mixtures of different strains of plant-growth-promoting bacteria (PGPB), PGPB-*Mycorrhizae* and PGPB-*Trichoderma* under normal and various stress conditions could be an alternative to inoculation with individual strains and facilitate best and consistent results (Çakmakçı et al., 2020; Santoyo et al., 2021).

Table 1. Effect of bio- and inorganic fertilizer, PGPR combinations on growth and yield parameters of three population of sweet basil

Popula-tions	Treat-ments*	Dried herb yield**		Dried leave yield		Leaf ratio (%)	Essential oil ratio (%)	Oil yield (L ha^{-1})
		(g plant^{-1})	(t ha^{-1})	(g plant^{-1})	(t ha^{-1})			
İspir	Control	92.0 e	4.38 e	49.e	2.32 e	53.1 c-e	0.50 e	12.7 g
	NP	104.3 ab	4.97 ab	58 a-c	2.74 a-c	55.1 a-e	0.57 a-e	17.0 b-e
	P	93.3 e	4.44 e	49 e	2.32 e	52.1 e	0.53 de	13.4 fg
	BF1	96.3 c-e	4.59 c-e	51 de	2.41 de	52.6 de	0.54 b-e	14.3 d-g
	BF2	101.4 a-d	4.83 a-d	56 a-d	2.67 a-d	55.4 a-e	0.61 a-d	17.8 a-c
	BF3	105.5 ab	5.02 ab	61 a	2.91 a	57.9 a	0.60 a-d	19.1 a-c
	BMG	101.3 a-d	4.82 a-d	56 a-d	2.68 a-d	55.6 a-d	0.62 a-d	18.0 a-c
	BMV	98.7 a-e	4.70 a-e	55 b-d	2.60 b-d	55.4 a-e	0.57 a-e	16.2 c-f
Average		99.1 b	4.72 b	54 b	2.58 b	54.7 b	0.57 b	16.1 b
Yusufeli	Control	92.9 e	4.42 e	49 e	2.31 e	52.2 e	0.50 e	12.7 g
	NP	106.3 ab	5.06 ab	61 a	2.91 a	57.6 a	0.65 a	20.2 a
	P	98.3 b-e	4.68 b-e	53 c-e	2.51 c-e	53.7 b-e	0.53 de	14.6 d-g
	BF1	102.9 a-c	4.90 a-c	57 a-c	2.70 a-c	55.1 a-e	0.64 ab	18.7 a-c
	BF2	105.5 ab	5.02 ab	59 ab	2.82 ab	56.2 a-c	0.62 a-d	19.0 a-c
	BF3	106.8 a	5.08 a	62 a	2.94 a	57.9 a	0.62 a-d	19.8 ab
	BMG	106.7 a	5.08 a	60 ab	2.84 ab	55.9 a-d	0.62 a-d	19.2 a-c
	BMV	106.3 ab	5.06 ab	61 a	2.89 a	57.1 ab	0.64 ab	20.3 a
Average		103.2 a	4.91 a	58 a	2.74 a	55.7 a	0.60 a	18.1 a
Kayseri	Control	92.1 e	4.38 e	49 e	2.31 e	52.6 de	0.56 a-e	14.2 e-g
	NP	106.8 a	5.08 a	59 ab	2.83 ab	55.7 a-d	0.62 a-c	19.4 ab
	P	92.2 e	4.39 e	51 de	2.42 de	55.0 a-e	0.54 b-e	14.3 d-g
	BF1	94.5 de	4.50 de	53 c-e	2.50 c-e	55.6 a-d	0.63 a-c	17.2 a-d
	BF2	99.4 a-e	4.73 a-e	57 a-c	2.70 a-c	57.0 ab	0.63 a-c	18.6 a-c
	BF3	101.4 a-d	4.82 a-d	59 ab	2.81 ab	58.2 a	0.63 a-c	19.3 a-c
	BMG	99.7 a-e	4.74 a-e	56 a-d	2.67 a-d	56.2 a-c	0.65 a	18.9 a-c
	BMV	103.7 a-c	4.93 a-c	59 ab	2.81 ab	56.9 ab	0.63 a-c	19.4 ab
Average		98.7 b	4.70 b	55 b	2.63 b	55.9 a	0.61 a	17.7 a
Average	Control	92.3 c	4.39 c	49 d	2.31 d	52.6 e	0.52 b	13.2 c
	NP	105.8 a	5.04 a	59 ab	2.83 ab	56.1 b	0.62 a	18.9 a
	P	94.6 bc	4.50 bc	51 cd	2.42 cd	53.6 de	0.54 b	14.1 c
	BF1	97.9 b	4.66 b	53 c	2.54 c	54.4 cd	0.60 a	16.7 b
	BF2	102.1 a	4.86 a	57 b	2.73 b	56.2 b	0.62 a	18.5 a
	BF3	104.5 a	4.98 a	61 a	2.89 a	58.0 a	0.61 a	19.4 a
	BMG	102.6 a	4.88 a	57 b	2.73 b	55.9 bc	0.63 a	18.7 a
	BMV	102.9 a	4.90 a	58 ab	2.77 ab	56.4 ab	0.62 a	18.6 a
Average		100.3	4.78	56	2.65	55.4	0.59	17.3

*Treatments are explained in Table 1; **Different letters within the same column indicate significant differences according to Duncan's Multiple Range Test ($P \leq 0.01$)

Conclusion

Positive effects of these selected strains formulations on fresh and dry herbage and leave yield, Essential oil ratio and yield, leaf length and width, plant height, branch number and trunk diameter of basil plants populations showed the beneficial role of these PGPR, which might be attributed to IAA production, N_2-fixation, P-solubilisation/mobilization, or even other non-

evaluated PGPR traits that stimulated the plant growth. The use of multi-featured effective free-living bacterial multiple consortia in basil is an important area of study to promote growth and yield, and to develop substitute strategies for the effective management of plant nutrients. Bacterial formulations can change the basil growth and can also affect the yield and growth parameters, but it was strongly dependent on the inoculant strain formulations and parameters evaluated. The experiment revealed that the bacterial consortia and commercial liquid bio-fertilizer inoculation, and NP supply were an effective treatment to improve the parameters measured of sweet basil.

Conflict of Interest

The authors declare no conflict of interest.

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FULL TEXT–ORAL PRESENTATION

AN IMPORTANT NATURAL RESOURCE FOR HEALTH AND NUTRITION: OLEASTER (*Elaeagnus angustifolia* L.)

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Abstract

Elaeagnus angustifolia L. belongs to the family of Elaeagnacea and is an important medicinal plant associated with numerous pharmacological activities, which can be used as natural antioxidant source. Oleaster is an herbal resource with the same name for both its tree and fruit. The roots, leaves, flowers, fruits, fruits peel, and seeds of the oleaster tree are used in food, medicine, pharmacy, and perfumery. It has ecological and economic use, suitable for adverse conditions such as drought, salinity, rocky, and alkalinity. It has diuretic and antipyretic effects. Fruit extracts can be used in traditional medicine as anti-inflammatory and analgesic. It is stated that it provides body resistance, protects against diseases such as cold and flu, and relieves cough and diarrhea. Oleaster consumption is recommended because smelling its flowers provides mental clarity and refreshment and reduces the possibility of developing cancer. Its flowers are used as a nectar source for bees and as a flavoring agent in liqueur production. Oleaster fruits are rich in nutritional value and contain natural antioxidant, phenolic and flavonoid compounds. Since it contains various compounds from different phytochemical categories, it could be proposed for the discovery of new drugs. Oleaster is rich in protein, sugar, amino acids, various vitamins (tocopherol, carotene, vitamin C, thiamine), mineral substances (calcium, magnesium, potassium, iron, zinc and manganese) and fatty acids. For this reason, it is seen that it is used as an additive to food products in recent studies. Although it has high nutritional value and many beneficial effects on health, its consumption is not common. Therefore, in recent years, research has focused on its use in various food formulations and successful results have been obtained. In this study, the subject will be explained in detail.

Key Words: *Elaeagnus angustifolia* L., oleaster, Russian olive, phenolic compounds, antioxidants, nutritional value.

1. Introduction

Elaeagnus angustifolia L. is a spiny, deciduous tree or shrub of the family Elaeagnaceae, grown in arid and semi-arid regions, and resembling an olive tree with small and reddish-brown oval-shaped, mealy, sweet, and edible fruits (Sahan et al., 2013; Safdari and Khadivi, 2021). This fruit-bearing shrub with fragrant flowers, also commonly known as oleaster, Russian olive, Persian olive, silver berry, or wild olive, is native to the southern Europe, central and northern Asia, and spread across from southern Russia and Kazakhstan to Turkey and the Himalayas. Dried oleaster fruits, which are generally eaten in autumn and winter in Turkey, are used in food products due to its rich in nutrients and antioxidants (Ayaz and Bertoft, 2001; Boudraa et al., 2010; Cansev et al., 2011; Çakmakçı, 2012). While the leaves of the plant are used as tea, fodder, wood pulp, the matured fruits could be consumed as fresh or dried snacks, jams, or beverages. Different parts of this tree, especially its fruit and flowers, have many medicinal uses and are used in the pharmaceutical and perfume industries.

Oleaster fruits are rich in chemical composition such as carbohydrates, protein, sugar, amino acids, vitamins and mineral substances beneficial to human health, as well as being one of the natural sources of phenolic, flavonoid and antioxidant compounds found in fruits, flowers, seeds and leaves. Plant polyphenols and flavonoids are widely found in the leaf and fruit extracts of oleaster plants, especially in the leaves, and the most significant biological functions of which are antioxidant (Carradori et al., 2020).

The fruits of oleaster are rich in nutritional value and contain protein, sugar, vitamins, minerals, chemical compounds, antioxidants, and fatty acids. The fruits and flowers are used as tonic, nutritious, anti-ulcerogenic and antipyretic, while various parts of the plant have many uses in some folk remedies and medicine, human and animal nutrition and cosmetics. Its use as a traditional medicine is widespread, for example, as reported by Xie et al. (2020), the flower of oleaster is used in Chinese Uyghur medicine to treat brain disease, thoracalgia, and asthma.

Fruits obtained from oleaster plants vary in fruit quality-related attributes and bioactive properties, which are appropriate for fresh consumption and health benefits, depending on genotypes, climate, environmental conditions, and growing location (Safdari and Khadivi, 2021; Simsek and Sufer, 2021). Although fruits of Russian olive contain significant antioxidant capacity and phytochemical compounds, these components are affected by genotype, different geographical features and climates (Hassanzadeh and Hassanpour, 2018).

2. Nutritional, Elemental and Phytochemical Compositions

The fruits of oleaster, which are consumed fresh and dry, are rich in vitamins such as vitamin C, tocopherol, vitamins A (provitamin A, β-carotene), vitamin E, vitamin K and thiamine B1, as well as minerals such as potassium, sodium, calcium, magnesium, manganese, iron, copper

and zinc (Abizov et al., 2008; Boudraa et al., 2010; Cansev et al., 2011). They have high in nutritional value and contain minerals, protein, sugar, and vitamins (Safdari and Khadivi, 2021). Carbohydrates such as galactose, glucose, mannose, rhamnose, sucrose, xylose and galacturonic acid, mainly glucose and fructose sugars, have been identified in oleaster fruits (Ayaz and Bertoft, 2001; Abizov et al., 2008). Ayaz and Bertoft (2001) indicated that 4-hydroxybenzoic, caffeic, benzoic, vanillic, 4-hydroxycinnamic, protocatechic, and ferulic acid were found in in maturity oleaster fruits in the northeast Anatolia.

