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6MESAP

The Sixth International Mediterranean Symposium on Medicinal and Aromatic Plants

October 15-17, 2020 / Izmir - TURKEY

Türkiye

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MESMAP – 6

PROCEEDINGS BOOK

ABSTRACTS & FULL PAPERS

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Dear Colleagues,

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

MESMAP-2 Symposium was held on April 22-25, 2015 in Antalya – TURKEY, which was organized by academicians from Gazi University (TURKEY), Gaziantep University (TURKEY), Kilis 7 Aralık University (TURKEY), Yüzüncü Yıl University (TURKEY), Association of Pharmaceutical Teachers of India (APTI – INDIA) joint with AMAPMED (Association of Medicinal and Aromatic Plants of the Mediterranean). INDUSTRIAL CROPS AND PRODUCTS JOURNAL with high impact factor from ELSEVIER group published a special issue covering some of the full papers selected after scientific evaluation.

MESMAP-3 Symposium which was held on April 13-16, 2017 in Girne (Kryneia) – Turkish Republic of Northern Cyprus (TRNC), was the third event of MESMAP symposium series on Medicinal and Aromatic Plants. After scientific evaluation selected full papers published in Indian Journal of Pharmaceutical Education and Research (IJPER), indexed with THOMSON REUTERS. MESMAP-4 Symposium, which was held on April 18-22, 2018 in Sherwood Breezes Resort Hotel Antalya – Turkey, was the forth 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 Cappadocccia 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.

This symposium was the sixth meeting series of MESMAP, and you can find abstracts of all the scientific works presented in MESMAP-6 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. MESMAP-6 has been supported by TÜBİTAK 2223-B National Scientific Meetings Grant Program. 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.

We would like to thank for their sincere supports of Turkish General Directorate of Forestry, TURKISH AIRLINES, TÜBİTAK, Kilis 7 Aralık University, Khon Kaen University, Kumamoto University, Rural Federal University of Rio de Janeiro (UFRRJ)-Brazil, AMAPMED, Association of Pharmaceutical Teachers of India, Cosmetic Producers and Researchers Associatons (KUAD), Talya Herbal Company, Torbalı (Izmir) Chamber of Commerce-Turkey, NS Herbals and all the other supporters.

Organizing Committee hope that MESMAP-6 Symposium participants would have an amazing experience and unforgettable memories to take back their homes. We would like to thank to all our participants from almost all over the world for their valuable attendance and scientific contribution to MESMAP-6, due to situation of COVID-19 pandemic. We are planning to organize the seventh meeting series of MESMAP in 2021autumn as face-to-face. It will be a big honor for us to see you again at MESMAP-7 internetional symposium.

Sincerely,
Symposium Chairman
Prof. Dr. Nazım ŞEKEROĞLU
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INVITED

SPEAKERS

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INVITED SPEAKER

MECHANISMS OF MULTIDRUG RESISTANCE AND ITS REVERSAL IN HEMATOLOGICAL MALIGNANCIES

Prof. Dr. Yusuf Baran

Izmir Institute of Technology, Faculty of Science, Department of Molecular Biology and Genetics, İzmir, Turkey
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Chemotherapy is the most widely used treatment strategy for cancer which is the highest second reason for humanbeing deaths after heart related diseases. However, cellular resistance mechanisms developed by cancer cells and tissues in the beginning or proceeding times to applied anticancer agents is a significant problem preventing succesfull therapy. Resistance developed by cancer cells to structurally and functionally different cytotoxic agents is called as multi drug resistance. The resistance can be observed in the beginning of the treatment or during the treatment known as intrinsic or acquired resistance, respectively. The resistance phenotype is associated with the tumor cells that gain a cross-resistance to large range of drugs that are structurally and functionally different.

Drug resistance mechanisms have different molecular genetics and biochemical reasons depending on the applied drug and the type of cancer. Secondary genetic alterations and disorders in cancer cells may also result in drug resistance. That is why it has vital importance to study and consider all signaling pathways, in multidrug resistance of cancer.

Multidrug resistance raises via many unrelated mechanisms, such as overexpression of energy-dependent efflux proteins, decrease in uptake of the agents, increase or alteration in drug targets, alterations in cell cycle checkpoints, inactivation of the agents, compartmentalization of the agents, inhibition of apoptosis, increases in DNA repair mechanisms, problems related with drug metabolism and aberrant metabolism of bioactive sphingolipids. Exact elucidation of resistance mechanisms and molecular and biochemical approaches to overcome multidrug resistance have been a major goal in cancer research. In this talk, we will explain the mechanisms contributing multidrug resistance in cancer chemotherapy and also touche on the approaches for reversing the resistance.

INVITED SPEAKER

ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME) OF DIETARY FLAVONES AND THEIR GLYCOSIDES

Prof. Dr. Randolph RJ Arroo

Leicester School of Pharmacy, De Montfort University, The Gateway, Leicester LE1 9BH, UK
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Epidemiological studies have long indicated a possible role for dietary flavonoids, notably flavones and flavonols, in the prevention of a range of degenerative diseases, e.g. cancer, diabetes, cardiovascular diseases and neurological disorders like Parkinson's and Alzheimer's disease. The flavonoids are a large and variable group of compounds, comprising thousands of different structures. The bulk of the dietary flavonoids occur as glycosides.

The effect of flavonoid aglycones and their corresponding glycosides on cell metabolism and aetiology of degenerative diseases has been a topic of interest for a number of decades. In contrast, the role of the metabolic products of dietary flavonoid that reach all parts of the human body through systemic circulation, has received much less attention.

Many detailed ADME studies focus specifically on quercetin and its glycosides, due to their abundant availability. Dietary quercetin occurs almost exclusively as β-glycosides, where the aglycone can be bound to a variety of sugars depending on the food source, e.g. apples are rich in galactosides, rhamnosides, and arabinosides, whereas in onions glucosides are the main glycosidic form. The sugar moiety was shown to be an important determinant of the bioavailability of quercetin from foods. The compounds that directly play a role in the prevention of degenerative diseases are most likely not dietary flavones themselves, but rather their metabolites and conjugation products.

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INVITED SPEAKER

AN INTEGRATED GIS STUDY OF MEDICINAL BARLEY FROM TURKEY AND HIMALAYA WITH PHYTOCHEMICAL EVALUATION TO CONTROL INTESTINAL FLORA OF HUMAN

Takashi Watanabe[1*], Faruk Karahan[2], Nazim Sekeroglu[3] and Raju Aedla[4]

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Recently, due to change the cultivation of vast crops in the Turkey, the overall existence of local inhabitants of the area has been poor soil condition. According to statistics of Turkey, the main crop Barley is changing with other crops. At the same time, lack of awareness of people about biodiversity, conservation, sustainable usage, *etc*. creating this non-repairable loss in agricultural system. Turkey is one among the hotspots of environmental degradation. The local people are economically disadvantage and completely depending on agriculture activities. Therefore, its urge to cultivate in a suitable areas where it's naturally grow for ecological stability. In this vision, we proposed this project for cultivation and preservation of medicinal crops in Turkey and Himalaya[1,2]. The research was carried with analyzing correlation between the growth environment and hereditary characteristics of "Barley" (*Hordeum vulgare* L.). Also the morphological differences among the plant leaves were examined with respect to the geographical variations.

The research on cultivation of Turkish crops, the root of native cereals (Barley in this presentation) will be conducted and monitored for natural farming. In particular, from the Turkish Mediterranean region to the Himalayan region will be carried out by examining the components (contents like resistant starch) using remote sensing and GIS technology. The spatial thematic maps will be prepared based on cultivation growth and component fluctuation in the investigated fields. Also, the anti-obesity and intestinal flora of the target component using the above-ground samples at each stage of harvest will be investigated. In addition, lifestyle-related diseases relating to various habitat Barley will be analyzed. Based on results of investigation, the origin of varieties of Turkish barley will be elucidated and cultivation in a suitable farmland survey will be conducted using remote sensing and GIS.

Keywords: Endemic barley, resistant starch, GIS, soil, Turkey

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INVITED SPEAKER

EFFECT OF MIROESTROL ON OVARIECTOMY-INDUCED BEHAVIORAL ALTERATION AND ITS UNDERLYING MECHANISMS

Assoc. Prof. Dr. Yaowared Chulikhit

Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, KhonKaen University, KhonKaen, 40002, Thailand
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Miroestrol (MR) is a phytoestrogen isolated from tuberous roots of *Pueraria candollei* var. *mirifica* (KwaoKrueaKhao), a Thai medicinal plant used for rejuvenating purpose in aged people, which correlated with its pharmacological activities reported by pre-clinical and clinical trials. MR is a unique chromene with potent estrogenic activity. Although this compound is only present in low amounts in the plant, it plays an important role in the estrogenic action of *P. candollei* products. In the present study, we examined the effects of MR on depressive behavior, cognitive function, serum estradiol level, size and volume of uterus, oxidative brain damage, and the expression of genes encoding brain-derived neurotrophic factor (BDNF) and cyclic AMP-responsive element-binding protein (CREB), factors implicated in neurogenesis and synaptic plasticity, in ovariectomized (OVX) mice. The ovariectomy induced depression-like behavior in forced swimming test (FST) and tail suspension test (TST) and also impaired object recognition performance in the novel object recognition test and spatial cognitive performance in the Y-maze test and the water maze test. MR treatment, as well as 17β-estradiol treatment, attenuated these symptoms. OVX mice showed the significantly decreased in serum 17β-estradiol level and uterine weight and volume. Moreover, OVX mice had a significantly increased thiobarbituric reactive substance, decreased antioxidant enzyme activities and down-regulated expression levels of BDNF and CREB mRNAs in the hippocampus and frontal cortex. MR treatment as well as hormone replacement therapy with 17β-estradiol significantly reversed these neurochemical alterations caused by estrogen deprivation. In addition, the miroestrol docking study on SERMs/ERα interaction was performed to investigate the potential binding modes of the test compounds; miroestrol, isomiroestrol, deoxymiroestrol and 17beta-estradiol. The alpha estrogenic receptor (ERα) template prepared from a crystal structure of Human ERα bound with a selective estrogen receptor modulator (SERM) dihydrobenzoxathiin analogue, 1XP1 was used in the docking study. Miroestrol had the interaction to the ERα with three hydrogen bonds and exhibited the free binding energy (ΔG^o)-10.33 Kcal/mol. Our findings raise the possibility that MR may have a beneficial effect on depression and cognitive deficits in which menopause/ovariectomy are implicated as risk factors. The effects of MR are mediated by attenuating the brain oxidative damage and synaptic plasticity-related signaling systems and neurogenesis in the hippocampus. MR also showed the strong interaction with ERα from the docking study.

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INVITED SPEAKER

MEDICINAL AND AROMATIC PLANTS (MAPS) DIVERSITY IN ALBANIA – CHALLENGES FOR THE FUTURE

Prof. Dr. Alban Ibraliu

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Abstract
Medicinal and Aromatic Plants (MAPs), which widely occur in Albania, comprise an important natural economic resource, not totally and sustainably exploited yet. More than 400 species of medicinal and aromatic plants (MAPs) belong to the Albanian flora which occurs in the wild state. Sixty-eight medicinal species are considered of the endangered status and 40 wild MAPs are included in the National Red Data Book About 182 of these species are rather widespread and many of them are harvested and exported. Cultivation of MAPs is a very effective means, if not the most promising one, to satisfy the market's expanding demand in future, while reducing or eliminating pressure on their wild populations. The exports, mainly to the EU and US, have increased over the years. Some of these challenges are related to evaluation of the MAPs genetic material, production and post-harvest technology and practices on the farm, safety and quality standards and traceability.

Keywords: Medicinal and aromatic plants, albania, cultivated medicinal and aromatic plants, value chain, market

INVITED SPEAKER

STABILITY OF QUERCETIN IN DULBECCO'S MODIFIED EAGLE'S MEDIUM

Prof. Dr. Jianbo Xiao

Institute of Chinese Medical Sciences, University of Macau, Macau, China
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Abstract

The bioavailability of most flavonoids is very low, posing concerns on their real efficacy in promoting health benefits. Most importantly for food industries, a low stability caused for example by heat, pH, light, oxygen, pre-systemic metabolism limits the exploitation of flavonoids as functional ingredients, natural antioxidants, and supplements. Quercetin with pyrogallol structure in ring B are immediately transferred to their stable dimers and oxidized products in PBS (pH=7.4) at 4 ºC in air within 5 seconds. Quercetin is evidently instable in Dulbecco's modified Eagle's medium (DMEM) at 37 °C. However, the underlying mechanism of this instability is not clear yet. The stability and new products of quercetin in DMEM at 37°C were investigated via an *in situ* analysis via UPLC-MS-MS. Quercetin was instable in DMEM and formed various degradation products derived from its dimer with increasing incubation time and there are many isomers formed during this process. Ascorbic acid (100 µM) improved the stability of quercetin by protecting quercetin from auto-oxidation in this medium. Ascorbic acid significantly improved the stability of quercetin when incubated with A549 cells. Ascorbic acid (100 µM) obviously enhanced the inhibition of quercetin against A549 cells via enhancing the stability of quercetin in cell culture.