Studies on phytochemicals have led to the identification of many compounds in the bark, leaves, fruit, flowers, young branches, seeds and essential oils of *E. angustifolia*, such as flavonoids, phenolics, carbohydrates, amino acids, fatty acids, phospholipids, glucosides, alkaloids, esters, ketones, phenolic acids, phenyl ether, polysaccharides, coumarines, terpenes, alcohols, tannins, steroids, carotenoids, vitamins, and others (Ayaz and Bertoft, 2001; Abizov et al., 2008; Tolkachev et al., 2009; Bucur et al., 2009a; Si et al., 2011; Wang et al., 2012; Okmen and Turkcan, 2014; Bendaikha et al., 2014; Chen et al., 2014; Gökbulut, 2014; Farzaei et al., 2015). Several flavonoids such as epicatechin, epigallocatechin, ısorhamnetin, kaempferol, rutin and quercetin and phenolic acids such as benzoic acid, 4-hydroxybenzoic acid, chlorogenic acid, ethyl cinnamate, gallic acid, ellagic acid, caffeic acid, ferulic acid, p-coumaric acid, protocatechuic acid and vanillic acid have been identified in this plant (Ayaz and Bertoft, 2001; Abizov et al., 2008; Bucur et al., 2009a; Wang et al., 2012; Carradori et al., 2020; Sun et al., 2021).

Fatty acids such as lauric, palmitic, palmitoleic, tridecanoic, pentadecanoic, myristic, oleic, linoleic, linolenic, stearic, and arachidonic acids (Kukina and Sal'nikova, 2012; Yıldırım et al., 2015), as well as amino acids such as aspartic acid, alanine, phenylalanine, arginine, leucine, histidine, isoleucine, lysine, methionine, threonine, proline, serine, valine, cysteine, glycine, tyrosine, glutamine and tryptophan (Abizov et al., 2008) have been identified in *E. angustifolia* fruits.

Phytochemical analysis of *E. angustifolia* fruits revealed the presence of chemical compounds such as phenolic acids, flavonoids, vitamins, carotenoids, lycopene, amino acids, organic acids, sitosteroles, glycosides, coumarines, tannins, and terpenoids, which may have many benefits in related industries such as food and pharmaceutical (Bekker and Glushenkova, 2001; Abizov et al., 2008; Faramarz et al., 2015). In addition, previous studies have reported the fruits of the oleaster could be used as a potential food and food supplement, and even in the pharmaceutical industry due to its rich and beneficial nutritional composition, phenolic content and natural antioxidant source (Cansev et al., 2011; Faramarz et al., 2015).

3. Pharmacological Activities

E. angustifolia recognize worldwide as a medicinal shrub or tree with an important multi-purpose folk remedy and modern therapeutic properties. Studies have shown that oleaster leaves, flowers, fruits and roots have various pharmacological activities such as antimicrobial (Khan et al., 2016; Okmen and Turkcan, 2013; Bahraminejad et al., 2015), insecticidal (Khan et al., 2016), muscle relaxant (Ramezani et al., 2001; Hosseinzadeh et al., 2003), analgesic (Ramezani et al., 2001; Karimi et al., 2010), anti-inflammatory (Taheri et al., 2010; Farahbakhsh et al., 2011; Nikniaz et al., 2014; Motevalian et al., 2017), hypoglycemic (Wang et al., 2018), anti-arthritic (Panahi et al., 2016), antitumour (Wang et al., 2013; Saleh et al., 2018), antimutagenic (Okmen and Turkcan, 2014), antiradical (Faramarz et al., 2015), gastrointestinal (Gürbüz et al., 2003; Monsefi et al., 2007; Eliassi et al., 2009; Huseini et al., 2013), cardioprotective (Belarbi et al., 2011; Wang et al., 2014), immunomodulatory (Du et al., 2016; Sun et al., 2018), antioxidant (Bucur et al., 2008; Çalışkan et al., 2010; Cansev et al., 2011, Wang et al., 2012; Okmen and Turkcan, 2014; Gökbulut, 2014; Yalcin et al., 2014; Hamidpour et al., 2017; Carradori et al., 2020; Simsek and Sufer, 2021) activities.

Oleaster is used as a traditional natural remedy or nutritional agent in the treatment of various diseases. On the other hand, different plant extracts of oleaster is used in gastric pain, dysentery, jaundice and diseases such as hepatitis A, B and C (Jabeen et al., 2015; Uzun et al., 2015), dyspepsia (Khan et al., 2011), cough and cold (Afzal et al., 2009), bronchial affections (Ajaib et al., 2014), improve osteoarthritis (Maghzi et al., 2015) and also in the treatment of diabetes as a food supplement (Wang et al., 2018). In addition, fruit and medulla powders of oleaster were effective in reducing total cholesterol and atherogenic indices in women to a certain extent (Nikniaz et al., 2016).

The gum of oleaster tree is used as shampoo and hair tonic for long and shiny hairs, as well as for the treatment of hair loss (Khan et al., 2011; Jabeen et al., 2015), and flower extracts are used in perfumery (Bucur et al., 2009b; Ajaib et al., 2014). On the other hand, as reported by Wang et al. (2021), polysaccharides extracted from the gum of oleaster showed an anti-inflammatory effect, as they promote a reduction in nitric oxide levels, which contributes to the pathogenesis of inflammation.

4. Food Products

Çakmakçı et al. (2015) reported that the milled crust and flour from oleaster fruits caused an increase in the dry matter, viscosity, acidity, first dripping time, complete melting time, and vitamin C concentration of the ice creams, providing nutritional and functional improvement, and thus can be used as natural antioxidants as a flavour resource in ice cream. In addition, it was emphasized that oleaster powder could be used as an ingredient in gluten free cakes due to its nutritional, fiber and functional properties (Zangeneh et al., 2021). In addition, it has been

reported by Çakmakçı et al. (2015) that oleaster fruit could be used as a functional ingredient in the food industry due to its floury structure, specific sweet taste, functional properties, rich and beneficial nutritional and chemical composition, and high pharmaceutical value. Previous studies have also reported that adding oleaster flour to set-type yoghurts improved the structural properties, increased total phenolic content and functionality (Öztürk et al., 2018). Besides, addition of oleaster flour increased the dietary fiber contents of cookies (Sahan et al., 2013). It has been reported that the addition of oleaster flour increases the calcium, potassium, crude fiber, fat and phenolic compounds of sponge cakes (Kouhanestani et al., 2019), while the fat, ash and fiber content of the doughnut increases with the level of oleaster flour substitution into wheat flour (Sarraf et al., 2017). Considering the sweet unique taste, structure, texture, mineral, phenolic and other components of oleaster flour, it could be used to alternative functional ingredient in the production of bakery products, ice cream, cookies, yoghurt, chocolate and others, however, this subject has not been studied sufficiently yet.

5. Others Uses

The *E. angustifolia* is an important plant, which is regarded as a useful species widely used in environmental protection due to its high ability and tolerance to cope with harsh stress and environmental conditions such as extreme drought, cold, frost, flooding, different pH, and rocky, alkaline and salt-affected soils (Wang et al., 2006; Akbolat et al., 2008; Asadiar et al., 2013; Liu et al., 2014; Hamidpour et al., 2017; Enescu, 2018). This plant has high ecological value due to its silver leaves, fragrant flowers, fruits and resistance to stress and adverse conditions and plays an important role in landscaping, shelterbelts and windbreak, sand stabilization, desertification control, soil and water conservation, restoration of vegetation and degraded lands, afforestation, and drought-resistant ornamental plant in poor soils (Zhang et al., 2018).

Different parts of oleaster such as root, wood, bark, young branches, juice extracts, flowers, leaves and fruits are used in the food, sanitary, pharmaceutical, perfume, forage, papermaking and wood industries. Suitable for papermaking, the oleaster tree has been used since ancient times for wood, food, shelter, hand tools and utensils (Akgül and Akça, 2020). It used in landscape architecture as an ornamental plant. It is also widely used as an ornamental plant in the rehabilitation of the environment due to its resistance to pollution, salinity tolerance, esthetic appearance and rapid growth ability (Kiseleva and Chindyaeva, 2011). It is suitable for degraded, eroded and landslide areas, saline- alkali soils, and steppes (Gokturk et al., 2006; Lai et al., 2012), as well as it fixes nitrogen and enriches the soil in terms of nitrogen and is used in the forest land reclamation (Ajaib et al., 2014; Enescu, 2018). Oleaster tree has even been described as the “treasure tree” of saline-alkali soils (Sun et al., 2021).

It is also a melliferous plant, with small, fragrant, odour and yellowish-white flowers, and is used as a well-known nectar source for honeybees and as a flavouring agent in liquor production (Zima, 2007; Kiseleva and Chindyaeva, 2011, Irimia et al., 2015; Yılmaz, 2016). Fruit of oleaster is a source of food for birds, so it plays an important ecological role in their habitats. While the oleaster is used as a a fragrant ornamental plant, its flowers are a good source of nectar to honeybees and are used in production of liqueur. As a nitrogen-fixing tree species that is salt-tolerant and used in fields such as fruit, wood, source of honey and medicinal purposes, it can increase soil nutrient resources and fertility (Khamzina et al., 2009). These plants have the potential to be used as windbreaks, wildlife attractants, snow traps, and live hedges, protection of river and ocean coastal areas, and erosion control. In addition, Aksoy and Şahin (1999) reported this plant could be used as a bio-monitor agent for screening the heavy metals, due to its ability to tolerate a variety of geographical and environmental conditions. Moreover, the application of compost produced from the oleaster tree by pyrolysis has significantly enriched soil fertility by improving biochemical properties such as available P, Mn, Fe, Zn, total N and organic C in calcareous sandy soils (Manirakiza et al., 2021).

6. Conclusion

The roots, leaves, flowers, fruits, fruits peel, and seeds of the oleaster tree are used in food, folk medicine, medicine, pharmacy, cosmetics, and perfumery. The fruits of oleaster are rich in nutritional value and contain protein, sugar, flavonoids, phenolics, vitamins, and minerals, and are one of the natural sources of especially phenolic, flavonoid and antioxidant compounds, as well as alternative functional ingredient in the food industry and production of food products. It has ecological and economic use, suitable for adverse conditions such as drought, salinity, rocky, and alkalinity, source of nectar for honeybees. In summary, considering the benefits of oleaster, it has emerged that its production and consumption should be increased.

Conflict of Interest

The authors declare no conflict of interest.

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ESSENTIAL OILS AS FOOD PRESERVATIVE: ENCAPSULATION, BIOLOGICAL ACTIVITIES, AND SENSORY IMPACT

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Abstract

This work evaluates the nanoencapsulation effect on essential oil potential to ameliorate food conservation. After extraction by hydrodistillation, tested essential oils *(Syzygium aromaticum, Cinnamomum zeylanicum*, and *Lavandula stoechas)* were analyzed by GC-MS, then mixed and encapsulated into nanoemulsion-based delivery system. The encapsulation effect has been evaluated, firstly *in vitro*, based on the antioxidant and antibacterial activities of the mixture. Then, deliberately contaminated milk (with *Staphylococcus aureus*) was chosen to evaluate the essential oil encapsulation effect in a real food model *via* the enumeration of surviving bacteria. Finally, a hedonic sensory survey studied the encapsulation effect on consumer acceptability of essential oil enriched milk. GC-MS obtained results showed a significant variability of volatile compounds depending on essential oils. Eugenol (75%), cinnamaldehyde (89%) and camphor (35%) were distinguished as majorcompounds of studied essential oils, respectively. The study of the *in vitro* encapsulation effect revealed that the nano encapsulated mixture had a lower DPPH inhibiting capacity, as compared to the bulk mixture. Indeed, for the same concentration (0.5 mg/ml) the inhibiting capacity significantly decreased from 65 to 61% (for bulk and nano encapsulated essential oil, respectively). Interestingly, the encapsulation has significantly improved the efficiency of the mixture for the iron reducing power test. Concerning the evaluation of antimicrobial activity, results revealed promising potential against *Escherichia coli* and *Staphylococcus aureus* with improved efficacy of the nano-encapsulated mixture. The study of the effect of enriching contaminated milk with bulk or nano-encapsulated mixture revealed a significant decrease in the bacterial load, compared with the negative control. The sensory evaluation revealed similar acceptances for control milk with the one treated bynanoemulsion, while milk supplemented with bulk essential oil was found unacceptable by panelist. In conclusion, gathered results conclude that the essential oil nanoencapsulation not only ameliorate its potential as natural food preservative, but also its sensorial acceptability.

Key Words: Essential Oils, Nano-encapsulation, Anti-oxidant Activity, Anti-bacterial Activity, Milk

1. Introduction

Milk is an important aliment in the human diet commonly known as a healthy and nutritious food because of its richness in proteins, calcium and vitamins. However, such richness in micronutrient renders this food vulnerable to microbial contamination (Smigic et al., 2012). In addition, milk is extremely perishable because most microbial and biochemical agents affect food quality especially in an aqueous environment. Milk contamination with pathogenic bacteria, viruses, harmful parasites or chemicals, not only render it unsuitable for human consumption, but also cause more than 200 diseases such as severe gastroenteritis.

In this context, finding appropriate protective measure is crucial to maintain milk safety through the elimination of pathogenic bacteria and the prolongation of milk shelf life. The use of natural products derived from plants, particularly essential oils (EOs), is getting increasing interest due to their high efficacy against foodborne pathogens and bacteria (Yap et al., 2014). However, the use of EOs as food preservatives could be limited due to their long and delicate extraction process, making them extremely expensive (Tajkarimi et al., 2010). It is therefore possible to overcome this problem by minimizing their concentration in the food matrix without losing their antimicrobial effectiveness. In this context, multiple approaches could be adopted, such the appeal of the experimental design methodology to design an efficient combination of EOs mixture (Gutierrez et al., 2008; Tserennadmid et al., 2010). In addition, the encapsulation of EOs into nanoemulsion based delivery system present an interesting approach. The objective of this manuscript is to evaluate the milk-protective-effect of nanoencapsulated EOs mixture (elaborated through an experimental design) made of *Syzygium aromaticum, Cinnamomum zeylanicum* and *Lavandula stoechas.*

2. Material and Methods

2.1. Plant sampling

Cloves (*Syzygium aromaticum*) and cinnamon bark (*Cinnamomum zeylanicum*) were purchased from a local market. Lavender (*Lavandula stoechas*) shoots were collected from Ouchtata region (GPS Point N 133, North 36° 57’ 8.30’’; East 8° 57’ 79.1’; Altitude 66 m), dried and cut into small pieces before extraction.

2.2. Essential oil extraction

EO extraction was performed using a Clevenger type device. A variable quantity, depending on the plant, was introduced into a 2L flask partially filled with distilled water. The assembly was brought to a boil using a flask heater. The water vapour driving the volatile compounds were condensed at the refrigerant and then recovered in the Clevenger siphon. To remove any trace of water, obtained EOs were treated with anhydrous sodium sulphate (Na_2SO_4) before storage in a refrigerator at a temperature of -18°C in opaque bottles.

2.3. The EO chemical composition identification

The chemical composition of tested EOs was identified referring to a gas chromatograph coupled to a mass spectrometer. The used column was an HP INNOWAX polar column (30 m × 0,25 mm, film thickness, 0.25 µm). Helium was injected at 1.2 ml/min flow set in the split mode (1/ 10). Injection and detection temperatures were set at 250 and 280°C, respectively. The ionization was made by electron impact (70 eV) and the ion source temperature was fixed at 175°C. For molecules mass determination, spectral data were obtained using the scan mode

in the m/z range of 50 to 550 (Ben Jemaa et al., 2017). The comparison of the retention times with authentic standards allowed component identification. Only peaks whose contribute by more than 0.01% of the total area were identified.

2.4.EO Mixture design

In this study, a plan of centered mixtures (Simplex-centroid designs) was used to assess the quaternary EOs antibacterial effect against *Staphylococcus aureus*. As detailed in Falleh et al. (2020), the mathematical model provided that the optimal formula against *S. aureus* is formed by 58.8% *C. zeylanicum* plus 34.4% *S. aromaticum* and 6.8% *L. stoechas*.

2.5. EO encapsulation

To improve its stability and activity, clove, cinnamon, and lavender EO mixture was encapsulated into oil in water nano-emulsion. This nanoemulsion was prepared by a two-step homogenization process.

Coarse emulsions were prepared in the first step by blending the two phases using a rotor–stator homogenizer (Polytron, PT-3000 Kinematica-AG, Littace, Switzerland) for 5 min at 7000 $rpm.min^{-1}$. Then, the final nanoemulsions were obtained in the second step by homogenizing the coarse emulsions through a high-pressure homogenizer (NanoVater, NV200, Yoshida Kikai, Nagoya, Japan) at 100 MPa for 5 passes. The continuous phase of the nano-emulsion was composed of an aqueous solution of Tween 80, while the remaining 10% was composed of the EO mixture and a vegetable oil. The average diameters of the droplets of the resulting nano-emulsion were analysed using a ζ-sizer (Falleh et al. 2021).

2.6. Biological activity assessment

- **Anti-oxidant activity.** The anti-oxidant activities of EO mixture "BM" and of nano-encapsulated EO mixture "NEM" were evaluated *in vitro* using two-method: the DPPH inhibition capacity measurement and the iron reducing power test.

DPPH inhibition capacity. 1 ml of samples, at different concentrations, was added to 250 µl of a $DPPH^{\cdot}$ solution (0,2 mM in methanol). The mixture was placed for 30 minutes in darkness to react (Hatano, 1988) and absorbance was measured at 517 nm against a negative control (without EO).

The results are expressed as a percentage of Inhibition Capacity (IC).

Iron reducing power test. Iron reducing power. The reducing powers of samples were determined through the transformation of Fe^{3+} to Fe^{2+}. Sample solutions at different concentrations were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%, w/v). The mixture was incubated at 50°C for 20 min. Afterwards, 2.5 ml of TCA (10%) were added and the mixture was centrifuged for 10 min at 1000 × g. Supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of ferric chloride (0.1%, w/v), and the absorbance was read at 700 nm against ascorbic acid as authentic standard. Higher absorbance of the reaction mixture indicates greater reducing power.

- **Antibacterial activity.** The evaluation of the antimicrobial activity of bulk or nano-encapsulated EO mixture was carried out by the disc method against 2 different bacterial strains *E. coli* (ATCC 35218) and *Staphylococcus aureus* (ATCC 25923). According to Hussain et al.

(2010), the first step in the disc method was to pour 20 ml of agar (Muller Hinton) into Petri dishes. After culture medium solidification, 0.1 ml of microbial suspension, of known concentration, was spread on the surface. Subsequently, and under aseptic conditions, sterile discs soaked with 10 µl of each EO samples were deposited on the agar surface. Finally, the Petri dishes were incubated at 37°C for 24 hours.

2.7. EO incorporation in milk

To evaluate the preservative effect of bulk "BM" and nanoencapsulated "NEM" EO mixtures (0.5 mg/ml) in food preservation, semi-skimmed UHT milk (purchased from a local supermarket) was chosen as food matrix for its greater frequency of consumption.

Milk contamination and treatment. A volume of 0.5 mg of the EO mixture (clove, cinnamon, and lavender) in the bulk form "BM" or in the nano-encapsulated form "NEM" were aseptically added to 1 ml of UHT milk. The bacterial strain, *Staphylococcus aureus* (ATCC 25923), was then added to the milk to obtain a final bacterial load of approximately 10^3 CFU/ml. After contamination, all milk samples are incubated for 24 hours at 37°C.
After incubation, 1 ml of suitably diluted sample was spread into empty Petri dishes, on which the Muller Hinton Agar was added. The whole was mixed with rotation in the form of eight and allowed to solidify. After solidification of the medium, the incubation was carried out at 37°C for 24 h. At the end of the incubation, the developed colonies, whatever their size, were counted and the results are expressed in units forming colonies (UFC/ml).

Sensory evaluation. To appraise the sensorial impact of bulk and nanoencapsulated EO mixtures incorporation into milk, supplemented UHT milk (with either BM or NEM) and standard milk were subjected to a blind sensory evaluation. The targeted characteristics (color, flavor, smell, taste, and aftertaste) were graded by 60 panellists of both sexes, aged from 20 to 50 years (students, supervisors and staff from Biotechnology Center of Borj-Cedria). The inquiry used five-point hedonic scale (1: unacceptable, 2: bad, 3: acceptable, 4: good, 5: excellent). The sensory profiles were conducted on coded samples served in plastic cups that did not impart any flavour or odour to the products.

2.8. Statistical analysis

At least three repetitions were performed for all analysis. Result variabilities were analyzed using means comparison using one way-ANOVA analysis (the Duncan test), and 5% of confidence level was designated for significant differences using the Statistical package SAS 9.1 (2002, 525).

3. Results and discussion

3.1. EO chemical identification

The data analysis of Table 1 revealed a significant variability on the chemical composition of the three-tested EOs. In fact, lavender EO was the most diverse sample containing more than 40 compounds with camphor (35%) and α-fenchone (32%) as the major compounds. For cinnamon EO, 18 volatile compounds were identified, with cinnamaldehyde (89%) as the major compound. Finally, only 4 components were detected in the clove sample and their percentages

ranged from 75% (eugenol) to 2% (beta-selenene). The subsequent result was in good agreement with those of Omidbeygi et al. (2007) working on the Iranian clove.

Table 1. The chemical composition of clove, cinnamon and lavender EO, expressed as %. The averages ±SD of three repetitions followed by the same letter, in the same column, are not significantly different ($p<0.05$).