Keywords: Quercetin; stability; dimmer; ascorbic acid; A549 cells.

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INVITED SPEAKER

BIOLOGICALLY IMPORTANT COMPOUNDS DERIVED FROM THE NATURAL FUNGAL METABOLITE "KOJIC ACID"

Prof. Dr. Mutlu Aytemir

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Kojic acid is a natural metabolic product commonly produced by many species of *Aspergillus*, *Acetobacter*, and *Penicillium* during aerobic fermentation using various substrates [1, 2]. It is also produced from the fermentation of some Asian foods (e.g soy sauce and rice wine), which acts as a primer for fungus or inoculum [3]. The presence of kojic acid was first reported during the researches on the fermentation of steamed rice "koji", where its name derived. Later, its chemical structure was extensively investigated and defined as 5-hydroxy-2-hydroxymethyl-γ-pyrone [1, 4]. Kojic acid is a good chelator of transition metal ions and a good scavenger of free radicals. In addition, it is the inhibitor of the tyrosinase, which is the key enzyme in pigment biosynthesis. It inhibits the tyrosinases of various *Aspergillus species*, *N. crassa* and mushrooms, as well as those of some plants and crustaceans by chelating copper at the active site [5]. Abnormal production of melanin causes many problems, especially malign melanoma, hyperpigmentation, post-inflammatory pigmentation, melasma and skin aging. Therefore, the inhibiton of tyrosinase is the main subject in hyperpigmentation disorders, as well. However, the cytotoxicity, chemical instability and low lipophilicity of kojic acid limited its use [6]. The hydroxypyrone scaffold of kojic acid has an excellent structure, which has been used as a powerful tool in drug discovery. Many derivatives have been discovered until today and have applications in medicine, cosmetics, agriculture, food and chemical industry. Mannich bases of kojic acid have been studied for many years in our laboratory. Their various biological actions including anticonvulsant, anticancer, antibacterial, antifungal, anti-mycobacterial, anti-aging, antioxidant, antityrosinase and antiviral activities with cytotoxicity were evaluated [7-13]. Promising results were submitted to Turkish and International Patent (TR2017/20155 and PCT/TR2018/050724). Further studies are ongoing to develeop more potent and safer derivatives of kojic acid.

Keywords: Kojic acid, mannich bases, antityrosinase, anticancer.

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INVITED SPEAKER

PLANT PHENOLICS AS ANTIOXIDANTS AND ENZYME INHIBITORS: CHARACTERIZATION AND BIOACTIVITY ANALYSIS

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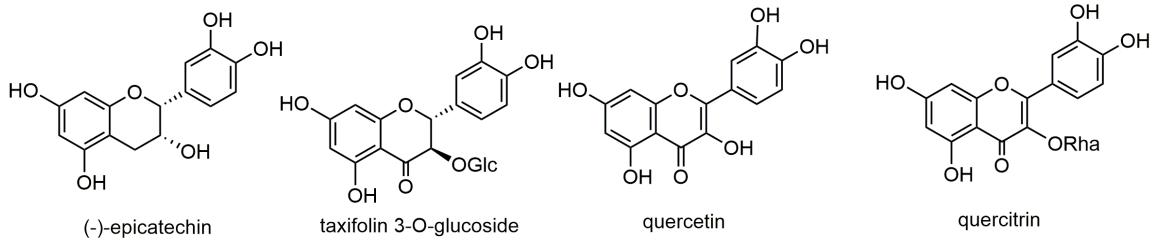
Abstract
Medicinal plants have played an important role in the maintenance of human health as the essential ingredients of traditional medicines for thousands of years. It is reported that more than 80% of the world population still relies on medicinal plants as a source of primary healthcare. Natural products isolated from medicinal plants have also played an important role as a source of modern drug discovery and development. Medicinal and aromatic plants are also widely used as foods, spices, and also used in cosmetics, aroma, perfumes. Phytochemicals isolated from plants also have more extensive applications as food preservatives, colourants, sweeteners, etc. In recent years, there is significant increase in the development of plant-based nutraceuticals and functional foods for maintaining proper health condition and prevention from diseases. Our current research is focused on the isolation and identification of potent bioactive molecules from medicinal plants and development of plant based functional foods.

During our preliminary survey of medicinal plants of Kyushu Island in Japan, extracts of various medicinal plants showed potent antioxidant activity and inhibitory activity against enzymes related to metabolic diseases. Detailed chemical analyses and characterization of bioactive compounds from these extracts have resulted into the isolation of several phenolic compounds with potent biological activities. For example, (-)-epicatechin, taxifolin 3-*O*-glucoside, quercetin and quercitrin from the leaves of *Lindera sericea* Blume (Lauraceaae) showed potent antioxidant activity and taxifolin 3-*O*-glucoside, quercetin and quercitrin showed potent α-glucosidase inhibitory activities. Similarly, petasiphenol, rosmarinic acid and quercetin from the flowers of *Farfugium japonicum* (L.) Kitam. var. *giganteum* (Siebold et Zucc.) Kitam. (Asteraceae) showed potent antioxidant activity and petasiphenol and quercetin showed potent tyrosinase inhibitory activity.



Keywords: Phenolics, flavonoids, functional foods, *Lindera sericea*, *Farfugium japonicum*

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INVITED SPEAKER

HONEY BEE PRODUCTS AND THEIR RELATIONSHIP WITH FLORA

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Abstract

A symbiotic relationship between plants and bees is a well-known fact: plants serve as food and shelter for bees, and bees help plant reproduction, and biodiversity. Diversity among species, including agricultural crops, medicinal and aromatic plants rely on bee pollination to some extent. Nearly 16% of the world's flowering plant species are pollinated by bees. Bees supply carbohydrates from nectar and proteins from pollen, and in return, they help plants to cross-pollination, increase the genetic variety, and ensure the generation. There are around 20,000 distinct bee species around the world. *Apis mellifera* L. (European/western honeybee) is particularly well known as a honey producer, but also as an important pollinator of many crops and wild plants. Although, it is referred to as honey bee, there are at least eight other honey bee species in the *Apis* genus. Apart from its native populations, *A. mellifera* is managed by beekeepers for mainly honey production, and other beehive products like bee pollen, royal jelly, propolis and beewax for years. Recently, bee bread, a diet for bee larvae and young bees, appears in the market as a functional food. All these products have beneficial effects on human nutrition and health, as they contain various useful and bioactive compounds arised from plant sources visited by bees. For instance, bee pollen (agglutinated form of flower pollens) made by worker honey bees, using nectar and salivary secretions, contain carbohydrates, crude fibers, proteins, lipids, basic amino acids, carotenoids, phenolics, flavonoids, sterols, terpenes, vitamins and minerals.

Honey bees provide main nutients from plants, carbohydrates and water from nectar, and proteins, lipids, vitamins and minerals from pollen. They also forage in compound-rich, 'dirty' water for their essential minerals since main floral diet only contains trace amounts of micronutrients. Composition and quality of both, diet of honey bees, and their attracting commercial products mainly depend on the floral and water resources, geographic origin, climate, soil, and to some extent, on beekeeping activities like handling, transportation and storage conditions. Besides, honey bees alter their foods, thereby quality of their products, according to the season. For example, phenolic and flavonoid contents, and antioxidant activity of autumn propolis are higher than those of spring propolis. Unfortunately, decline or disappear (colony collapse disorder) of honey bees arising from combination of various diseases, climate change, loss of habitats, environmental pollution, and farming practices mainly due to toxic phytosanitary products and large monoculture croping, is a serious threat. As a result, yield and quality of honey bee products may decline and/or contaminants arised from environmental pollution and detrimental beekeeping practices may exist in these products. This presentation covers the close relationship between honey bee products and flora, with all dimensions. Urgent implementations to create more sustainable ecosystems, and improve food security and nutrition will be discussed in detail.

Keywords: Honey bee, honey bee products, bee flora, nutrition, pollination, beekeeping

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INVITED SPEAKER

ANTIMICROBIAL AND IMMUNE STIMULATING USE OF PLANTS IN VETERINARY MEDICINE

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Objective / Purpose: The relatively recent "One Health, One Welfare" concept connects humans and animals in the same health sphere, stressing the similarities between them as hosts for infectious agents, also shearing species specific, but to some extent overlapping immune responses to pathogens. Phylogeny depending structure, integrity and responsiveness of the immune system is essential in preserving the health of the host during microbial aggression and along history, drugs were sought amidst natural resources. Medicinal and aromatic plants have been used for millennia in traditional healing practices in humans. In some countries, ethno-botanicals represented the only easily accessible treatment for small communities, which had to turn to their living environment for medicines showing antimicrobial and in some cases, immune-enhancing activity (Mbuni et al., 2020). Similarly, once effective in treating humans, plants as such, or their extracts were used to treat animals raised by those communities.

This work aimed to comparatively assess the effects of different plant families, containing various chemical compounds and review their antimicrobial and immunological activities. The plants with such synergistic effects could be of help, especially in areas where the microbiome is resistant to antibiotics, with and increased MAR (multiple antibiotic resistance) index, as it is the case in farmed animals or pets. Similarly, the immune stimulating activity of certain plant-derived compounds could support their use as adjuvants for veterinary vaccines. Such plants are ranked for their overall medicinal usefulness.

Conclusion / Discussion: The investigation of biological effects of plants in veterinary medicine is far from being closed, the antimicrobial and immunological activity of different plants, as facets of the same complex healing potential plants could offer, need further in-depth interdisciplinary studies.

Keywords: medicinal plants, antimicrobial effect, immunity enhancers, veterinary medicine

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INVITED SPEAKER

IMMUNOMODULATORY EFFECTS OF MEDICINAL PLANTS AND NATURAL PHYTOCHEMICALS IN COMBATING COVID-19

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SARS-CoV-2 (severe acute respiratory syndrome caused by coronavirus-2), a newly discovered coronavirus, has led a worldwide pandemic named as COVID-19 by WHO. From ancient times, nature has always been attractive to scientists for the treatment of many diseases and disorders as alternative therapy. Taking into consideration that many medicinal plants and secondary metabolites with antiviral activity may shed a light on dealing with COVID-19 and effective drug development. The most effective medicinal plants known as immune system boosting are *Acanthopanax gracilistylus* W.W. Smith (Araliaceae) cortex, *Aesculus hippocastanum* L. (Hippocastanaceae), *Angelica archangelica* L. (Apiaceae), Astragalus *membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (Fabaceae) radix, *Bupleurum chinensis* DC. (Apiaceae) radix, *Cedrela sinensis* Juss. (Meliaceae), *Cimicifuga racemosa* (L.) Nutt. (Ranunculaceae), *Cinnamomum verum* J.S. Presl. (Lauraceae) cortex, *Cupressus sempervirens* subsp. *pyramidalis* (O. Targ. Tozz.) Nyman (Cupressaceae), *Forsythia suspensa* Vahl. (Oleaceae), *Geranium macrorrhizum* L. (Geraniaceae), *Glycyrrhiza uralensis* Fisch. and *Glycyrrhiza glabra* (Fabaceae), *Hyssopus officinalis* L. (Lamiaceae), *Juniperus oxycedrus* L. subsp. *oxycedrus* L., Laurus nobilis L. (Lauraceae), *Melia azedarach* L. (Meliaceae), *Melissa officinalis* L. (Lamiaceae), Mentha piperita L. (Lamiaceae), *Nepeta cataria* L. (Lamiaceae), *Nigella sativa* L. (Ranunculaceae) seeds, *Origanum vulgare* L. (Lamiaceae), *Pistacia palaestina* Boiss. (Anacardiaceae), Potentilla arguta Pursh. (Rosaceae), *Rhodiola rosea* L. (Crassulaceae) roots, *Salvia officinalis* L. (Lamiaceae), *Salvia officinalis* L. (Lamiaceae), *Sambucus nigra* L. (Adoxaceae), *Sambucus racemosa* L. (Adoxaceae), *Sanguisorba officinalis* L. (Rosaceae) radix, *Satureja thymbra* L. (Lamiaceae), *Sophora flavescens* Aiton (Fabaceae) radix, *Thuja orientalis* L. (Cupressaceae), *Thymus vulgaris* L. (Lamiaceae), *Torilis arvensis* (Huds.) Link (Apiaceae), and *Tribulus terrestris* L. (Zygophyllaceae). Most of the active compounds and molecules (e.g. quercetin, lycorine, hesperetin, emodin, glycryrrhizin, curcumin, chrysanthemum B, betulinic acid, hirsutenone, xanthoangelol E, myricitrin, licoleafol, methyl rosmarinate, calceolarioside B, glucopyranoside, amaranthine, and etc.) isolated from medicinal plants seem to be quite promising towards SARS-CoV-2 in both enhancing immune system and developing novel antiviral drugs. This presented review has emphasized that herbs and natural bioactive compounds already reported with antiviral effects against various types of viruses including coronaviruses could help to strengthen the immune system towards SARS-CoV-2 infections.

Keywords: Coronovirus, COVID-19, phytochemicals, herbs, antiviral, immune booster, phytotherapy

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INVITED SPEAKER

STANDARDIZED *Litsea glutinosa* (Lour.) UPREGULATES mRNA EXPRESSION OF ANTIOXIDATUVE ENZYMES AND NORMALIZES IgE LEVELS TO CONTROL DIARRAHEAL INCIDENCES

Prof. Dr. Atiar Rahman

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Medicinal plants have been paid an utmost attention for treating several different diseases due to their accessibility, availability, inherited practice, economic feasibility, and perceived efficacy. This research investigated how the antioxidative potentials of *Litsea glutinosa* controls the diarrheal incidences in albino rat model. Extraction, standardization, purification and GC-MS analysis of *L. glutinosa* leaf were undertaken followed by an evaluation of antioxidative properties of the extract. Extract was further subjected to an inhibition assay for diarrhea-causing microorganism especially *Salmonella paratyphi*, *Shgella dysenteriae*, *E. coli* and *Vibrio cholera*. Apart from these, we endeavored for an intervention study on castor oil induced diarrhea and $MgSO_4$ induced gastrointestinal motility tests in animal models of normal control, treatment and reference control. Compounds from *L. glutinosa* were interacted through a ligand-receptor interaction in chemico-biological simulation study. Results showed an worthy contents of total phenolic, total flavonoid, tannin and a pure oxoaporphine-derivative in association with promising DPPH free radical scavenging capacity, superoxide radical scavenging ability and iron chelating capacity which were statistically significant ($p<0.05$) compared to ascorbic acid and rutin. *S. paratyphi*, *V. cholera* and *S. dysenterae* were significantly inhibited showing very remarkable inhibition zones compared to Ciprofloxacin and Kanamycin. The extract significantly ($P<0.05$) rendered the antidiarrheal effect decreasing the total number of wet feces produced upon administration of castor oil, total length of small intestine, and intraluminal fluid intake. Decrease of IgE and increase of electrolytes especially Na^+, K^+ and Cl^- in the treatment groups were found to be normalized compared to Loperamide group. Expressions of mRNA for superoxide dismutase (SOD1) and catalase (CAT) have been found to be increased multifold while glutathione peroxidase (GPx) remains unchanged. Occurrence of a pure oxoaporphine derivative and few antidiarrheal compounds from L. glutnosa support its antidiarrheal potential which has been justified with low binding energy for a Ligand-receptor interaction. These findings thereby demonstrate that L. glutionsa extract might potentiate in vivo antioxidative enzymes which play pivotal role to normalize the markers responsible for causing *hypersecretory* diarrhea.

Keywords: Superoxide dismutase, catalase, castor oil, diarrhea, gastrointestinal motility

INVITED SPEAKER

REGULATORY EXPRESSION OF MURINE CYP1A1 BY ANDROGRAPHOLIDE AN ACTIVE CONSTITUENT IN *Andrographis paniculata*

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Cytochrome P450 1 (CYP1) is a member of hemethiolate monooxygenase superfamily, which majorly participates in metabolic activation of chemical procarcinogens from daily exposure, e.g. tobacco smoke, grilled food, and automobile exhaust. CYP1 activity is a critical aspect for risk assessment determining carcinogenicity and a safety criterion for drug development. *Andrographis paniculata* has a board range of pharmacological benefits, e.g. anti-inflammatory, antidiarrheal, antiviral, hepatoprotective, and immunostimulant activities (1,2). *A. paniculata* increased CYP1A1 (ethoxyresorufin *O*-deethylase) and CYP1A2 (methoxyresorufin *O*-demethylase) activities in mouse microsomes (3). Andrographolide, a major diterpenoid in *A. paniculata*, concentration-dependently induced CYP1A1 in primary mouse hepatocytes (4). Co-treatment of andrographolide and benz[*a*]anthracene synergistically enhanced CYP1A1 and the synergism was blocked by an aryl hydrocarbon receptor (AhR) antagonist resveratrol, suggesting the synergistic induction associated with an AhR-mediated transcriptional activation. Andrographolide plus 3-methylcholanthrene synergistically enhanced CYP1A1 in C57BL/6 male mice (5). Neither single treatment with 3-methylcholanthrene or andrographolide nor co-treatment modified CYP1A1 in male DBA/2 mice. Andrographolide-enhanced induction of CYP1A1 was not observed in intact or ovariectomized C57BL/6 females, or in orchiectomized C57BL/6 males. The effect in both orchiectomized males and ovariectomized females was restored by testosterone, indicating a male hormone as a crucial mediator of the sexually dimorphic modulation of CYP1A1 by andrographolide plus a typical CYP1 inducer. Besides andrographolide, three diterpenoids isolated from this plant, including 14-deoxy-11,12-didehydroandrographolide (DHA), andrographiside (AS), and neoandrographolide (Neo) modified CYP1A1 (6). DHA induced CYP1A1, though it demonstrated lower enhancing activity than andrographolide. Co-treatment of DHA with β-naphthoflavone showed a synergistic induction of CYP1A1. Neo suppressed β-naphthoflavone-induced CYP1A1. AS did not modify the induction. Moreover, andrographolide up-regulated 18 metabolism/oxidation/reduction-related genes among 28,853 genes, and in combination with β-naphthoflavone, the 45 genes were modified (7). Therefore, herb-drug interaction and risk assessment with the use of andrographolide or *A. paniculata* are of strong concern.

Keywords: AhR, C57BL/6, diterpenoids, orchiectomy, testosterone, typical CYP1 inducer

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INVITED SPEAKER

POISONOUS PLANTS FOR CATS AND DOGS KEPT IN HOUSE 1: *DIEFFENBACHIA* SPP., *MELIA AZEDARACH*, *RICINUS COMMUNIS*, *EUPHORBIA PULCHERRIMA*, *NARCISSUS* SPP.

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Abstract

Poisonous plants are responsible for many poisoning events. These plants are commonly grown in homes and gardens and are eaten by dogs and cats. In this context, it was dealt with poisonous effects of especially *Dieffenbachia* spp., chinaberry tree (*Melia azedarach*), castor bean (*Ricinus communis*), poinsettia (*Euphorbia pulcherrima*) and daffodil (*Narcissus* spp**.)** from plants leading to poisoning, grown commonly in parks, gardens, and homes, in our country. The plant species leading to poisoning for both dogs and cats were presented in Table. In addition, comprehensive information was presented on their adverse effects in different organ and tissues occurring in the result that they are eaten by dogs and cats, their clinical signs, and their treatments. The concise knowledge was given on the required measures for preventing poisoning of animals kept in home with these plants in also our country, as in world countries.

Keywords: Pet animals, poisonous plants

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INVITED SPEAKER

DISCOVERY OF PLANT SECONDARY METABOLITES AS LEAD FROM IN VITRO CONSERVED INDIAN THREATENED MEDICINAL PLANTS IN HEALTH CARE MANAGEMENT

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Abstract
The present situation of COVID-19, World is going under traumatic situation. Still neither proper medication nor approved vaccination developed against COVID-19. Hence, millions of people relay on alternate medicine system for their healthcare needs. In this context, antiviral herbals play a highly significant role in the drug discovery and development process for the man kinds. A vast number of plant sources are available in the Universe and all the plants have some of other therapeutic properties but all are definitely impossible to make an account for the same. There are many gray areas where very important plant secondary metabolites are available but those are rarely known to the people. Such plants are become red labeled due to increased environmental damage by global warming, habitat loss, and over exploitation which further create an impact on the threat of plant extinction. Further, many plant species are being destroyed due to their prevalent removal. However, India is a rich source of plant biodiversity. Of the 3,00,000 estimated angiospermic species of the world, about 2,50,000 has been described till now. India is one of the mega diversity regions of the world and has 18,000+ species of flowering plants occupying ca. 7% of world species. Out of the total twelve biodiversity hotspots in the world India has two, one is the north east region and other is western ghats from where discovery of drugs are possible from the threatened plants which needs to be conserved for future research. These constituents are present in many of the unknown plants which are become red labeled. As per the International Union for Conservation of Nature (IUCN), about 3654 endangered plant species and about 17% of all evaluated plant species are endangered in all over the world. The IUCN also listed 99 subspecies and 101 varieties as endangered. An attempt to compile drug discovery some of threatened plant species so as to give scientific account on future use as well as could be a source of a new class of miracle drugs against chronic diseases. An alternate method, in vitro plant tissue culture technique using modern 3-D printing is recent trend for mass propagation of any type of plants easily. Furthermore bioactivity guided fractionation is recommended to identify lead compounds from the resources for various activities. The main interest of this investigation is due to multiple chemical constituents in the same species due to biodiversity. A vast number of chemical compounds isolated from natural sources are currently undergoing clinical and preclinical studies and maximum from the plant sources. Very few herbal pharmaceutical companies are involved in drug discovery screening from natural sources, may be due to improper authentication of raw herbals, excessive cost in isolation and identification of pure phytoconstituents, complex nature of plants due to diversity and thereafter ambiguities in regulatory guidelines for natural products. Hence the issue of evaluation, efficacy and safety for herbals still remains. Therefore, development of a new phytoconstituents as lead through proper protocol as well as in vitro conserved herbal plants is most essential in many thrust areas.

Keywords: Biodiversity, drug discovery, threatened plants, phytoconstituents, Western Ghats region

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INVITED SPEAKER

NANO- AND MIKRO- LIPOSOMAL DELIVERY SYSTEMS FOR PLANT DERIVED BIOACTIVE COMPOUNDS

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Abstract
Liposomes are lipid-based delivery systems for protection and carrier of bioactive agents used in pharmaceutical, cosmetic and food industries since they are well characterized, easily made, highly versatile in their carrier properties, highly biocompatible and GRAS materials. Liposomes are structures enclose both aqueous (core) and lipid (bilayer) phases, so they can be used for entrapment, delivery, and release of hydrophilic and lipophilic compounds such as polyphenols and carotenoids, respectively. Oral bioavailability is often improved with liposomes in the case of such bioactive compounds *in-vitro* and *in-vivo*. Liposomes can increase solubility and stability of the bioactive agent, resulting in improved bioavailability and therapeutic benefit. For these reasons, the objective of this review is to focus on the liposomal encapsulation of these hydrophilic and lipophilic bioactives. Sonication, high pressure homogenization and supercritical fluid treatment are suitable for liposomal encapsulation of hydrophilic compounds (polyphenols) while ethanol/ether injection, reverse phase evaporation method are suitable for liposomal entrapment of lipophilic compounds (carotenoids). However, all lipophilic compounds require organic solvents such as ethanol, isopropyl ether at high temperature in these methods. Among these organic solvents, ethanol is less harmless accepted by the authorities. Therefore, new solvent free liposome preparation methods are necessary. In this concept, methods including generating liposomes from proliposomes or using supercritical carbondioxide may have potential to prepare liposomal vesicles with desirable characteristics and free of organic solvents. In our previous study, liposomal encapsulation for hydrophobic phenolics has been performed successfully using proliposomes. Several authors also demonstrated that lipophilic carotenoids are encapsulated using super critical carbondioxide. In conclusion, nano and micro sized liposomes are promising delivery systems for both hydrophilic and lipophilic bioactives. Liposome preparation methods as well as lipid composition and the surface modification of liposomes should be intensively researched to incorporate insoluble compounds into liposomes.