Compounds	Clove	Cinnamon	Lavender
4-terpineol	-	-	0,07±0,1**i**
tricyclene	-	-	0,3±0,0**h**
α -pinene	-	0,6±0,0**c**	2,8±0,1**e**
camphene	-	3,5±6,5**b**	4,3±0,0**d**
Ni	-	-	1,9±3**e**
camphenilone	-	-	0,1±0,1**i**
β-pinene	-	0,1±0,0**c**	-
α-terpinolene	-	0,08±0,0**c**	-
α -copaene	-	0,1±0,0**c**	0,05±0,0**i**
isopinocarveol	-		0,1±0,1**i**
Ni	-	-	0,2±0,2**i**
β- cymene	-	-	2,7±4,6**i**
d-limonene	-	0,3±0,0**c**	0,2±0,3 **i**
1,8-cineole	-	1,6±0,1**b**	7,4±0,06 **c**
β-selinene	2±0,1**c**	-	0,06±0,1**i**
(+)-calarene	-	-	0,07±0.1**i**
α-cadinene	-	-	0,05±0,1**i**
α-calacorene	-	-	0,06±0,1**i**
Ni	-	-	0,08±0,1**i**
Ni	-	-	0,07±0,1**i**
Ni	-	-	0,04±0,0**i**
Ni	-	-	0,06±0,12**i**
1edol	-	-	0,04±0,0**i**
linalool oxide cis	-	-	0,7±0,0**j**
α -fenchone	-	-	32,5±1,8**b**
l-fenchone	-	0,3±0,0**c**	-
Ni	-	-	0,06±0,1**i**
linalool	-	-	0,8±0,08**f**
d-fenchyl alcohol	-	-	0,2±0,0**h**
α -campholenal	-	-	0,3±0.0**h**
camphor	-	0,2±0,0**c**	35,4±0,1**a**
caryophyllene	20,4±1,1**b**	-	-
p-mentha-1,5-dien-8-ol	-	-	1,1±0,0**f**
terpinen-4-ol	-	0,8±0,0**c**	-
α-terpineol	-	1,6±0,0 **b**	-

myrtenol	-	-	1,1±0,0**f**
verbenon	-	-	0,5±0,0**h**
Ni	-	0,1±0,1**c**	--
Ni	-	0,2±0,2**c**	-
cinnamaldehyde	-	89±0,3**a**	-
(-)-caryophyllene oxide	-	-	0,2±0,2**i**
bornyl acetate	-	2±0,0**b**	2,4±0,0**e**
eugenol	74,5±0.65**a**	-	-
cycloisosativene	-	-	0,7±0,9**j**
aceteugenol	2,6±0,29**c**	-	-
δ -cadinene	-	-	0,7±0,1**j**
calamenene	-	0,1±0,0**c**	-
α- muurolol	-	-	1,1±0,1**f**
α-cadinol	-	-	0,3±0,08**h**
Total (%)	99.75	99.99	99.7

3.2. Nanoencapsulation effect on the EO specific mixture anti-oxidant activity

The encapsulation effect of the EO specific mixture was evaluated through the DPPH and the iron reducing power tests both for the bulk and the nano-encapsulated EO specific mixture.

Table 3. The inhibition capacity of bulk "BM" and nano-encapsulated EO "NEM" for both DPPH and the iron reducing power at 0.5mg/ml EO. The average of three repetitions followed by the same letter, in the same line, are not significantly different ($p<0.05$).

	%IC	
Tests	**BM**	**NEM**
DPPH	65 ^{b}±2.18	61^{a}±0.62
Iron reducing power	36 ^{a}±1.95	41 ^{b}±3.4

DPPH Test. Results presented in Table 3 demonstrated that the bulk EO mixture has an interesting efficacy in inhibiting the DPPH free radical. Indeed, the use of 0.5 mg/ml of BM is capable of producing 65% inhibition. This interesting anti-oxidant activity may be due to a synergy between the different majority molecules of each EO, especially since they are known in the literature by their anti-oxidant efficiency. Indeed, Subash-Babu et al., (2014) confirmed the antioxidant potential of Cinnamaldehyde (the majority of cinnamon EO) *via* the DPPH test. For its part, Gulcin (2011) has shown that eugenol (the majority of clove EO) is highly active against the DPPH radical (IC_{50} =16.06 µg/ml). Regarding lavender, several studies have demonstrated the ability of its EO to inhibit efficiently the DPPH radical (Barkat and Laib, 2012).

The encapsulation of the EO mixture into a nano-emulsion based delivery system induced a reduction in the mixture ability to reduce the DPPH radical. Indeed, for the same concentration (0.5mg/ml) the IC decreased significantly from 65 to 61% (for BM and NEM, respectively). This decrease in the DPPH-radical reduction is likely related to the slowing down of the reaction

due to encapsulation of anti-oxidant molecules within the nano-emulsion. In fact, Ha et al. (2015) suggested that the speed of this reaction is faster for non-emulsified compounds than for encapsulated compounds, which are drowned in the continuous phase and thus protected against oxidation.

Iron reducing power test. The analysis of Table 3 showed that the iron reduction potential of the EO mixture in the bulk state "BM" or nano-encapsulated "NEM" is quite strong. In addition, the encapsulation of EO allowed a significant improvement ($p<0.05$) of this potential with a 36% to 41% increase in inhibition at a concentration of 0.5mg/ml (for BM and NEM, respectively).

3.3. Encapsulation effect on EO specific mixture anti-bacterial activity

The evaluation of the antimicrobial activity of bulk "BM" and nano-encapsulated EO mixture "NEM" against *E. coli* and *S. aureus* was performed using the disc method. Obtained results (Figure 1) have revealed an interesting potential of tested mixtures, and this against the two pathogenic tested strains.

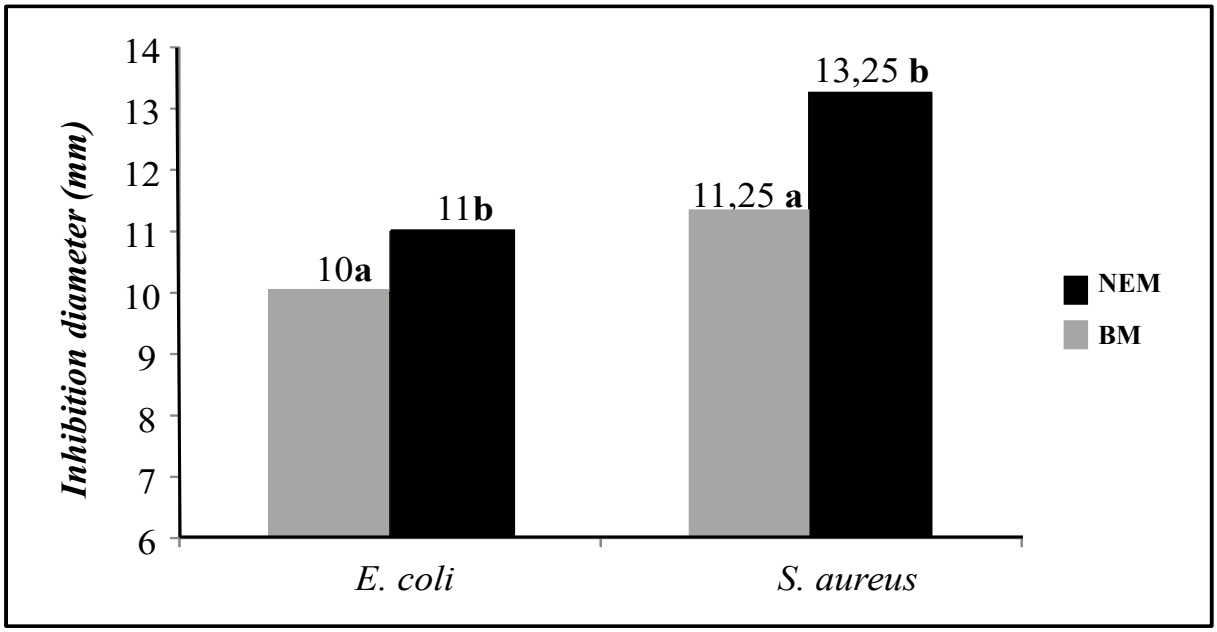

Figure 1. The anti-bacterial activity of bulk "BM" and nano-encapsulated "NEM" EO mixture against the two pathogenic strains *E. coli* and *Staphylococcus aureus* at a concentration of 0.5mg/ml EO. The average of three replicates followed by the same letter, for the same bacterium, are not significantly different ($p<0.05$).

The bacterial growth inhibition diameters generated by "BM" at 0.5 mg/ml exceeded 10 and 11.25 mm (against *E. coli* and *S. aureus*, respectively). The obtained results are probably related to the synergism between the majority compounds of the different used EOs in the mixture (*Syzygium aromaticum*, *Cinnamomum zeylanicum* and *Lavandula stoechas*).

Actually, Falleh et al., (2019) who investigated the improvement of the anti-bacterial activity of EO specific mixture confirmed this result. The mode of action of EOs against pathogenic

bacteria has been extensively described in the bibliography, and the majority of research has agreed that EOs benefit from their activity through the disintegration of the bacterial outer membrane, followed by the release of lipo-polysaccharides, resulting in increased permeability of the cytoplasmic membrane ATP (Burt 2004).

Interestingly, obtained results showed that *S. aureus* strain was more sensitive than *E. coli* strain to both forms of the EO mixture. In fact, the inhibition diameter generated by "BM" and "NEM" was always significantly larger in the case of *S. aureus* than in the case of *E. coli*. For *E. coli*, the inhibition diameters were limited to 11 mm (for "NEM"), while for *S. aureus*, inhibition diameters exceeded 11.25 mm (for "BM"). This resistance generated by *E. coli* may be related to the difference in the cytoplasmic membrane between $Gram^+$ and $Gram^-$ bacteria, such as the presence of an important hydrophilic barrier for $Gram^-$ bacteria (Hyldgaard et al. 2012).

Figure 1 showed that the encapsulation of EO specific mixture into a nano-emulsion based delivery system was able to amplify its ability to inhibit the growth of the two tested strains. For example, "NEM" generated a larger area of *S. aureus* growth inhibition (13.25 mm) than that generated by "BM" (11.25 mm). This improvement in the effectiveness of the EO mixture after its nano-encapsulation can be explained by the fact that the EO hydrophobicity (as bulk) can cause a limitation of their effectiveness against pathogenic strains that bathe in an aqueous medium. Indeed, Ben Jemaa et al. (2017) stated that EO nano-encapsulation can facilitate the transfer of bioactive molecules mechanisms through the cell membrane of the target micro-organism.

3.4. EO incorporation in milk

Evaluation of milk enrichment effect

The objective of this work is to evaluate the conservative effect of the EO mixture in bulk form "BM" and nano-encapsulated form "NEM" after its incorporation at a concentration of 0.5mg/ml in milk deliberately contaminated with *S. aureus*. Obtained results (Figure 2) showed that the enrichment of milk deliberately contaminated with *S. aureus* by either bulk or nano-encapsulated EO mixture significantly reduced the bacterial load, in comparison with the negative control (contaminated and not enriched milk).

After incubation for 24h to 37°C, the bacterial load of the negative control exceeded $3 * 10^5$ CFU/ml, while it did not exceed $1.5 * 10^5$ CFU/ml for the milk enriched with "NEM", and was reduced to $3 * 10^4$ CFU/ml for the milk enriched with "BM".

In fact, milk, as well as dairy products, represent an excellent medium for the growth of many spoilages and pathogenic microorganisms. Thus, in the absence of "BM" and "NEM", the pathogenic strain *S. aureus* had the advantage of degrading the nutrients available in this rich environment, which may be responsible for the profound deterioration of the health and sensory quality of milk (Smigic et al., 2012). Incorporation of the bulk "BM" or nano-encapsulated "NEM" EO mixture into the contaminated milk has demonstrated a good ability to control bacterial growth and therefore protect the milk from spoilage and extend its shelf life.