Keywords: Liposome, encapsulation, bioactive compounds, polyphenols, carotenoids

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INVITED SPEAKER

ACTIVE SUBSTANCES ACCUMULATION OF CHAMOMILE VARIETIES UNDER GROWING CONDITION IN CHULLCUISA, PERU

Prof. Dr. Ivan Salamon[1], Gabriela Sabelli[2]

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Abstract
Slovakia is one of the European countries in which particular attention has been devoted to research of medicinal, aromatic and spice plants in all its aspects, including the breeding and selection. Based on the study of pharmacodynamics properties of chamomile (*Matricaria recutita* L.), the new chamomile variety '*LIANKA*' was bred at the University of Presov, Slovakia, between the years 2008 – 2013. Currently, the variety '*LIANKA*' has the certificate by the Community Plant Variety Office in Angers, France (No. 46937) [1].

Thanks of Lush Ltd. Company (Dorset, UK), a comparative investigation of four chamomile varieties ('*LIANKA*', '*PERUVIAN*', *'BODE GOLD*' and *'ZLOTY LAN*') carried out in Chullcuisa (S 13°40'47.3", W 73°15'15.3", Altitude: from 3,500 to 3,811 m), Province: Andahuaylas, District: San Jerónimo, Peru in 2019. The Slovakian chamomile variety was characterized by its highest percentage of sequiterpenes (/-/-α-bisabolol [60 ±2.00 %], chamazulene [13±1.00 %], the low contents of /-/-α bisabololoxides A and B [< 2.5 %] and essential oil content was 0.55 ±0.05 % of the dry inflorescences. The mean flavonoid content (Σ apigenines) was 0.80 ±0.05 %; at which the apigenine-7-glucoside had its amount 0.40 ±0.02 %. Pharmaceutical practice and clinical studies have generally used chamomile extracts from high bisabolol varieties. Given the pharmacological properties of /-/-α-bisabolol and chamazulene it is important to use these varieties which are high in designate active constituents and are suitably processed. There is a strong premise that the cultivation of chamomile variety '*LIANKA*' throughout the EU and outside it will be expanded in the coming years.

Keywords: South America, chamomile, essential oil, large-scale cultivation, varieties

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INVITED SPEAKER

LOW TEMPERATURE ATMOSPHERIC PLASMA: A TOOL FOR PROMOTING EARLY SPROUTING, IMPROVING PLANT GROWTH AND YIELD OF TURMERIC

Prof. Dr. Srinivasa Rao Mentreddy[1], Lam Duong[1], K.G. Xu[2] and R. Gott[2]

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Non-thermal, or low-temperature Plasma (LTP) is a weakly ionized noble gas or ambient air comprised of positive and negative ions, electrons, excited and neutral atoms, free radicals, molecules in the ground and excited states and the UV photons. Dielectric barrier discharges, plasma jets, and corona jets generated using noble gases, mainly He and Ar, are increasingly used in several agricultural applications, including microbial disinfection, enzymatic inactivation, improving the cooking quality, starch modification, and for enhancing the seed germination and plant growth. Turmeric (*Curcuma longa*), a popular condiment, has proven anti-cancer, anti-inflammatory, and anti-Alzheimer properties, among many other benefits. There is growing potential for turmeric production in southern US due to a conducive climate. Most farmers save a portion of the turmeric harvest for planting a crop the next season. Sometimes, immature rhizomes that were harvested early to season limitations often do not sprout and result in poor stand establishment and low yields. The objective of the study was to assess the effects of LTP on sprouting and plant growth of turmeric varieties known for slow or lack of sprouting. Six-month (6MG) and 7-month (7MG) old rhizomes of turmeric variety CL9 were exposed to Helium low-temperature pulse jet plasma for 0s (Untreated Control), 60s, 90s, or 120s; and four varieties VN12, VN18, VN 23, VN24, and VN 44 were exposed to the same plasma parameters for 90 s. Each treatment was replicated four times. The untreated and treated rhizomes were planted in pots containing potting mix at the rate of one rhizome per plant and placed in a complete randomized block design in the greenhouse. The date of sprouting and plant heights were recorded at weekly intervals for 63 days after treatment (DAT). The control untreated 6MG rhizomes never sprouted, whereas the untreated 7MG rhizomes sprouted 49 DAT. The 6MG rhizomes sprouted 49, 28, and 21 days at exposure times of 60, 90, and 120s, respectively. The 7MG rhizomes exposed to LTP for 60s and 90 or 120s, sprouted 14 and 28 days earlier than the untreated, respectively. In the 7MG, the rhizomes sprouted 49, 35, 21, and 21 DAT after exposure to 0, 60, 90, and 120s, respectively. All plasma-treated rhizomes of the five varieties sprouted six to twenty-eight days earlier and achieved 100% sprouting a week to four weeks earlier than the untreated control. The plasma-treated plants grew faster and were taller (37 – 39 cm) than the untreated control (26-33cm) at 67 days after planting. The study showed that cold or low-temperature plasma offers potential for improving turmeric plant stand establishment and crop performance.

Keywords: Turmeric, *Curcuma longa*, Cold Plasma, rhizome, sprouting, growth, stand establishment

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INVITED SPEAKER

CHEMICAL PROFILE, ANTIOXIDANT ACTIVITY AND CYTOTOXICITY ASSESSMENT OF *Tagetes erecta* FRACTIONS

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Objective: *Tagetes erecta* is a popular ornamental plant of the *Asteraceae* family, being widely cultivated not only for its decorative use, but also for the extraction of lutein, which can be used for the production of different ocular nutritional supplements as well as a food additive [1,2]. Besides carotenoid representatives, which have been extensively studied, other important classes of secondary metabolites are present in the plant, such as polyphenols and terpenoids, that can possess substantial therapeutic effects. Therefore, we decided to investigate its potential use as a resource of such constituents with biological actions. **Material and Methods:** The initial chromatographic analysis of phytoconstituents present in a methanolic extract obtained from *Tagetes erecta* inflorescences was achieved using high-resolution UHPLC-PDA-CAD-MS/MS technique. The extract was further subjected to reversed-phase C_{18} solid phase extraction and gel permeation column chromatography on Sephadex LH-20 column eluted with MeOH, which allowed for different fractions to be obtained. One representative fraction was subjected to two different antioxidant assays (metal chelating activity and lipoxygenase inhibition) and to *in vitro* cytotoxicity assessment. **Results:** The obtained results indicate that the investigated fractions contain several polyphenolic compounds such as hydroxybenzoic and hydroxycinnamic acid derivatives - syringic, gallic, ellagic and chlorogenic acids, flavonols - quercetagetin glycosides (quercetagitrin) as well as several aglycons (e.g. quercetin, patuletin). Generally, the antioxidant and cytotoxicity assays showed promising results for the investigated fraction containing mostly quercetagitrin, compared to the initial extract. **Conclusions:** Given the promising results obtained in the *in vitro* antioxidant and cytotoxicity assays of extracts and fractions obtained from *Tagetes erecta* inflorescences, further purification and structural analysis of compounds as well as further *in vivo* investigations are justified.

Keywords: *Tagetes erecta*, HR-MS, quercetagitrin, antioxidant, cytotoxicity

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References:

INVITED SPEAKER

AGRONOMICAL AND CHEMICAL CHARACTERIZATION OF SOME SPECIES OF AROMATIC AND MEDICINAL PLANTS FROM SICILIY, ITALY

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Abstract

Profesor Bella will speak on biodiversity Sicilian Rosemary, Oregano, Thyme and Myrtle, focusing on biomorphological characteristics, production, qualitative chemical evaluation and antioxidant activity evaluation of extracts and essential oils, in order to identify those biotypes best suited for the local development.

Given its central position in the Mediterranean region and its vast plant heritage, the island of Sicily is ideally placed for a planned resource development of this kind. Furthermore, this study and its results contribute to generating interest in the biodiversity of the Mediterranean area, in which Sicily plays a crucial role. On the basis of the above mentioned considerations, the cultivation and technological utilization of medicinal plants are emerging as a profitable alternative income in the crop-production sector.

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INVITED SPEAKER

INVESTIGATION OF PREPARATIONS OBTAINED FROM *Juglans regia* L. FROM THE FOLK AND TRADITIONAL MEDICINE OF REPUBLIC OF MACEDONIA

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Abstract

This study was carried out concerning ethnomedicine on preparations obtained from *Juglans regia* L. from the folk and traditional medicine of Republic of Macedonia, an area so far less frequently studied from the perspective of plant folk traditions. The district, from the ethnobotanical point of view, shows traces of the influences of the neighbouring regions. Some medicinal uses are linked to beliefs or residual forms of magic prescriptions. Amongst the more notable uses the most interesting are those of conserving of the juglandis immaturi fructus in honey and in rakiya. Walnuts in these recipes are not heat, and there is no harmful effect from heat. These useful customs are continuing to be expanded at every step by the people and valued as good old medical receipts. It should be chemically and clinically examined how vitamins are preserved in that can. In this way "sweet" (vitamin C 165.16 mg/100 g, vitamin D 7 mg/100g, sodium 142.54 mg/100g, calcium 3.01 mg/100g) and rakiya (vitamin C 6.15 mg/100 g, vitamin D 1.7 mg/100g, sodium 119.55 mg/100g, potassium 58.65 mg/100g, calcium 1.12 mg/100g) of the walnuts is our best folk can of vitamins and other medicinal ingredients.

Keywords: Honey, vitamins, rakiya, minerals, walnut

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ORAL

PRESENTATIONS

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ORAL PRESENTATION

PREVENTION OF ADVANCED END GLYCATION PRODUCTS BY THE SEED EXTRACTS OF *Nigella sativa*

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Diabetes is one of the most prevalent non-communicable disorder. Most of the developing and developed counties have seen a several fold increase in the number of people suffering from this this disorder. Several approaches have been used for the management of Diabetes. Although some medicines have helped in controlling the blood glucose level and other consequences of Diabetes but they have led to some other complications due to their side-effects. Plant extracts and formulations are being explored for their antidiabetic and other health potentials. *Nigella sativa* has been one of the most commonaly used medicinal plant for its various therapeutic potentials. In the present study we have analysed the therapeutic potential of this plant extract in preventing the formation of advanced glycation end products (AGEs). These AGEs are produced in large amount during the diabetes as a result of nonenzymatic interaction between sugars and proteins. Early and advanced glycation products have been quantified using NBT and carbonyl methods respectively. Similarly, glycation induced aggregation and glycoxidative damage were assessed using Congo Red method and agarose gel electrophoresis. These results indicate that aqueous extract of *Nigella sativa* seeds prevent the formation of early as well as advanced glycation products. The extent of glycation-induced aggregation and glycoxidative damage of DNA were also inhibited significantly. It can be concluded from the results obtained in the present study that the phytoconstituents of *Nigella sativa* seeds have substantial antiglyucating and antiaggregation properties. Further characterization of the nature and properties of phytoconstituents present in the aqueous extract need for the purpose of drug development to prevent Glycation and its consequences.

Keywords: Advanced glycation end products (AGEs), diabetes, glycoxidation, *Nigella sativa*, protein aggregation

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ORAL PRESENTATION

ANTI-DIABETIC ACTIVITY OF COMMERCIAL ETHANOLIC PROPOLIS EXTRACTS FROM ANATOLIA

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Diabetes mellitus is one of the most common diseases today and continues throughout a person's life. It develops when the pancreas cannot produce enough insulin or when the blood sugar level increases because of a defect in the use of insulin produced. Its prevalence is increasing worldwide, and this rate of increase is also supported by obesity. It is predicted that the number of diabetic patients in the world will reach 592 million in 2035 due to obesity and sedentary lifestyle. Propolis is a resinous natural mixture that is collected by bees from leaves and buds of trees and plants and processed by enzymatic reactions. Bees use this material for the repair of the hive, protection against foreign substances, stability of the hive environment. Propolis has a wide variety of biological activities and contains various components for human health. Propolis has been reported to treat *diabetes mellitus* in recent studies. In this study, the use of propolis as an agent to treat diabetes was investigated. For this, a commercial ethanolic extract was taken from Anatolia and its activity was examined both in vivo and in vitro conditions. The effect of Anatolian propolis extract on streptozotocin (STZ)-related diabetes was investigated. Twenty Wistar rats (250–300 g) were formed four groups under in vivo conditions. These groups were marked as GC control rats, GD diabetic rats, GDP30 and GDP15 treated with propolis extract. Two different ethanolic propolis extracts (30% and 15%) were applied by gavage for four weeks. Anti-diabetic activity was monitored from both blood glucose level and pancreatic tissue histology. To support these in vivo data, analysis of total phenolic and flavonoid content, antioxidant capacity, radical scavenging activity, phenolic composition and α-amylase and α-glucosidase inhibition analyses were performed on Anatolian propolis extract under in vitro conditions. According to the findings obtained, it was observed that ethanolic Anatolian propolis extract with high biological activity decreased blood sugar levels in diabetic rats. This result indicates that propolis extract is a potential agent in the treatment of diabetes. Anatolian propolis extracts of our findings have a preclinical anti-diabetic role and the data are important for clinical studies.