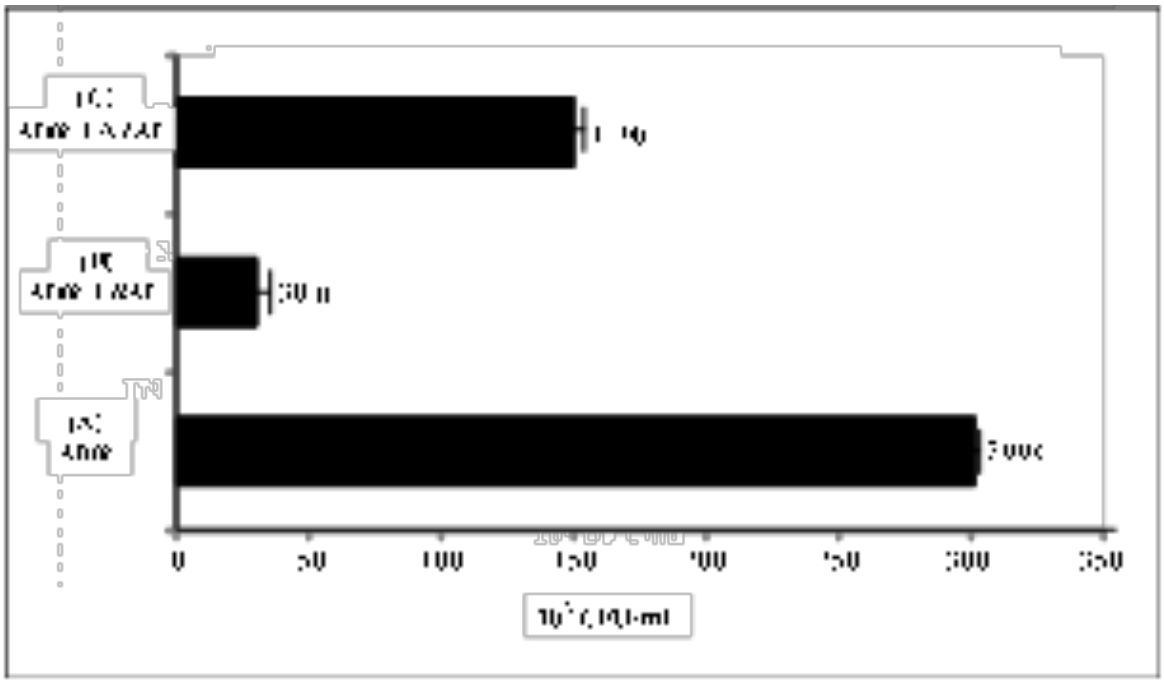

Figure 2. Bacterial load of milk samples deliberately contaminated with 10^3 CFU/ml of *S. aureus* bacteria, whether enriched or not with EO mixture at a concentration of 0.5mg/ml EO. The average of three replicates followed by the same letter, for the same bacterium, are not significantly different ($p<0.05$).

(A) Negative control: milk contaminated with S. aureus and not enriched.
(B) Milk +BM: milk contaminated with S. aureus and enriched with "BM"
(C) Milk+NEM: milk contaminated with S. aureus and enriched with "NEM"

Sensoriel acceptability analysis of enriched milk

Milk sensorial properties are crucial for the dairy industry because the consumer directly relates them to the product quality and therefore its acceptability. The sensorial evaluation of the three milk samples (Figure 3) showed that the incorporation of bulk or nano-encapsulated EO specific mixture significantly ($p<0.05$) influenced the assessment of milk by the panel.

Figure 3 results exhibited that the incorporation of the EO specific mixture did not alter the panelists' assessment of milk colour. Indeed, the panellist stated a similar appreciation for the colour of the three samples tested. However, milk enrichment by "BM" resulted in a significant decrease in milk appreciation with respect to its aroma, taste, aftertaste and its overall appreciation, as the "BM" enriched milk sample had the lowest scores in each of the last listed attributes. In fact, the control (standard milk) and the milk enriched by «NEM» presented a better appreciation of the aroma, and higher overall appreciation notes, in comparison with the milk enriched by «BM».

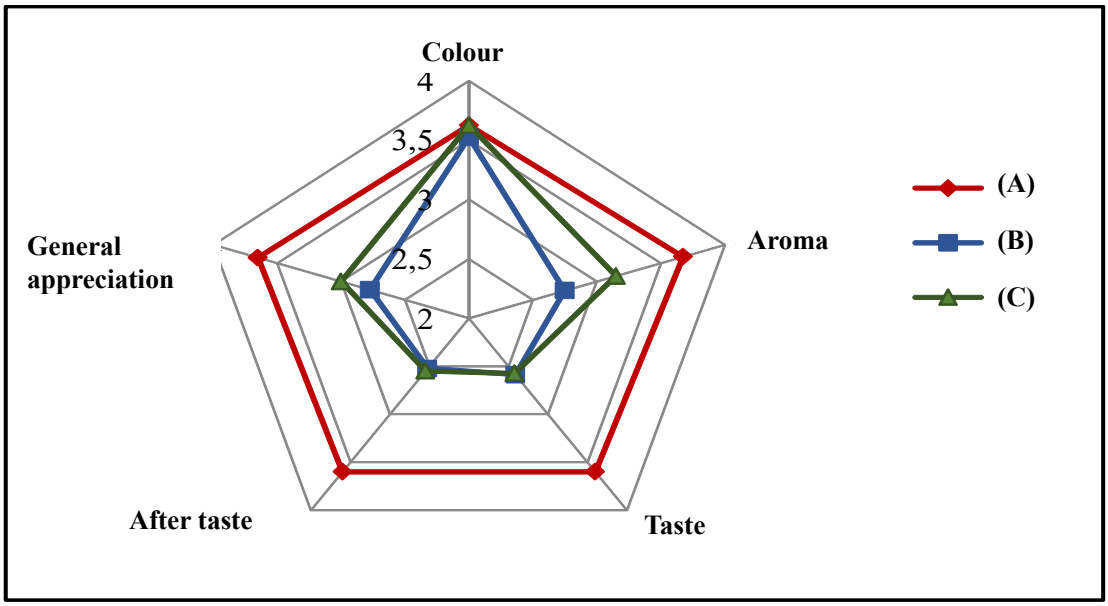

Figure 3. Sensorial assessment of the effect of milk enrichment (0.5mg/ml) by the bulk EO mixture "BM" or by the nano-encapsulated EO mixture "NEM". For the same sensory attribute, two samples with the same letter are not significantly different ($p<0.05$).

(A) Negative control: not enriched milk.
(B) Milk +BM: milk enriched with "BM"
(C) Milk+NEM: milk enriched with "NEM"

The nano-encapsulation of the EO mixture has improved the sensorial acceptability of enriched milk. In fact, the assessment of the flavour of milk enriched by "NEM" was higher than 3 on a scale of 5, while it was limited to 2 for milk enriched by "BM". Similarly, the overall appreciation of milk enriched by "NEM" had higher scores compared to milk enriched by "BM" (3 and 2.7, respectively). This improvement in the sensory appreciation of the finished product was one of the objectives of EO nano-encapsulation. In fact, despite their biological efficacy, the enrichment of aliments with EO may be limited by their high sensorial impact, which can lead to the consumer refusal. In the same context, EO encapsulation into nano-emulsion based delivery systems allows the trapping of highly odorous bioactive molecules inside the capsule surrounded by water molecules, which helps to mitigate their olfactory impact.

Conclusion

The chemical analysis results showed the presence of a variable and significant number of volatile compounds as a function of essential oil. In addition, the study of the effect of nano-encapsulation of the mixture (2.4% *S. aromaticum*, 59.4% *C. zeylanicum* and 38.2% *L. stoechas*) revealed that the nano-encapsulated essential oil mixture has a lower inhibition capacity of DPPH radical than the bulk mixture. In addition, the iron reducing power test showed that bulk and nano-encapsulated essential oil mixture had quite strong potential with a significant improvement for the nanoencapsulated form. The estimated antimicrobial activity of bulk and nano-encapsulated essential oil mixture has shown interesting potential against *E. coli* and *S. aureus*. Finally, the sensory evaluation of the three milk samples showed that the incorporation of bulk or nano-encapsulated essential oil mixture significantly influenced the

assessment of milk by the panel. In conclusion, the current results have improved the potential use of essential oil as a natural and healthy preservative for milk.

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FULL TEXT–POSTER PRESENTATION

EFFECTS OF WALL MATERIALS ON AROMATIC PROFILE OF SPRAY DRIED CLARY SAGE OIL (*Salvia sclarea*)

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Abstract

Clary sage (*Salvia sclarea*) oil is a valuable essential oil due to its aromatic properties, bioactive constituents and beneficial health effects. Spray drying is the most common method used for microencapsulation of essential oils and aromas providing the opportunity to differentiate the taste and aroma of foods, to mask undesired tastes and odors, to ensure the stability of food components and to increase their bioavailability. The most important point to be considered in the encapsulation process of essential oils is the preservation of the natural composition of the essential oil. The aim of this study was to maintain flavor retention of spray-dried clary sage oil. Therefore, effects of wall material composition (80-100% maltodextrin, 0-10% gum Arabic, and 0-10% modified starch-EmCap®) on encapsulation of clary sage oil were investigated. The D-optimal design was used with 3 factors and 2 levels. Spray dried samples were characterized in terms of retention rates of volatile compounds in the clary sage oil using GC-MS. The retention rates of linalyl acetate (LA), linalool (LO) and α-terpineol (α-T) the main constituents of sage oil in powder products were compared. Encapsulation efficiency, moisture content, water activity (a_w), and glass transition temperature (T_g) were determined as well. The main volatile organic compounds (VOCs) of clary sage oil were identified as LA, LO, and α-T which constituted ~85% of the oil. Encapsulation efficiency of spray dried samples varied between 94.1 %- 98.6% whereas their T_g ranged from 54.9°C to 87.6°C. Moisture content values varied between 0.8 and 1.7%, while water activity (a_w) values were in the range of 0.11-0.20. According to the retention rates of linalyl acetate, linalool, α-terpineol, as well as the ratios of LA/LO and LA/α-T, a mixture of 95:5 maltodextrin:gum Arabic could be suggested as the most suitable wall material for microencapsulation of clary sage oil, which could best preserve the natural aromatic composition of clary sage oil.

Key Words: Clary sage, essential oil, spray drying, maltodextrin, gum Arabic

1. Introduction

Nowadays, nutritional habits of individuals are changing due to the fact that health awareness is improving and life standard is rising gradually. Recently, foods taken within daily diet have become highly essential in terms of consumer preference due to the importance of their quality and the positive effect of their functional components on physical and mental health.

With the increasing demands of consumers, the widespread use of functional ingredients in the food or drink formulations has gained importance in the food industry. In recent years, essential oils which have been used for thousands of years in various cultures for medicinal and health purposes has gained popularity due to mainly their antioxidant, antimicrobial and anti-inflammatory properties. These oils are produced from various sections of plants and herbs (Bigliardi and Galati, 2013).

Salvia sclarea is one of the most interesting Mediterranean herb species due to its aromatic properties and beneficial health effects. Various *Salvia* species have been reported to have many biological activities such as antimicrobial, antioxidant, anti-inflammatory, antifungal, antiplasmodial, antiseptic, hypoglycemic and anticancerogenic and expectorant effects (Esmaeili and Sonboli, 2004; Kamatou et al., 2008; Gürsoy et al., 2012;) The so-called functional and medicinal properties of clary sage oil arise from the beneficial effects of volatile bioactive components of the essential oil. Therefore, there is an increasing interest in the use of clary sage oil in food and medicinal applications in recent years.
Peana et al. (2002) reported that linalool and linalyl acetate-containing *Salvia sclarea* and *Salvia desoleana* species were potentially anti-inflammatory. Duarte et al. (2016) investigated the antibacterial, antibiotic, and antioxidant potential of linalool and reported that it showed an exceptional ability to inhibit lipid peroxidation. These results have shown that these components may be potential alternatives to synthetic antioxidants and can extend the shelf life of various food products (Duarte et al., 2016).

To date, a majority of studies investigated the antimicrobial and antioxidant effect of clary sage oil in various meat products (Simitzis et al., 2008; Ahmed and Ismail, 2010). In a study of Ahmed and Ismail (2010), clary sage oil was tested for the effectiveness of prolonging shelf-life and increasing microbial quality in minced meat. It was observed that the microbial quality and shelf life of minced meat were increased with the use of 0.3-0.5% sage oil at 4 ° C for 7 days.

Another study reported that during the cooling process, sage oil reduced the oxidation in veal and pork, and in addition, sage oil delayed fat oxidation in frozen meats (Simitzis et al., 2008). In addition to the fresh meat products, Salem and Ibrahim (2010) used sage oil in the formulation of dry fermented sausages made from buffalo meat to prevent or delay oxidation. It was shown that sage oil could be used in sausages as a natural antioxidant (Salem and Ibrahim, 2010). On the other hand, like edible oils, essential oils including sage oil are not

chemically stable and are susceptible to oxidative deterioration. Further effects of oxidative degradation cause loss of nutritional quality and development of undesirable off-flavors and dark color formation, loss of flavours, deterioration of pigments, thereby affecting stability and sensory properties of the oil (El Ghannam et al., 2010). Therefore, encapsulation technology has been increasingly attracting the attention of food ingredient manufacturers to retard lipid oxidation and increase the range of applications of essential oils (Turasan et al., 2015).

Encapsulation is a technique that physically encloses the active ingredients which can be liquid droplets or solid particles, in a matrix to protect the active ingredients from any undesirable reactions and to provide stabilization and controlled release of the active ingredients. There is a number of techniques available for encapsulation of food ingredients. Spray drying is one of the oldest and the most extensively applied encapsulation technique for encapsulation of oils and flavours since it is flexible, continuous, but more importantly, it is an economical operation (Partanen et al., 2008; Lim et al., 2012). In addition, powder products obtained after spray drying process have good quality, low water activity, they are easier to handle and the coated ingredient is protected against undesired reactions. Microcapsules with low surface oil and high encapsulation efficiency can be obtained by appropriate processing conditions and with the choice of proper wall materials (Carneiro et al., 2013). In general, size and shape of the formed microcapsules depend on wall materials and the methods used to prepare them. Generally, a single coating material is not expected to meet all the features. It is, therefore, possible to mix more than one material. The most commonly used wall materials in spray drying are maltodextrin and gum Arabic (Carneiro et al., 2013). Maltodextrin has cost advantages compared to the other wall materials. It has a neutral taste, high solubility, and low viscosity and can be used in combination with other surface-active wall materials such as gum Arabic and modified starch in spray drying (Fernandes et al., 2008; Carneiro et al., 2013). Gum Arabic is the dried gummy exudation of high molecular polysaccharides obtained from the stems and branches of Acacia Senegal. Gum Arabic is a highly branched complex heteropolyelectrolytes formed principally by L-arabinose and D-galactose, and minor proportions of 4-omethyl-D-glucuronate, and L-rhamnose differing only in the ratio between 4:2:1:1 (Ray et al., 1995; Román-Guerrero et al., 2009). Moreover, gum Arabic contains a small amount of protein (1.0 % to 2.0 %) which contribute to the emulsifying and film-forming properties (Gonzales et al., 2012). EmCap® is a modified starch produced with spray-dried waxy maize starch ester. The properties of EmCap® include cold water dispersibility, providing emulsion stability, and low viscosity.

A few studies have been conducted recently to investigate the effect of wall materials on spray drying of various active ingredients (Gonzales et al., 2012; Ixtaine et al., 2015; Bakry et al., 2016). However, to the best of our knowledge, current study is the first research evaluating the effects of wall materials for spray drying of clary sage oil (*Salvia sclarea*). The main goal of this study was to investigate and optimize the effect of wall material composition containing maltodextrin, gum Arabic and modified starch-EmCap® on encapsulation efficiency, glass

transition temperature (T_g) and flavor retention of spray-dried clary sage oil. Optimum wall material combination yielding a volatile composition of the spray dried sage oil closest to the free oil was determined.

2. Material and Methods

2.1. Materials

Clary sage oil (Salvia sclarea) was provided from R.C. Treatt &Co. Ltd (Netherland) and stored at +4°C until use. The volatile composition of clary sage oil is presented in Table 1. Maltodextrin DE 12 (Tate & Lyle plc, UK), gum Arabic (Alland & Robert, France), and modified starch (EmCap®) (Cargill, FR) were used as wall materials. Moisture content, aw and Tg values of wall materials used in this study are presented in Table 2. Powder products which were obtained by using spray drying were packed with vacuum packaging and stored at +4°C. All chemicals used were analytical grade (Merck, Darmstadt, Germany).

Table 1. Composition of volatile organic compounds of free clary sage oil.

Ingredient	Area (%)
Linalyl Acetate	56.84
Linalool	26.89
α-Terpineol	3.45
β-Caryophyllene	2.33
Geranyl Acetate	1.85
Geraniol	1.06
Neryl Acetate	0.93
Myrecene β	0.73
Limonene D	0.67
Germacrene D	0.62
Cis-Ocimene	0.56
Trans-Ocimene	0.56
Nerol	0.48
β-Pinene	0.26
α-Pinene	0.25

Table 2. Tg, moisture content and a_w values of wall materials.

Wall Material	Moisture Content (%)	a_w	T_g (°C)
Maltodextrin	6.7	0.8	86.7
Gum Arabic	7.0	0.6	77.3
EmCap®	4.7	0.5	93.7

2.2. Emulsion preparation

Wall material solutions were prepared by mixing distilled water and wall materials using magnetic stirrer occupied with a temperature controller. The total soluble solid concentration was adjusted to 40 ± 1°Brix. Clary sage oil was added to solutions and emulsions were prepared by using a homogenizer (IKA T25 Ultra-Turrax®, Staufen, Germany) operating at 12,000 rpm for 5 min. Oil ratio was fixed in all emulsions as 8% and only the wall material composition was changed according to the experimental design (Table 3).

Table 3. Experimental design, glass transition temperatures (Tg) and composition of volatile organic compounds of spray dried clary sage oil samples.

Run	A: MD (%)	B: GA (%)	C: EmCap® (%)	T_g (°C)	Linalyl Acetate (Area%)	LA/ LO	LA/ α-T
1	92.5	5.0	2.5	72.1	44.1	1.4	11.3
2	100.0	0.0	0.0	56.4	48.8	1.6	12.8
3	90.0	5.0	5.0	73.5	34.8	1.1	8.5
4	80.0	10.0	10.0	87.6	38.5	1.2	9.4
5	95.0	2.5	2.5	54.9	40.0	1.3	9.8
6	85.0	10.0	5.0	57.2	36.8	1.1	9.0
7	100.0	0.0	0.0	56.9	45.9	1.4	10.4
8	95.0	5.0	0.0	62.6	51.6	1.6	12.0
9	90.0	10.0	0.0	56.7	45.3	1.3	10.1
10	95.0	0.0	5.0	56.9	42.7	1.1	9.1
11	90.0	0.0	10.0	57.2	39.2	1.0	7.3
12	80.0	10.0	10.0	57.2	38.9	0.9	6.9
13	85.0	10.0	5.0	57.7	33.2	0.8	4.7
14	90.0	10.0	0.0	56.7	37.0	0.9	6.9
15	85.0	5.0	10.0	55.7	43.2	1.1	7.6
16	90.0	0.0	10.0	64.5	41.9	1.1	8.4

Abbreviations: LA: Linalyl acetate, LO: Linalool; α-T: α-Terpinol

2.3. Microencapsulation by spray drying

Spray drying of emulsion was carried out using a laboratory-scale dryer (Mini Spray Dryer B-290, BÜCHI, Flawil, Switzerland) with a water evaporation capacity of 1 kg/h. Emulsion was fed into the drying chamber by a peristaltic pump and the flow rate was adjusted by the pump setting. Inlet and outlet temperatures were 150 ±2°C and 92±2°C, respectively and flow rate was 9±2 g/min. Spray dried powder accumulated in the collection vessel and brushed from the drying chamber were collected, packaged and stored at 4°C in air-tight containers until further analyses.

2.4. Experimental design

The D-optimal design was used with 3 factors and 2 levels, for investigation of the effect of wall material composition on encapsulation of clary sage oil with spray drying. Studied factors and working intervals were: maltodextrin ratio (80-100%), gum Arabic ratio (0-10%) and the modified starch ratio (0-10%) (Table 3). The levels of the factors studied were chosen on the basis of previous experiments and findings of the studies from the literature (Carneiro et al., 2013; Sachin et al., 2015; Ferreira et al., 2016;).

2.5. Powder analyses

2.5.1. Encapsulation efficiency

Total oil and surface oil were analyzed for determination of encapsulation efficiency.

2.5.2. Total oil

Total oil content in the powders was determined using a Clevenger hydro-distillation apparatus. In brief, 20 g of powder sample and 225 g of salt were dispersed in 500 g distilled water in 500 mL flask. A Clevenger oil trap and water cooling condenser were attached. The solution was slowly brought to boil and allowed to distill for 3 h (Beristain et al., 2001). The oil volume, read directly from the oil collection arm, was converted to weight by multiplying with its density. The analyses were performed in duplicate.

2.5.3.Surface oil

Surface oil extraction was carried out according to the method of Turasan et al. (2015) with minor modifications.[11] Surface oil in the spray-dried powder was determined with Soxhlet apparatus. Twenty grams of powder was placed in an extraction thimble, covered with glass wool and extracted with petroleum ether for 4 h. Each extract was completely dried under the fume hood and the surface oil was determined by weighing the residue. Encapsulation efficiency was calculated using the following formula (Jafari and Bhandari, 2007):

$$Encapsulation\ efficiency\ (\%) = \frac{Total\ oil\ content - Surface\ oil\ content}{Total\ oil\ content} \times 100$$

2.5.4. Moisture content and water activity (aw)

The moisture content of spray dried powder samples was measured gravimetrically, after drying the samples in a vacuum oven (EV018, Nüve, Ankara, Turkey) at 105°C for 24 h. Water activity (a_w) of powders was measured using an AquaLab VSA water activity meter (Decagon Devices, Inc., Pullman, WA, USA) at 25 ± 0.5°C.

2.5.5. Glass transition temperature determination (Tg)

T_g was analyzed using a differential scanning calorimeter (Q2000 DSC, TA Instruments, New Castle, DE, USA) which was equipped with a refrigerated cooling system. The equipment was calibrated by checking standard temperatures and enthalpies of fusion for indium and sapphire. The purge gas was dry nitrogen (50 mL min^{-1}). About 10 mg powders were weighed in Tzero™ aluminum pans and sealed hermetically. For the reference, an empty sealed pan was used in each test. The samples were heated from room temperature to 50°C and then cooled to −30°C. A second scan was conducted between −30 and 140°C with a heating rate of 10°C min^{-1}. The midpoint values for T_g of the samples were calculated using data analysis software (Universal Analysis 2000, TA Instruments) (Can Karaca et al., 2015).