Keywords: Anti-diabetes, antioxidant, glycemia, propolis, α-amylase, α-glucosidase

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ORAL PRESENTATION

EFFECT OF *Lawsonia inermis* OINTMENT ON EXCISIONAL WOUND HEALING IN WISTAR RATS

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Abstract

Aim: *Lawsonia inermis,* a shrub naturally grown or cultivated from north-east Africa to India, has been used traditionally as a potent cure for various injuries. Presently there are few scientific reports on its use as a cicatrizing agent. The aim of this study is to evaluate the effect of a daily application of an ointment prepared with *Lawsonia inermis* leaves' powder on wound healing in Wistar rats. **Material and methods:** 20 female Wistar rats were used in this study. Excisional wound model was realized by removing skin from the dorsal nape of the neck of each animal. Wounds have been then treated by a daily application of *Lawsonia inermis* ointment prepared by mixing leaves' powder to petroleum jelly in test group and by simple application of petroleum jelly in control group. Evaluation of wound healing activity was then based on calculating the percentage of wound contraction, period of epithelialization and wound index every three days until complete healing. **Results:** Treatment with *Lawsonia inermis* has shown excellent wound healing activity, since it has increased percent of wound contraction, and reduced period of epithelialization and wound index as compared to control group. **Conclusion:** Therefore, the topical application of *L. inermis* ointment showed remarkable excision wound healing in Wistar rats thus showing its potential as a promising wound healing agent.

Keywords: Wound healing, *Lawsonia inermis*, petroleum jelly, wistar rats.

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ORAL PRESENTATION

INFLUENCE OF ENVIRONMENTAL FACTORS ON INHIBITORY ACTIVITY AGAINST PSF1 GENE EXPRESSION AND HABITAT SUITABILITY MAPPING OF *Cardiocrinum cordatum* (THUNB.) MAKINO IN CHIBURIJIMA ISLAND, JAPAN

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Environmental factors may influence the biological and chemical composition in the plants. The study of functionality and correlation of plants with environmental factors is very significant to increase the quality of food or medicinal products from the plants. Thorough understanding of this relationship will impulse the research in identifying and developing new cultivation areas to preserve many valuable rare plant resources. In this study, *Cardiocrinum cordatum* (Thunb.) Makino, a plant with rich chemical and nutrient contents from lily family, was investigated for correlation of inhibitory activity against PSF1 gene expression with environmental factors in Chiburijima Island, Shimane prefecture, Japan. Based on substantial correlation, the habitat suitability map was prepared with soil, geographical and meteorological data using MaxEnt software package, a species distribution/environmental niche modeling. Bioactivity analysis was examined by using crude extracts of the collected 12 – plant samples. Photosynthetically active radiation (PAR) was estimated using discolouration rate (DR) from initial and final absorbance values of 3 - day OptoLeaf - solar radiation film. The soil bearing capacity, and moisture content were recorded, and also samples were collected for chemical analysis. As a result, the phosphorus content showed a negative correlation with the inhibitory concentration ($R^2 = 0.75$, p-value <0.05) whereas iron content exhibited positive effect ($R^2 = 0.82$, p-value <0.05). The final suitability map was categorized into very high, high, moderate and low suitability zones. The very high and high regions are recommended for future cultivation of *Cardiocrinum cordatum.*

Keywords: Medicinal plant, bioactivity, PSF1 gene expression, soil moisture content, spatial analysis, interpolation, habitat suitability, MaxEnt, geographic information system

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ORAL PRESENTATION

BIOLOGIC ASSESSMENT OF ALCOHOLIC EXTRACTS OF *Equisetum pratense, Equisetum sylvaticum* AND *Equisetum telmateia*: ANTIOXIDANT AND NEUROPROTECTIVE ACTIVITY

Denisa Batîr Marin[1], Cornelia Mircea[2], Lucian Hriţcu[3], Oana Cioancă[2], Monica Boev[1], Andreia Corciovă[2], Răzvan Ştefan Boiangiu[3], Monica Hăncianu[2]

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Objectives: The *Equisetum* species are perennial plants which have spontaneously developed and are frequently found in forests, wet meadows and swamps. Sterile stems belonging to the *Equisetum* genus are often used in traditional medicine. They are highly efficient in treating urinary tract infections, cardiovascular diseases, respiratory tract infections and medical skin conditions due to its content of polyphenolic derivatives that have been isolated. The purpose of this work is to highlight the chemical composition of the extracts obtained from the *Equisetum* species and to investigate the biological action *in vitro* and *in vivo*. **Methods:** *In vitro* evaluation of the antioxidant activity of the plant extracts (methanolic and ethanolic) obtained from three species of *Equisetum* genus (*E. pratense, E. sylvaticum, E. telmateia)* was determinated using four tests: the chelating capacity of ferrous ion, the lipoxygenase inhibition capacity, the scavenger action of the hydroxyl radical and the scavenger capacity of the superoxide anion. *In vivo,* we determined the neuroprotective activity of those three extracts (ethanolic samples) using zebra fish tests. The behavioral tests made were the swimming pool test for the anxious behavior and the Y labyrinth test for short-term memory determination. **Results:** The obtained results indicate that methanolic and ethanolic extracts of *E. sylvaticum,* have the greatest antioxidant activity for all four *in vitro* tests made, followed by the ethanolic extract of *E. telmateia* and *E. pratense*. The results for the *in vivo* tests regarding the effect on short-term memory, significant differences are observed that confirm the great effectiveness of the *E. pratense* and *E. telmateia* extracts compared to *E. sylvaticum*. Also, the 1 mg/L concentration of *E. pratense* ethanolic extract had anxiolytic and antidepressant effects higher than the other two extracts used. **Conclusion:** Given the promising results obtained in the antioxidant *in vitro* tests and neuroprotective activity of *in vivo* tests further isolation of certain flavonosids subfractions and the evaluation of their complex biological potential are justified.

Keywords: *Equisetum* species, antioxidant, neuroprotective, polyphenolic derivatives

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ORAL PRESENTATION

PRE-FORMULATION STUDY FOR NANOEMULSION PREPARATION OF ESSENTIAL OILS FOR TREATMENT OF CANDIDIASIS

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Objective: Candidiasis is the leading infection of skin and nails caused by *Candida* species. Essential oils are natural compounds obtained from aromatic plants consisting of complex mixtures of volatile and non-volatile compounds as metabolites. Essential oils have been studied for their activity against fungal growth and are proved to be active against fungal strains. This study was aimed to pre-formulate a nanoemulsion using cinnamon essential oil or clove oil. Based on assay, cinnamon oil was used as an oil phase as well as an active ingredient in prepared optimum nanoemulsion. **Materials and Methods:** Cinnamon oil **(***Cinnamomum zeylanicum* L.*)* and clove oil (*Eugenia caryophyllata*) were extracted by gas chromatography and analysis was done by using GC-MS (Agilent technologies, U.S). Assays for antifungal activity of oils was done by disc diffusion method for fungal growth inhibition and broth dilution to get minimum inhibitory concentration of oil against candida. Non-ionic surfactants will be use for nanoemulsion formulation by BASF® (Ludwigshafen, Germany). **Results:** The constituents found in cinnamon oil as cinnamaldehyde 61.5 % , In clove oil euogenol was found 79.37 %. The assay for antifungal activity shows, cinnamon oil to have wider zone of inhibition 66mm as compared to clove oil 27mm and minimum inhibitory concentration of cinnamon oil was obtained as 0.001µg/ml while for clove oil as 0.008µg/ml. Nanoemulsion formulation was prepared using cinnamon oil as oil phase, non-ionic surfactant Kolliphor RH40® and purified water as an aqueous phase. Optimum nanoemulsion formulation was prepared and characterized with droplet size 154.6nm, poly-dispersity index as 0.206 and zeta potential as -10.0mV. **Conclusion:** Nanoemulsion pre-formulation studies were done and an optimum nanoemulsion formulation with cinnamon essential oil was prepared and characterized for droplet size, pdi and zeta potential, obtained results ensures the effectiveness of formulation against candida species.

Keywords: Cinnamon essential oil, candidiasis, nanoemulsion, topical pre-formulation, antifungal

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ORAL PRESENTATION

ANTIOXIDANT AND PHENOLIC PROFILE OF MAHALEB PLANT AS A FUNCTIONAL FOOD

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Prunus mahaleb L. (syn. *Cerasus mahaleb* L. Mill.) (Mahaleb) is a member of medicinal plants and grows naturally in Tokat and in some other regions of Turkey. Seed and flesh of mahaleb fruit are used as mahaleb vine, mahaleb flour, mahaleb oil and as puree. Wild fruits contain many bioactive compounds, such as phenolic compounds, anthocyanins, flavonoids, and vitamins C and tocopherols. Depending on its health promoting effects it is usually used in folk medicine, such as for diabetes. It is shown that wild fruits and other parts of the mahaleb tree possess free radical scavenging, antioxidant, anti-inflammatory, and anticancer activities, and classified as functional foods in preventing several chronic diseases. The present study evaluates the phenolic contents and antioxidant activities of the methanolic extracts of the fruits, leaves and barks of mahaleb plant that is collected from Tokat. The total phenolic content (170.21 mg GA g-1) in barks and the total flavonoid (260.5mg QE g-1) and anthocyanin (38.54 mg CA g-1) contents in fruits were greater than the other parts of the plants. Antioxidant activity of the samples was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). The antioxidant activity was the highest with the fruit, which showed 90.2% in DPPH assays. This study showed that all parts of mahaleb have high phenolic, flavonoid, and anthocyanin contents, potentially. These findings suggest that mahaleb plant (fruits can be consumed; the other parts not consumed but can be used in other ways) could serve as a source of bioactive compounds and as being rich in anthocyanin pigments could be used as a natural food colorant and antioxidant component in the utilization formula of functional foods.

Keywords: *Prunus mahaleb* L., phenolic compounds, anthocyanins, flavonoids, antioxidant, functional foods

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ORAL PRESENTATION

ANTIOXIDANT PROFILE OF A MEDICINAL HALOPHYTE: *Portulaca oleraceae* L. UNDER SALINE CONDITIONS

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Purslane (*Portulaca oleracea* L.) is an annual C_4 species belongs to *Portulacaceae*, is the eighth most common globally-distributed weed with a long history of use in folk medicine and cooking, throughout the world. It is a succulent medicinal plant, is grown rapidly, with self-compatible producing large numbers of seeds that have a long survivability, is distributed in temperate and tropical regions world-wide, with a broad physiological adaptability. Although, purslane is considered as one of the world's most aggressive weeds in many agroecosystems, it is listed in the World Health Organization as one of the most used medicinal plants, providing biologically active substances and essential compounds for human nutrition. Owing to its rich omega-3 fatty acids, antioxidant and radical scavenger properties, it is used in wound-healing, as analgesic, antimicrobial, muscle-relaxant, anti-inflammatory agent. As an important drought-tolerant vegetable crop, purslane is developed as a model system for exploring plant responses to stress, particularly saline stress. Due to its salinity tolerance purslane is listed as halophyte in the Haloph database. Medicinal uses of halophytes are reported while describing the economic importance of plants. Growing salt-tolerant crops such as *P. oleracea* is also one of the most cost-effective strategies for coping with soil salinity and for agricultural sustainability, suggesting to be used to combat salinity and rehabilitate saline soils. In this study, *P. oleracea* was grown in 0, 50 and 100 mM NaCl solutions. 20 days old seedlings were detected for prolin, total phenolics, peroxidase enzyme activity, DPPH activity and betalain pigment content. All parameters were increased due to higher NaCl concentrations. Based on these results, *P. oleracea* has the capacity to grow in salt stress conditions and could be considered as a medicinal herb and vegetable for growth in harsh environments.

Keywords: *Portulaca oleracea* L., weed, medicinal halophyte, NaCl stress, phenolics, antioxidant

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ORAL PRESENTATION

ANTIOXYDANT ACTIVITIES OF SOME POLYPHENOLIC EXTRACTED FROM *Marrubium vulgare* LEAVES FROM ALGERIA

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The *Marrubium vulgare* from the labiate family is one of the medicinal plants for ancestral therapeutic use. This plant is very popular in the Mediterranean region. It is a perennial herbaceous plant of grayish color that can reach 25 to 45 cm in height and which bears a slight resemblance to mint. In ancient Egypt *M. vulgare* was already recognized for its soothing properties against coughs. *M. vulgare* is also used for the treatment of inflammation, gastroenteritis and respiratory disorders. It has also hypotensive, antioxidant and insecticidal properties.

In the present study the phytochemical tests and antioxydant activities of some polyphenolic compounds extracted from *M. vulgare* leaves have been done. The phytochemical tests were carried out on various extracts obtained after maceration of *M. vulgare* leaves with different solvents such as water, methanol and ethanol. Then the antioxydant activities were evaluated on the polyphenolic compounds extracted from *M. vulgare* leaves.

Phytochemical tests revealed the richness of this plant on tannins; terpenoids flavonoids, steroids and saponins. The results show that the level of polyphenols is proportional to the polarity of the solvent used for maceration. Thus, the aqueous extract exhibited the highest rate, with 18.88%, followed by the methanolic extract, 16.16%, then the ethanolic extract estimated at 15.22%. Indeed, we recorded contents of 2.2 mg eq of acid Gallic par gramm of extract in total polyphenols and 1.321mg eq of Quercetine per gramm of total flavonoids extract. The antioxidant activity evaluated by the reduction method of DPPH free radical shows that different polyphenol extracted from *Marrubium* Vulgare presented good results.

The results suggest that *M. vulgare* leaves are rich in polyphenols with interesting antioxidant activities. The polyphenolic compunds extracted from *M. vulgare* leaves can be used to increase the antioxidant activity of some food, pharmaceutical and cosmetic products.

Keywords: *Marrubium vulgare*, polyphenolic compounds, antioxydant activity, DPPH

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ORAL PRESENTATION

THE EFFECTS OF *Tribulus terrestris*, *Avena sativa* AND WHITE GINSENG ON ADIPONECTIN, LEPTIN, RESISTIN, FATTY ACID BINDING PROTEIN 4, HOMOCYSTEINE AND PARAOXONASE-1 LEVELS IN HYPERCHOLESTEROLEMIC RATS

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The present study was aimed to assess the effects of *Tribulus terrestris* (TT), *Avena sativa* (AS), white ginseng (WG), and a triple combination (TC) powder on adipokines, homocysteine and paraoxonase-1 levels in hypercholesterolemic rats. Hypercholesterolemia in rats was induced by diet added 2% cholesterol. Herbal treatment groups consisted of Group III (TT), Group IV (AS), Group V (WG) and Group VI (triple combination of TT, AS, and WG). Significant increase in total cholesterol, LDL-C, homocysteine, leptin and resistin levels ($P<0.05$) and insignificant decrease in adiponectin, and paraoxonase-1 levels ($P>0.05$) were found in hypercholesterolemic rats. The treatment combination with TT, AS and WG significantly decreased total cholesterol, LDL-C, homocysteine, leptin and resistin levels in hypercholesterolemic rats ($P<0.05$). In conclusion, it was determined that TT, AS and WG had positive effects on reversing the effects of hypercholesterolemia in rats. The combination treatment with TT, AS and WG may have therapeutic potential in the treatment of hypercholesterolemia.

Keywords: Hypercholesterolemia, adipokines, homocysteine, paraoxonase-1, herbal treatment

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ORAL PRESENTATION

THE ANATOMICAL AND PALYNOLOGICAL CHARACTERISTICS OF MEDICINAL PLANT SPECIES OF *ECHINOPHORA* L. (APIACEAE)

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The members of *Echinophora* genus is a widely used herbal medicine and food seasoning in Turkey. In this study the medicinal species of Echinophora genus *E. tournefortii* Jaub. & Spach and *Echinophora tenuifolia* subsp. *sibthorpiana* (Guss.) Tutin species were analysed based on the anatomy and palynomorphology. The genus *Echinophora* (Apiaceae) is represented in the flora of Turkey by six species including three endemics. These are: *E. tenuifolia* subsp. *sibhthotpiana*, *E. tournefortii*, *E. orientalis* and three endemic species: *E. chrysantha*, *E. trichophylla* and *E. lamondiana*. The plant materials were collected from campus of Selçuk University and stored in 70% ethyl alcohol. The paraffin method was applied for cross sections of vegetative organs. If paraffin method was not successful, we then cut the cross sections using a razor blade by hand or a cryostat. On average, 10 preparations were made for each type of section and 30 cell groups were measured. The pollen morphology of *Echinophora* genus was analysed by Wodehouse method using light microscope and also using scanning electron microscope. The anatomical analysis showed that the cross sections of stem had an epidermis layer in outermost surface. Cortex which characterized with parenchymatic cells was 6 layered and has many secretory channels. The collenchymatic tissue had 9 layered. The pith covered by parenchymatous cells and also had many secretory channels. The leaves of studied species had between two epidermis layers with mesophyll tissue. Mesophyll was bifacial and had many secretory channels. The sections of bractea had like leaf cross sections but differ from it with shape of sections. The pollen grains were tricolporate and isopolar. Pollen shapes of studied species were prolate. The sculpture of pollen grains was rugulate in examined species. *E. tournefortii* had bigger pollen grains than *E. tenuifolia*. The anatomical and palynological characteristics of *Echinophora tenuifolia* and *E. tournefortii* were described for the first time in this study.

Keywords: Anatomy, Apiaceae, pollen morphology, spiny-leafed, secretory

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ORAL PRESENTATION

THE EFFECT OF PHOSPHORUS AND ZINC FERTILIZATION ON LYCORINEAND GALANTAMINE ACCUMULATION IN GIANTSNOWDROP

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Giant snowdrop (*Galanthus elwesii* Hook) is an important bulbous species that contains the acetylcholinesterase inhibitor galantamine and lycorine alkaloids with anticancer activity in all plant parts. This research was carried out to determine the effect of phosphorus (0, 30, 60, and 120 kg ha^{-1} P_2O_5) and zinc (0, 25, 50, and 100 kg ha^{-1} $ZnSO_4$) applications on lycorine and galantamine accumulation in *G. elwesii* in two growing seasons. Above- and underground plant organs were sampledat the stages of flowering and fruit ripening and the quantities of galantamine and lycorine were determined with Reversed Phase-High Performance Liquid Chromatography (RP-HPLC). The highest amount of lycorine (26.90 µg/g) was determined in underground organs at flowering stage, while the highest amount of galantamine (69.66 µg/g) was found in underground plant parts at fruit ripening stage. These findings provide the first comprehensive galantamine and lycorine alkaloids picture of a Giant snowdrop with regard to soil P and Zn availability.

Keywords: Alkaloids, Amaryllidaceae, *Galanthus elwesii* Hook, RP-HPLC

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ORAL PRESENTATION

HEAVY METAL ACCUMULATION AND CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *Juniperus oxycedrus* L. (CUPRESSACEAE) GROWN ON SERPENTINE SOILS IN BULGARIA

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In this study, the heavy metal concentrations and chemical compositions of the leaves and berries essential oils of *Juniperus oxycedrus* L. (Cupressaceae), growing on serpentine soils in Bulgaria was investigated. The content of heavy metals in leaves and berries of *Juniperus oxycedrus* L. was determined by ICP. The oils were obtained from the leaves and berries of the plant by hydrodistillation and analyzed by gas chromatography mass spectrometry (GC-MS) technique. Elevated Ni content do not affect the development of Juniperus oxycedrus L and the quality and quantity of oil obtained from it. The essential oils were obtained from leaves, and berries by yielding 0.1%, and 2.2%, respectively. Sixty components representing 98,10-98,92% of the total leaves oil, and 39 compounds representing 98,09-98,85% of the total berries oil were identified. The major compounds were determined limonene (12,10-15,62%), γ-himachalene (7,47-12,58%), manoyl oxide (6,60-13,59%), α-pinene (6,11-8,78%), dibutyl phthalate (1,48-8,14%), δ-cadinene (2,93-6,33%), γ-cadinene (3,64-5,47%), β-bisabolene (2,98-4,29%) in leaf oil, and β-myrcene (21,16-25,32%), germacrene d (20,62-30,32%), α-pinene (16,22-20,16%), limonene (5,41-6,72%), δ-cadinene (5,20-6,47%), β-caryophyllene (2,38-3,56%), α-caryophyllene (2,40-2,98%) in berry oil. The *Juniperus oxycedrus* L. can be considered as "excluder plant", containing relatively low metal concentrations in the aerial parts (leaves, and berries) even in cases of high elemental concentrations in the soils. Metal concentrations for toxic elements in plants and oils were below the permissible limits for pharmaceutical purposes. Therefore, *J. oxycedrus* L. found on serpentine bedrock are recommended to be collected for pharmaceutical purposes.

Keyewords: Serpentine soils, essential oil composition, heavy metals, *Juniperus oxycedrus* L.

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ORAL PRESENTATION

HEAVY METAL ACCUMULATION AND CHEMICAL COMPOSITION OF ESSENTIAL OILS OF BASIL (*Ocimum basilicum* L.) CULTIVATED ON HEAVY METAL CONTAMINATED SOILS

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Comparative research has been conducted to allow us to determine the content of heavy metals and chemical composition of basil oils, as well as to identify the possibility of basil growth on soils contaminated by heavy metals. The experimental plots were situated at different distances of 0.5 km, and 15 km, respectively, from the source of pollution the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. On reaching flowering stage the basil plants were gathered. The content of heavy metals in leaves of basil was determined by ICP. The essential oils of the basil were obtained by steam distillation in laboratory conditions which were analyzed for heavy metals and chemical composition was determined. Basil is a plant which is tolerant to heavy metals and can be grown on contaminated soils. Heavy metals do not affect the development of basil and the quality and quantity of oil obtained from it. Thirty components were identified in the oils. The quantity of identified compounds was shown correspond to 98,37% and 98,42% of the total oil content. Among the detected compounds, β-linalool (36,60-37,11%), methyl chavicol (27,55-28,74%), germacrene D (5,28-6,58%), δ-cadinol (3,90-3,93%), β-caryophyllene (2,50-2,77%), (+)-camphor (2,49-2,75%), β-elemene (2,49-2,75%), eucalyptol (1,82-2,02%), tau.-cadinol (1,67-1,85%), γ-cadinene (1,46-1,61%), borneol (1,26-1,39%), β-pinene (1,21-1,34%), limonene (1,17-1,29%) were the major compounds. The results clearly showed that the composition of oil is not affected by soil contamination with heavy metals. The essential oil of *Ocimum basilicum* L. can be a valuable product for the farmers from the polluted regions.

Keywords: Contaminated soils, essential oil composition, heavy metals, basil.

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ORAL PRESENTATION

PHYTOCHEMICAL ANALYSIS AND HABITAT SUITABILITY MAPPING OF *Glycyrrhiza glabra* L. COLLECTED IN THE HATAY REGION OF TURKEY

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The growth and quality of licorice depend on various environmental factors, including the local climate and soil properties; therefore, its cultivation is often unsuccessful. The current study investigated the key factors that affect the contents of bioactive compounds of *Glycyrrhiza glabra* L. root and estimated suitable growth zones from collection sites in the Hatay region of Turkey. The contents of three bioactive compounds (glycyrrhizic acid, glabridin, and liquiritin), soil factors (pH, soil bearing capacity, and moisture content), and geographical information (slope, aspect, curvature, elevation, and hillshade) were measured. Meteorological data (temperature and precipitation) were also obtained. An analysis of variance (ANOVA) and multivariate analysis of variance (MANOVA) were performed on the data. The soil parameters (soil bearing capacity and moisture content) and geographical parameters (slope, aspect, curvature, and elevation) of the study area showed statistically significant effects on the the glycyrrhizic acid and liquiritin contents. In contrast, these parameters exhibited no effect on the glabridin content. A habitat suitability zone map was generated using a GIS-based frequency ratio (FR) model with spatial correlations to the soil, topographical, and meteorological data. The final map categorized the study area into four zones: very high (15.14%), high (31.50%), moderate (40.25%), and low suitability (13.11%). High suitability zones are recommended for further investigations and future cultivation of *G. glabra*.

Keywords: Frequency ratio, glabridin, glycyrrhizic acid, habitat suitability map, liquiritin, soil moisture content.

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ORAL PRESENTATION

NEW DICHOTOMOUS IDENTIFICATION KEY FOR TURKISH LAMIACEAE GENERA

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In recent years, the flora of Turkey, while adding many new taxa, as a result of rapidly increasing systematical and molecular research, the status of some taxa is being changed and become synonyms. Since this situation is also reflected in the Lamiaceae family, the current key is not sufficient to identify the genus. For this reason, a new revised identification key is necessary in Turkey. Although the key was initially arranged according to the classical system, it was updated whenever a new taxon was added or the status of a taxon was changed and then adapted to the APG system. It was tested variously herbaria in Turkey. While Lamiaceae family consists of approximately 8602 taxa of the 242 genera worldwide, with a 44% rate of endemic in Turkey are represented by 48 genera and 782 taxa [1-3]. In this study, as an alternative to the key in Flora of Turkey, which has lost its functionality since 1982 when it was prepared [4], a more useful diagnostic key was prepared for the Lamiacee family today.