2.6. GC-MS analysis of clary sage oil before and after spray drying

The chemical composition of clary sage oil was analyzed by GC-MS device (Agilent 7890B GC; Agilent 5977A MSD, USA). Quantitative analyses were conducted using FID with a capillary column (INNOWAX with the size 60m-0.25mm-0.25mm). Following GC-MS conditions were used during the analyses: carrier gas was Helium with a flow rate of 1 mL/min (constant pressure mode); split ratio of 1:80; 250°C injector temperature with 0.1 µL injection volume. Oven temperature was programmed from 70°C to 240°C with a ratio of 5°C/min; MSD transfer line temperature was 280°C; MSD quadrupole temperature was 150°C and MS source temperature was 230°C. Before the analyses, spray dried powder samples were dispersed in distilled water and mixed until complete powder dissolution. Oil was then extracted by adding 100 mL pentane/ether solution (1:1 v/v) for 1 min and triplicate extractions were performed. The clear organic phase was separated and filtered through anhydrous Na_2SO_4 on a Whatman Gr.1 filter. Pentane/ether was then evaporated off under a N_2 gas.

2.7. Statistical analysis

Statistical analysis was performed by applying ANOVA for mixture quadratic, linear and cubic models at 5% significance level ($p \leq 0.05$). Experimental design, data analysis, and response surface plots were prepared with Design-Expert 7 software (Stat-Ease, Inc., Minneapolis, MN, ABD).

3. Results and Discussion

3.1. Encapsulation efficiency

Encapsulation efficiency is an expression of the amount of clary sage oil incorporated into the microcapsules. A successful microencapsulation operation is expected to result in a product with minimum surface oil and maximum retention of the core material. Surface oil on microcapsules is undesirable as it can easily be oxidized and lead to the formation of undesirable off-flavors and decrease the stability and consumer acceptance of the product (Najafi, 2011). Higher encapsulation efficiency indicates a better protection of the core material by the wall material (Bakry et al., 2016). It has been reported that the choice of wall materials is very important for encapsulation efficiency and microcapsule stability in spray drying of essential oils (Ixtaine et al., 2015). Charve and Reineccius (2009) compared the performance of sodium caseinate, whey and, soy protein isolates with gum Arabic and modified starch for encapsulation of flavor compounds. The authors reported that the materials yielding the highest flavor retention during spray drying were gum Arabic (94%), modified starch (88%) and whey protein isolate (87%). In our study, the surface oil of powders varied between 0.2-0.7% and encapsulation efficiency ranged between 94.1% and 98.6%. There was no significant difference in encapsulation efficiency of the samples under the experimental conditions used (p>0.05) (Charve and Reineccius, 2009).

3.2. Moisture content and water activity (aw)

Moisture content is a critical parameter for spray dried oils as high moisture content may result in off-flavors in the product by promoting lipid oxidation (Klinkesorn et al., 2006). In our study, moisture content of spray dried clary sage oil varied between 0.8-1.7% whereas a_w changed between 0.11-0.20. For spray dried powders, moisture content below 3% and a_w lower than 0.3 indicate a stable product (Drusch and Schwarz, 2006).

3.3. Glass transition temperature (Tg)

Glass transition temperature is an important parameter for determining optimum processing conditions, storage stability and formulation of dried products (Roos and Karel, 1991). The suggested T_g of a stable product should be at least 20°C higher than the ambient storage temperature. T_g of spray dried clary sage oil powders varied between 54.9°C and 87.6°C (Table 3) which indicates that the spray dried clary sage oil will be stable at ambient storage conditions. Changes in wall material composition did not have a significant effect on T_g value of the products under the experimental conditions used in our study (p> 0.05).

3.4. GC-MS analysis of clary sage oil before and after spray drying

GC-MS analysis was performed to determine the effect of spray drying process on the concentrations of volatile organic compounds of clary sage oil. The volatile composition of free (unencapsulated) clary sage oil used in our study is presented in Table 1. The major aromatic

components of clary sage oil were determined to be linalyl acetate (56.8%), linalool (26.9%) and α-terpineol (3.5%) which constituted ~87% of the oil. Volatile composition of essential oils is affected by various factors such as geographical origin, climate and soil conditions, stage of the vegetative cycle, and seasonal variation (Callan et al., 2007). For example, Koutsaviti et al. (2016) reported that linalool acetate (37.6%), linalool (35.8%), and α-terpineol (11.0%) were the most important volatile components of clary sage oil from Greece. On the other hand, Kumar et al. (2017) reported the major volatile compounds of clary sage oil from the western Himalayas as linalool (31.2%), linalyl acetate (26.8%), linalyl propionate (10.0%) and geranyl acetate (5.1%); whereas Šulniūtė et al. (2017) reported the major volatile compounds of clary sage oil from Lithuania as sclareol (4240.6 mg/kg), linalyl acetate (410.9 mg/kg), and linalool (296.2 mg/kg).

Volatile composition of clary sage oil extracted from spray dried samples is presented in Table 3. Volatile compounds in free and encapsulated clary sage oil were found to be similar; however, there were some variations in the concentrations of individual components. Baranauskiene et al. (2006) encapsulated peppermint (*Mentha piperita* L.) oil by spray drying and investigated the properties of several commercial food starch based coating matrices. They determined the main volatile compounds in peppermint oil before and after spray drying. Similar to our study, they reported that the compositions of pure, emulsified and encapsulated peppermint oil were quite similar; however, they observed changes in concentrations of individual compounds.

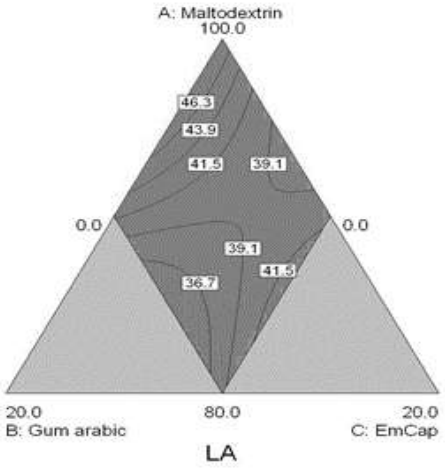

Figure 1. Model graph based on LA retention rates. (LA: linalyl acetate)

In our study, the amount of linalyl acetate (LA) in spray dried clary sage oil varied between 33.2-51.6% (Table 3). Retention of LA was affected by the composition of wall materials under the experimental conditions used (p<0.05). The model graph for retention rate of LA is presented in Fig. 1. Formulation which provided the highest retention rate for LA contained 95% maltodextrin and 5% gum Arabic.

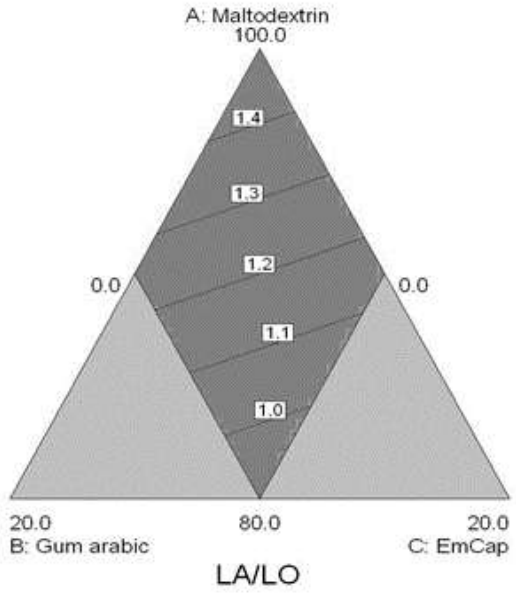

Figure 2. Model graph based on LA/LO retention rates. (LA: linalyl acetate, LO: linalool)

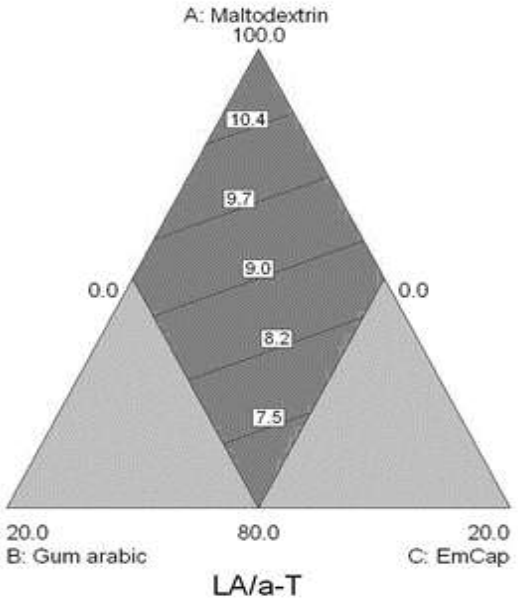

Figure 3. Model graph based on LA/α-T retention rates. (LA: linalyl acetate, α-T: α-terpineol)

In order to investigate the effects of the spray drying process on the major aromatic components of clary sage oil, not only their individual concentration but also the ratio between each other were examined. In free clary sage oil, LA/LO ratio was determined as 2.1, whereas LA/α-T ratio was 16.5. In case of clary sage oil extracted from spray dried samples, LA/LO ratio changed between 0.8-1.6 and was significantly affected by the wall material composition

(p<0.05). The model graph based on the LA/LO ratio of the spray dried powders is shown in Figure 2. Formulations which provided the closest ratio of LA/LO to the free clary sage oil contained >99% maltodextrin, <0.6% gum Arabic and <0.4% modified starch.

It was observed that the LA/α-T ratio was affected by the composition of the carrier material according to the results of the variance analysis (p<0.05). The model graph for LA/α-T ratio of the encapsulated powder products is shown in Figure 3. Formulations which provided the closest ratio of LA/α-T to the free clary sage oil contained >99% maltodextrin, <0.6% gum Arabic and <0.4% modified starch as also observed for LA/LO.

The proportions of LA/LO and LA/α-T in powder products were compared and the formulations closest to the natural composition of sage oil were obtained in Run 8. The formulation yielding to spray dried powder with volatile composition closest to the free sage oil was determined as Run 8 (95:5 mixture of maltodextrin:gum Arabic). Considering the retention rates of linalyl acetate, linalool, α-terpineol, as well as the ratios of LA/LO and LA/α-T; the formulation closest to the natural composition of clary sage oil after spray drying was obtained in Run 8. Based on these findings, it was determined that the carrier material composition that best preserve the aromatic composition of clary sage oil in the spray drying process was 95:5 maltodextrin:gum Arabic mixture.

4. Conclusion

In this study, it was aimed to evaluate the performance of different wall materials combinations in the clary sage oil microencapsulation by spray drying and to optimize the formulation that best protects the natural volatile composition of the clary sage oil. Amount of volatile compounds in free and encapsulated clary sage oil were evaluated. Volatile compounds in free and encapsulated clary sage oil were found to be similar; however, there were some variations in the concentrations of individual components. Moreover, moisture content, water activity, encapsulation efficiency, and glass transition temperature of the spray dried powder products were compared.

Considering the retention rates of linalyl acetate, linalool, α-terpineol, and the ratios of LA/LO and LA/α-T, the use of maltodextrin:gum Arabic (95:5) could be suggested as the most suitable wall material for microencapsulation of clary sage oil, for maintaining the natural aromatic composition of clary sage oil.

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Conflict of Interest

All authors declare that there is no conflict of interest.