Keywords: Lamiaceae, identification key, Turkey

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ORAL PRESENTATION

POTENTIAL APPLICATIONS OF PLANT LECTINS AGAINST SARS-CoV-2

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Severe acute respiaratory syndrome coronavirus 2 (SARS-CoV-2) is a novel virus responsible for the human pandemic (COVID-19). There is a huge universal effort to develop diagnostic reagents, vaccines and antiviral drugs in order to slow down the spread of the disease and save lives. Lectins are powerful tools in modern glycobiology research. Plant lectins, which are proteins that can bind specifically and reversibly to carbohydrate groups, contributed to many glycoconjugate studies and have potential for SARS-CoV-2 treatment. These lectins have shown also to be promising as antiviral agents against other viruses such as influenza and herpes simplex virus. Up to date activity of a broad range of plant lectins have been screened against SARS-CoV by using a cytopathicity assay. Although the exact mechanism was not determined, activity at the stage of viral attachment or the end of infectious cycle were considered to be most likely targets. These compounds may be toxic at certain levels, and hence *in vitro* and *in vivo* testing is required to determine safe and therapeutic levels of them before clinical trials in humans. In this presentation, plant sources, structures of antiviral lectins and their biological action mechanism will be discussed in detail.

Keywords: SARS-CoV-2, COVID-19, plant lectins, antiviral agents, viral attachment, glycobiology.

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ORAL PRESENTATION

INVESTIGATION ON EFFECTS OF ONTOGENETIC VARIATIONS OF MARITIME PINE (*Pinus pinaster* AIT.) ON VOLATILE COMPONENTS

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In this study, it is aimed to identify volatile components of needle and cones of Maritime Pine which is fast growing exotic species in our country and used in lots of industry as timber, furniture, paper and investigate the seasonal differences of these volatile components in the Maritime Pine. The needle, cone and bark samples of the Maritime Pine in the Bartın Region had been collected on four different seasons (2015 November, 2016 February, May and August). Essential oil of collected samples had been obtained by hydrodistillation method. And volatile components had been determined by Gas Chromatography- Mass Spectroscopy (GC-MS). 67-75 components in needle and 61-71 components in cones had been identified for all seasons in our study. In general, it was found that needle had more components than cones for all seasons. In needle samples α-pinene, germacrene-D and trans-β- caryophyllene, also in cone samples α-pinene, β-pinene and junipene had been identified as main components. α-pinene, germacrene-D and trans-β- caryophyllene, which were main component in needle samples had been detected at highest level respectively in November, February, February. On the other hand, α-pinene, β-pinene and junipene which were main component in cone samples had been detected at highest level respectively in May, August, August. When the results were examined, it was observed that both needle and cone volatile components had seasonal differences. Generally, in needle, total percentage of monoterpen components in November, total percentage of sesquiterpen and diterpen components in February had been determined at highest level. On the other hand, in cones, total percentage of monoterpen components in May, total percentage of sesquiterpen and diterpen components in August had been found at highest level.

Keywords: Maritime Pine, *Pinus pinaster*, GC-MS, volatile components

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ORAL PRESENTATION

MEDICINAL AND AROMATIC PLANTS AS A SOURCE OF ANTIOXIDANT DURING COVID-19 PERIOD

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Today, increased consumption of ready-made food, crowded city life and stressful living conditions cause the formation of free radicals in our body. Antioxidants appear as valuable compounds that can reduce or eliminate the harmful effects of these free radicals. Antioxidants are generally classified in two groups as natural and artificial. Vitamins (C, E and A vitamins), phenolic compounds (flavonoids), terpenoids (carotenoids) and sulphur containing (allicin) compounds, can be counted as the leading of natural antioxidants. Today, natural and synthetically derived antioxidants are used to preserve the freshness of foods for a long time, or in other words to extend their shelf life and preserve their quality. However, even though synthetic antioxidants are affordable over time, the emergence of negative health aspects made natural antioxidants to be preferred. Medicinal and aromatic plants, like many vegetables and fruits, are also powerful sources of antioxidants. The best examples of these herbs include rosemary, thyme, sage and turmeric. In this review, medicinal and aromatic plants that can be used as natural antioxidants will be mentioned.

Keywords: vitamin C, flavonoids, carotenoids, polyphenols, Covid-19.

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ORAL PRESENTATION

COMPUTATIONAL BOTANY – A REVIEW

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Technology is a part of human being, due to its diversity. Being such an important part, people have started using it as a more viable resource in different areas. One of the areas is botany, in which technology was implemented for helping the researches, due to the low-level errors, compared to the high rates which we encounter in the human errors. In this paper are mentioned some of the digitalized techniques implemented in botany, also the valuable contribution for the plant field. Automated species identification has become a reality due to utilization of technologies such as digital cameras, some new databases and new techniques improving the processing of images. The aim of this review is synthesis and discussion of studies involving computer technology for plant species identification, also to provide an overview of the literature surrounding this subject. The objective of the present work is to identify the importance of using technology among plants, the impact of digital techniques in botany and the future of computer technology in the sustainability of plant wealth.

Keywords: Technology, botany, review

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ORAL PRESENTATION

QUALITY CHARACTERISTICS OF SOME ESSENTIAL OIL PLANTS GROWN UNDER CONVENTIONAL AND ORGANIC FARMING CONDITIONS

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This study was carried out to determine the quality characteristics of some medicinal and aromatic plants grown under conventional and organic farming conditions. In the study, Anatolian sage (*Salvia fruticosa* Mill.), Turkish oregano (*Origanum onites* L.), lavandin (*Lavandula xintermedia* L.), lavander (*Lavandula angustifolia* L.), spearmint (*Mentha spicata* L.) and pepermint (*Mentha piperita* L.) species are used as a plant material. Among the plant materials included in the study, organic farming ones were grown in accordance with the principles in the organic farming regulation. Plants were harvested in full bloom, dried in an oven, and their essential oils were extracted by water distillation. The chemical composition of the essential oils obtained was analyzed by GC/MS.

Essential oil yield in conventional and organic farming conditions varied between, 3,2% -2,8% in Anatolian sage, 3,8% -3,5% in Turkish oregano, 6,3%-5,4% in lavandin, 3,4%-3,1%, in lavander, 2,2%-1,7% in spearmint and 2,4%-2,1% in pepermint respectively. In the content analysis of essential oils, 21 components in Anatolian sage, 24 in Turkish oregano, 16 in lavandin, 20 in lavander, 19 in spearmint and 19 in pepermint were determined. The main components and ratios of essential oils in samples obtained from conventional and organic agriculture; 1,8-cineole 42,92%-44,52% in Anatolian sage, 46,84-42,47% carvacrol in Turkish oregano, linalool 34,69%- 35,84%, in lavandin, linalyl-acetate 38,03-36,28% in lavander, carvone 59,51-58,64% in spearmint and menthone 39,32-40,86% in peppermint respectively. As a result, it was determined that the essential oil yields of all the species studied were decreased in organic farming conditions. However, no significant change occurred in the proportions of the main components in essential oils in organic and conventional agricultural conditions.

Keywords: Organic, conventional, essential oil, yield, quality

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ORAL PRESENTATION

DIVERSE BIOLOGICAL ACTIONS OF UROLITHINS, THE METABOLITES OF ELLAGITANNIN METABOLISM

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Urolithins (i.e., hydroxylated benzo [c] chromen-6-one derivatives) are the bioavailable metabolites of ellagitannins, abundantly available in many diets, including but not limited to nuts, berries, and particularly pomegranate. Exposure to ellagitannin-rich food generates ellagic acid within the gastrointestinal tract, the precursor metabolite of urolithins. Through the aid of gastrointestinal microflora, ellagic acid is stepwise converted to urolithins D, C, A, and B, respectively standing for tetra-, tri-, di, and mono-hydroxylated benzo [c] chromen-6-one analogues. Studies have indicated the significance of these molecules for the diverse biological actions of many herbals containing ellagitannins, since ellagic acid and particularly ellagitannins have negligible absorption characteristics. The Urolithin B (i.e., 3-hydroxy-benzo [c] chromen-6-one), and Urolithin A (i.e., 3,8-dihydroxy-benzo [c] chromen-6-one) have been shown to be the major circulating molecules observed in circulation. It is noteworthy to state the significance of phase II metabolism reactions as well for the generation of glucuronide, sulfate, and methyl ether metabolites of urolithins. Particularly within the last two decades, many research studies have been conducted on these molecules to describe the mechanistic background of biological activities obtained through exposure to ellagitannin-rich plants. These studies clearly demonstrated enzyme inhibitor, antioxidant, anti-inflammatory, and antimicrobial features of urolithins. This presentation has aimed to display the the main components of ellagitannin metabolism yielding out urolithins and the diverse biological activities of urolithins pointed out so far.

Keywords: Urolithin, ellagitannin metabolism, biological activities, CNS effects, antioxidant activity, enzyme inhibition.

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ORAL PRESENTATION

NATURAL AND SYNTHETIC UROLITHIN ANALOGUES AS MULTI-TARGET LIGANDS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

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The current treatment of Alzheimer's Disease (AD) employs cholinesterase inhibition and NMDA receptor partial antagonism as validated strategies considering the current drugs in the market. Nevertheless, the pathophysiology of AD is much more complex indicating a necessity for multi-target drug development. Pomegranate juice and extracts have long been known for their characteristics to possess beneficial effects on the prevention and treatment of neurodegenerative diseases, particularly AD. Pomegranate is a rich source of ellagitannins. These molecules are subject to gastrointestinal microflora induced biotransformation reactions to generate hydroxylated benzo(c)chromen-6-one derivatives, also referred to as urolithins. Regarding the bioavailability of urolithins, the biological effects of pomegranate related to AD have been attributed to the function of urolithins. Single urolithin compounds, mainly urolithin A and B (i.e., dihydroxyl, and monohydroxyl-substituted benzo(c)chromen-6-one, respectively), have been assessed in certain studies to display their activities on various validated and non-validated targets of AD. However, there is deficiency in the explanation of structure activity relationship and identifying mechanism of action of urolithins with respect to their effects on AD. This presentation aimed to provide a recent finding on the AD related effects of major urolithins (i.e., Urolithin A and B), their methyl ether metabolites, as well as some synthetic urolithin analogues. Mainly, synthesis studies, screening enzyme inhibition (acetylcholinesterase, butyrylcholinesterase, monoamine oxidase B, cyclooxygenase 1, and cyclooxygenase 2), and antioxidant (DPPH radical scavenging) activities of the compounds obtained will be presented. The outcomes of the study are very critical, since, the possible mechanisms of the activities of the urolithins for the treatment of AD with the corresponding structure activity relationships have been displayed. Docking studies were also performed to investigate the possible interactions with the corresponding receptors.

Keywords: Urolithin, Alzheimer's Disease, cholinesterase, monamine oxidase B, cyclooxygenase, antioxidant.