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FULL TEXT–ORAL PRESENTATION

A NOVEL HERBAL COFFEE: OLIVE STONE COFFEE

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Abstract

Herbal coffees, which refer to the coffee product enriched with herbs, spices or other herbal ingredients, have become of the most popular types of coffee generally accepted by consumers, as the consumer's perspective on consuming the coffee product has begun to change, not only for "pleasure" but also by considering its health benefits such as reducing high caffeine consuming. Terebinth coffee (*Pistachia terebinthus* L.), black cumin coffee (*Nigella sativa* L.), carob coffee (*Ceratonia siliqua* L.), date kernel coffee (*Phoenix dactylifera*), Gundelia coffee (*Gundelia tournefortii* L.), chicory root coffee *(Cichorium endivia*), and dandelion root coffee (*Taraxacum officinale* L.), are of the widely consumed herbal coffees as a caffeine-free and healthy substitute to regular coffee. Herbal coffees help prevent certain illnesses thanks to several health benefits including reduced inflammation, decreased blood sugar, improved digestive health, increased the metabolism, stimulated cell growth, weight loss etc.

Along with the interest in consuming of herbal coffees, there is currently a huge demand for producing products from industrial and agricultural wastes around the world. In this regard, olive stone, an important by-product produced in the olive oil extraction and pitted table olive industries, is an attractive source of bioactive and rich source of valuable compounds e.g., cellulose, hemicellulose, lignin, polysaccharides, polyphenols, protein, fat, minerals and fibers. Olive stone coffee is a product innovation that consists of a mixture of special ingredients such as carob (*Ceratonia siliqua* L.), terebinth fruit (*Pistacia terebinthus* L.), cinnamon (*Cinnamomum ceylanicum* L.), clove (*Syzygium aromaticum* L.), vanilla (*Vanilla planifolia*) , as well as olive kernel (*Olea europaea* L.). This coffee has flavorful aroma and rich nutritional value, and also provides many health benefits in reducing the risk of stomach pains, gastritis, ulcers, hemorrhoids, intestine disorders, diabetes and autoimmune diseases which are well known for using in Traditional Anatolian Medicine for olive kernel.

Keywords: Herbal coffee, olive stone, novel product, by-product, *Olea europaea*

Introduction

Coffee is an excessively important agricultural product that provides the livelihood of an estimated more than 125 million people almost worldwide, especially in Latin America, Africa and Asia. More than 50 countries around the world, almost all of them in developing countries, produce and export approximately 9 million tons of coffee seeds annually. Although coffee is still extremely significant in the economies of many countries, dependence on coffee as the main foreign export earner has decreased in nearly all coffee exporting countries (Krishnan, 2017; ICO, 2021).

Figure 1. Coffee beans of *Coffea arabica* and *Coffea robusta*

Prepared from roasted coffee beans (*Coffea arabica* and *Coffea robusta,* in the Rubiaceae family), coffee is the most second consumed beverage after tea throughout the world (https://besttoppers.com/top-10-widely-consumed-drinks/) and has been a tradition for all the cultures on the worldwide since the first it explored in Yemen. In a short time, coffee containing addictive caffeine has become the indispensable beverage of humanity. However, various preparation techniques have been developed such as espresso, French press, cappuccino, frappe, cafe latte, Turkish coffee or brewed canned coffee and served like hot coffee, cold

coffee, and iced coffee (https://besttoppers.com/top-10-widely-consumed-drinks/). Related to huge interest and demand for coffee beans, production area throughout the coffee belt after the 17th century and global market size have astonishingly increased in this period. Nowadays the biggest coffee producer countries are Brazil, Vietnam, Colombia, Indonesia, Ethiopia, Honduras, Uganda, India, Mexico and Peru. Among the top ten producers, Brazil, Vietnam and Colombia together produce and export about 60% of the total globally (Alasmari et al., 2020; Gupta et al., 2020; ICO, 2021).

Coffee production and its trade has been cut or interrupted by climatic changes, wars and the political reasons during the its short history. Because of restrictions and shortage, however; people have tended to discover alternative coffee substitutes with similar taste. Apart from their distinguished taste and aroma of the novel coffee substitutes, they were rich in bioactive compounds, having low in caffeine content and no addictive compounds. This tendency has crucial contributed to emerged the term of "Herbal Coffee" among the people. Herbal coffee was the first time defined as hot drinks, which are prepared by roasting different parts of various plant parts, other than coffee beans, till brown color, grinding and cooking like Turkish coffee by Sekeroglu (Sekeroglu, 2012; Sekeroglu et al., 2012; Gezici and Sekeroglu, 2019; Sekeroglu et al., 2020; Sekeroglu and Gezici, 2020; Gezici and Sekeroglu, 2021).

Herbal Coffees: Functional and Healthy Hot Drinks

Plant-based, caffeine-free and healthier hot drinks as coffee substitutes, herbal coffees have gained increasing popularity around the world, recently. These novel coffee substitutes are produced from different plants parts such as root, tuber, fruit, seed, kernel etc., and they are made and served like traditional methods in similar to true coffee; they are roasted, ground, cooked and served. Up to now, there are many different herbal coffees have traditionally been discovered such as acorn coffee (*Quercus coccifera* fruits), black cumin coffee (*Nigella sativa* seeds), brown rice coffee (*Oryza sativa* seeds), carob coffee (*Ceratonia silique* pods), chicory coffee (*Cichorium intybus* roots), date kernel coffee - Café de noyaux de dattes (*Phoenix dactylifera* kernels), Gundelia coffee (*Gundelia tournefortii* fruits), terebinth coffee (*Pistachia terebinthus* fruits), chickpeas coffee (*Cicer arietinum* seeds) and barley coffee - Caffè d'orzo (*Hordeum vulgare* seed) around the world. Herbal coffees are natural, healthy and functional drinks which are produced by traditional methods with no chemical conservatives and additives. Herbal coffees mentioned above, except brown rice and chicory coffees, have been traditionally produced and consumed in Turkey for a long time. These novel, healthy and functional drinks, for example terebinth coffee, have been produced in industrial scale in Turkey and marketing from Turkey to throughout the world in recent years (Sekeroglu et al.,

2012; Febrianto et al., 2016; Sekeroglu et al., 2017; Gezici and Sekeroglu, 2019; Gezici and Sekeroglu, 2021).

Figure 2. Herbal coffee as caffein free hot drinks

Olive Tree and Olive Stone

Olive tree (*Olea europaea* L., fam: *Oleaceae*) is an ancient tree that is also called as a natural drug. It is a well-known medicinal plant used traditionally for medicinal purposes thanks to its wide range of biological properties including antioxidative, antimicrobial, antidiabetic, anti-inflammatory, antinociceptive, gastroprotective, and enzyme inhibition activities. Olive fruit, olive-oil and the leaves of olive tree are also used in food additives, pharmaceuticals, nutraceuticals, cosmetic purpose, aromatherapy, and other industries (Moubarik et al., 2015; Gouvinhas et al, 2017). Even though various studies have been conducted to reveal medicinal properties of olive fruit, olive-oil and olive leaves, a few studies have performed with olive stones that contains valuable bioactive components. The olive stone and seed are important

products for olive industries. As a lignocellulosic material, the hemicellulose, cellulose and lignin are the main components of olive stone as well as protein, fat, phenols, free sugars and cellulose, hemicellulose, lignin and polyphenols. Potential uses of olive stone are summarized as given below (Alcaide and Nefzaoui, 1996; Rodríguez et al., 2008; Moreno-Castilla et al., 2001; Gouvinhas et al., 2017; Cheboub et al., 2020);

- a metal biosorbent
- a plastic filled
- an abrasive
- a dietary animal supplementation
- a source of phenols for phenol–formaldehyde resins
- a source of fiber and antioxidant
- a source for cosmetic industries
- s source for furfural production

Figure 3. Olive tree, olive fruit and kernel

Although by-product of the olive fruits after olive oil or breakfast olive production, olive kernel is one of the most valuable part of this medicinal plant. The olive fruit can structurally be separated into the following three parts (Rodríguez et al., 2008):

- the skin, called epicarp (1.0–3.0% of the drupe weight), which contains the chlorophyll, carotenoids and anthocyanins that account for the color;
- the pulp or flesh, called mesocarp (70–80% of the whole fruit), is the major part of the olive and is the reserve supply of all the constituents;
- and the stone, called the woody endocarp (18–22% of the olive weight), which contains the seed.

Olive Stone Coffee

The Mediterranean region supplies 97% of the total olive production worldwide, with the olive oil industry a momentous activity that produces 95% of the world's olive oil. Over the past decade, olive oil production has been growing globally at a rate of about 4% per year, and the International Olive Council predicts that this rate may raise in the coming years. The growing will be raised from increasing demand for olive oil and other olive products in both existing and new markets as their health benefits become more widely recognized. However, large amounts of waste and by-products such as olive kernels and flowers are formed in the olive oil production process. In other words, the direct disposal of olive stones (kernels) causes a major environmental problem since its high organic and polyphenol content, and this is also toxic to water and soil ecosystems. Therefore, the production and re-evaluation of new products from these by-products is of great importance (Rodríguez et al., 2008; Elbir et al., 2015, Moubarik et al., 2015; Chanioti et al., 2016; Farag et al., 2020).

Olive kernel is a rich source of valuable compounds e.g., cellulose, hemicellulose, lignin, polysaccharides, polyphenols, protein, fatty oil, minerals and fibers have attracted scientists (Rathinavelu and Graziosi, 2005; Elbir et al., 2015; Echeverria et al., 2017). However, polyphenols are one of the most important groups of natural antioxidants and they help protect the organism to provide an effective defense system against free radical attacks through different mechanisms. Thus, olive kernel has been an important folk medicine in the Mediterranean cultures. The kernel has been swallowed for many ailments such as gastrointestinal disorders, stomach and intestine problems, mainly. Recent scientific data have revealed the presence of bioactive component such as oleuropein, leuropein, hydroxytyrosol, verbascoside, lutein, and rutin in the olive kernels, which could be a novel natural source in order to develop novel natural drugs (Rathinavelu and Graziosi, 2005; Rodríguez et al., 2008; Moubarik et al., 2015; Echeverria et al., 2017).

Figure 4. An innovative novel product: olive kernel coffee

However, some of the entrepreneurs developed a novel functional drink from this by-product, and this novel coffee substitute was Olive Kernel Coffee. Olive kernels (*Olea europaea* L.) were separated from fruit parts, roasted and some herbal ingredients such as carob (*Ceratonia siliqua* L.) powder, terebinth fruit (*Pistacia terebinthus* L.), cinnamon (*Cinnamomum*

ceylanicum L.), clove (*Syzygium aromaticum* L.), and vanilla (*Vanilla planifolia*) were added into olive kernel coffee in order to soften and add flavor. Thus, this caffeine-free novel herbal coffee with its distinguished aroma has been produced in industrial scale, marketed and soon after it won the appreciation of the consumer. Besides flavorful aroma and rich nutritional value, this coffee has a remarkable potential to provide many health benefits in reducing the risk of stomach pains, gastritis, ulcers, hemorrhoids, intestine disorders, diabetes and autoimmune diseases.

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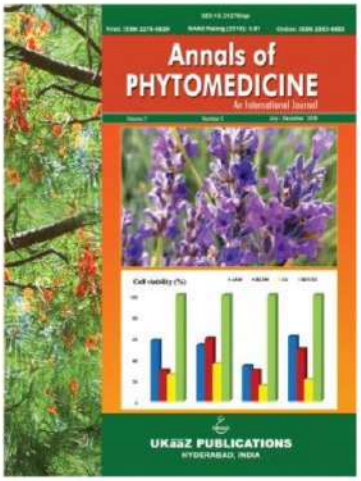

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