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ORAL PRESENTATION

THE EFFECTS OF DIFFERENT NITROGEN FERTILIZER APPLICATIONS ON THE ESSENTIAL OIL COMPONENTS AND CHLOROPHYLL CONTENTS OF OREGANO (*Origanum vulgare* SUBSP. *hirtum*)

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In this study, Origanum vulgare subsp. Hirtum plant has been studied. The experiment was planned with 4 replications according to randomized parcels. Four different doses of nitrogenous fertilizers (0, 0.5, 1 and 1.5 kg N ha^{-1}) were applied in the experiment. Plants grown in greenhouse conditions were harvested during the flowering period. At the end of the harvest, the effects of different doses of nitrogen fertilizer on the essential oil components and chlorophyll contents of *O. vulgare* were researched. The effects of nitrogen applications on the essential oil quantity and chlorophyll contents lead to important changes. The quantity of *O. vulgare* essential oil increased with nitrogen application and this increase was statistically significant ($p<0.01$). The essential oil quantity in plant were determined as 1.5 kg ha^{-1} (1.8 %), 1 kg ha^{-1} (1.69 %), 0.5 kg ha^{-1} (1.53 %) and control (1.02 %). The effect of nitrogen fertilizer applications on total chlorophyll contents and colour values of *O. vulgare* leaves were not found to be statistically significant but the nitrogen fertilizer applications created a difference at 5 % significance level on the chlorophyll concentrations

Keywords: Nitrogen, *Origanum vulgare* subsp. *hirtum*, essential oil, chlorophyll

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ORAL PRESENTATION

ADJUVANT POTENTIAL OF PLANT EXTRACTS IN SHEEP VACCINATED AGAINST CLOSTRIDIA

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Objective: Vaccination represents one of the most efficient procedures to prevent clostridioses in sheep. This research aimed at investigating the potential immune enhancing activity of alcoholic plant extracts in vaccinated local sheep breeds. **Methods:** The experiment was carried out in a sheep flock of 580 individuals, injected with a booster vaccine containing toxoid from *Clostridium perfringens, C. septicum, C. tetani, C. sordelii* and *C. haemolyticum*. Blood was randomly sampled (n=10) on heparin before and two weeks after the vaccination, and subjected to an *in vitro* blast transformation test in 96 well plates, using classical mitogens (PHA, LPS and ConA) and also alcoholic extracts of *Calendula officinalis*, *Echinacea angustifolia, Mentha pipperita, Betula pendula*, *Salvia officinalis* and *Symphitum officinale* (1.5 µl/well), most of which were common for Romanian pastures. The plates were incubated for 63 h at 37ºC, and the glucose residue was quantified by an orto-toluidine colorimetric test. Blast transformation indices were calculated versus untreated controls (%). The statistical significance of the differences between the two samplings was interpreted by Student-s t test. **Results:** The overall activity of the investigated plant extracts proved to exert a blast transformation inhibiting effect two weeks after the booster vaccination ($p<0.001$). The stimulation index recorded for the *E. angustifolia* extract was maximal during the first sampling and minimal post-vaccination (60.8±15.91 and 12.96±9.51, respectively). **Conclusion:** Of all the extracts involved, the maximal efficiency was recorded for the birch (*Betula pendula*) extract ($p<0.001$), which could be used as an adjuvant.

Keywords: Clostridioses, vaccination, immune blastogenesys, plant extracts

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ORAL PRESENTATION

WILD AROMATIC AND MEDICINAL PLANTS USED IN THE MOUNTAINOUS AREAS OF SICILY (ITALY) – A COMPARISON

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Wild plant species have always played an important medicinal role in the history of man, a role which has progressively diminished as intensive agriculture has grown and with the development of the pharmaceuticals industry. However aromatic and medicinal plants (MAPs) represent an important source of raw material for pharmaceuticals and traditional health care system due to a variety of their complex chemical compounds. Therefore, the conservation and valorization of MAPs is fundamental in a global world. Sicily (Italy) is a region of great interest for plant biodiversity and also for MAPs. This paper illustrates the results of a study carried out in four Regional Parks in Sicily, Madonie Regional Park, Nebrodi Regional Park, Sicani Regional Park and Etna Regional Park. These Parks are all located in mountainous areas of the island. The main aim of the study was to compare the level of traditional knowledge of MAPs by local people of the four study areas in order to show analogies and differences. A further aim was to identify MAPs that are already cultivated by farmers or could be of potential agricultural interest in order to increase their income. The information was obtained using semi-structured interview format performed on a sample of more 800 people over the age of 60 who were considered experts in rural and agricultural traditions. Local aromatic and medicinal plant uses were evaluated using quantitative indices (e.g. cultural importance index, informant consensus factor). The results highlight the existing differences in the way of use of MAPS among people of the four Regional Parks, but also show an ongoing process of cultural erosion. Particularly, Lamiaceae is the family most represented concerning the wild species which are considered of great agricultural interest by farmers.

Keywords: plant genetic resources, aromatic and medicinal uses, cultivation, potentiality, Sicilian highlands

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ORAL PRESENTATION

KILIS YAĞLIK VIRGIN OLIVE OIL: A NOVEL SOURCE FOR OMEGA-7 FATTY ACIDS, ESPECIALLY PAULLINIC ACID

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Abstract

Olive is an ancient food and medicinal plant that is mentioned in all the holy books. All the plant parts with rich and unique phytochemicals are also useful for human diet and health. Virgin olive oil produced from its unripe fruits by cold press is quite different from other vegetable oils by means of its distinguished fatty acid composition and phenolic compounds. Besides food purposes, virgin olive oil is also used in the cosmetic industry for natural cosmetic products. Recent scientific studies showed that fatty acid composition of the virgin olive oils is affected by many internal and external factors, olive variety, growing conditions, soil, climate, harvest time etc. In our study, Kilis Yağlık olive variety commonly grown in Kilis district, that claimed to be the homeland of olive trees, located in Southeastern part of Turkey, was evaluated in terms of its olive oil yield and quality in two years' study. According to results, unlike previous studies, Paullinic Acid, also known as (C20:3) Eicosenoic acid (cis-13), one of the recently most popular Omega-7 fatty acids, was found first time in the early harvest cold pressed Kilis Yağlık virgin olive oil. Omega-7 fatty acids are recently preferred in the cosmetic industry because of their moisturizing and firming properties. These fatty acids have many beneficial health effects, such as strengthening the immune and nervous system, increasing levels of HDL cholesterol and lowering levels of LDL cholesterol. An important member of Omega-7 fatty acids, Paullinic Acid is mainly found in Guarana seeds. In this study, unlike previous studies, Paullinic Acid, one of the Omega-7 fatty acids rarely found in the olive oil, also known as Eicosenoic acid (cis-13), was detected for the first time in Kilis Yağlık olive oil. It has been established that Paullinic Acid may be a distinguishing feature in Early Harvest - Cold Press Kilis Yağlık olive oil and a novel data has been added into scientific literature.

Keywords: Fatty acids, Kilis, Paullinic acid, Virgin Olive oil

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ORAL PRESENTATION

COMPARATIVE ANALYSIS OF TOTAL PHENOLICS, FLAVONOIDS AND ANTIOXIDANT POTENTIAL OF METHANOL AND ACETONE EXTRACTS OF *Sideritis niveotomentosa* HUB. -MOR.

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Phenolic compounds are secondary metabolites of plants which are containing flavonoids and phenolic acids. The increasing interest in the powerful biological activity of plant phenolics and flavonoids outlined the necessity of determining their content in medicinal herbs. In this study, total phenolic and flavonoid contents and antioxidant capacities of different extracts obtained from *Sideritis niveotomentosa* Hub. -Mor., an endemic species for our country, were investigated comparatively. For this purpose, extracts with acetone and methanol were prepared using the leaves and flowers of the plant. Total phenolic contents of extracts were determined by the Folin-Ciocalteu method and total flavonoid contents were determined by aluminium chloride method. Also, the free radical scavenging activities of extracts was measured by using diphenyl - picrylhydrazyl (DPPH). The results show that methanolic extracts contain more phenolic content than acetone extracts. According to DPPH assay results, IC_{50} doses were lower in methanolic extracts than acetone extracts. This is consistent with the phenolic content of the extracts. In conclusion, *Sideritis niveotomentosa* extracts, especially methanolic extracts, are rich in phenolic content and have a strong radical scavenging effect. The findings from this study are promising for hereafter studies.

Keywords: Duvaklı çay, endemic, Turkey.

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ORAL PRESENTATION

THE KARYOTYPE ANALYSES IN SUBTAXA OF *Centaurea pseudoscabiosa* FROM TURKEY

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In this study the karyotypes of five populations in subtaxa of *Centaurea pseudoscabiosa* were investigated by aceto-orcein method in the root tips. All populations have the same chromosome number (2*n*=22) and basic chromosome number (*x*=11). Karyotype formulas of *C. pseudoscabiosa* subsp. *pseudoscabiosa* are 22m, *C. pseudoscabiosa* var. *sipikorensis*, 20m+2sm and *C. pseudoscabiosa* subsp. *glehnii* 16m + 6sm. Five quantitative asymmetric indices were used to evaluate the karyological properties of the subtaxa. According to the asymmetry indices, *C. pseudoscabiosa* subsp. *glehnii* has the most asymmetric chromosomes (CV_{CI}: 10, M_{CA}: 16). In addition, karyological results support that *C. pseudoscabiosa* var. *sipikorensis*, regarded as synonym in Flora Turkey, could be appropriate to revive in subspecies category.

Keywords: Asteraceae, *Centaurea*, endemic, karyomorphology, Turkey.

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ORAL PRESENTATION

EFFECTS OF FERMENTATION ON ANTIOXIDANT ACTIVITY OF PLANT-BASED MILK SUBSTITUTES

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Recently, there is a regular growth towards the minimization of animal-based foods and the maximization of plant-based foods, in the dietary habits of consumers due to disapproval of health effects of meat products, environmental worry related to animal production, and animal welfare. Likewise, fermented foods are currently gaining popularity because of the beneficial health effects of food additives such as probiotics and prebiotics on gut microbiota composition. Plant-based milk substitutes include nuts and seeds which are commonly soy, almond, hazelnut, cashew etc. The rich oligosaccharide content of nuts and seeds as prebiotic are favourable substrate sources for fermented kefir-like beverages. Phenolic compounds found in nuts and seeds may be lost during the production of plant-based milk. The reduced antioxidant activity can be developed by the fermentation process. In this review, comparisons of fermented kefir-like and non-fermented plant-based milk substitutes were conducted in terms of antioxidant activity.

Keywords: Plant-based milk substitutes; kefir-like beverages; fermentation; antioxidant activity; phenolic compounds.

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ORAL PRESENTATION

A STUDY ON THE CULTIVATION AND ADAPTATION OF *Stevia rebaudiana* BERTONI PLANT TO ANTALYA CONDITIONS

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Objectives: *Stevia rebaudiana* (stevia, sweetleaf, sugarleaf) is the homeland of South America, was discovered in 1887 by a South American natural scientist, Antonio Bertoni. *S. rebaudiana* is member of Asteraceae family which likes moist conditions, temperatures mean 25°C and grows up to 60-90cm high. The plant, detected 79 species in North America is stated to be found more than 200 species in South America. The main components of stevia extract are stevioside, rebaudioside-A and steviol molecules. The stevia plant is known to have been used as a source of sugar by the locals for centuries. Dried form of stevia leaves is 10-15 times sweeter than sugar and the glycoside extract obtained by purification from the stevia plant is 200-300 times sweeter than refined sugar. The existence of some research, showing that non-calorie stevia plant does not increase blood sugar levels, has an effect on increasing insulin sensitivity and even insulin secretion, lend support its use in the treatment of diabetes. With this research, it was aimed to determine the favourable genotype or genotypes and the appropriate sowing frequency by investigating the adaptation of *Stevia rebaudiana*, *Stevia rebaudiana* Candy and *Stevia rebaudiana* Organic genotypes, imported as an introduction material, for Antalya plain conditions. **Methods:** Compared with the varieties used in the research, *Stevia rebaudiana* Candy genotype comes to the fore in terms of single plant dry leaf weight and rebaudioside-A/stevioside ratio which is an important quality criterion. While there was no significant difference in rebaudiosideA/stevioside ratios from three different plant densities (30x30cm, 40x40cm and 50x50cm) applied in the experiment, single plant/herb, fresh leaf and dry leaf weights were found high in 50x50cm application. **Results:** Considering the number of plants in the unit area, the yield per decare is higher with the application of 30x30cm, but as the plant density increases, the risk of fungal disease increases depending on the temperature and humidity. **Conclusion:** When the features which are examined are taken into consideration; Plant height, number of side branches, single plant dry leaf weight values vary depending on year, application and genotype. The values of single plant fresh herb weight and single plant age leaf weight vary depending on application and year. Rebaudioside-A content varies depending on the genotype, stevioside content and rebaudioside-A/stevioside ratio.

Key words: *Stevia rebaudiana*, stevia, sweetleaf, sugarleaf, medicinal and aromatic plants

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ORAL PRESENTATION

ANTIMICROBIAL PROPERTIES OF THE SEED EXTRACTS OF *Ceratonia siliqua* L. AND *Olea europaea* L.

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In this study, we aimed to investigate the effects of the seed extracts of *Ceratonia siliqua* L. and *Olea europaea* L. against some pathogen microorganisms. The seeds were removed from fruit and powdered. Fifteen grams of the materials was added separately in 150 mL of ethyl acetate, acetone and methanol. The extraction was carried out for 6 hours using the Soxhlet. Then, the solvent was removed from the active ingredient by evaporation. Under aseptic conditions the extracts were filtered through 0.45µ-pore size diameter filters and stored at 4°C. These extracts were experienced on seventeen bacteria and four yeasts. The agar well diffusion method is used for the antimicrobial effects of extracts. The extracts of the methanol, ethyl acetate, and acetone of *Ceratonia siliqua* L. and *Olea europaea* L. were found to be most effective against tested bacteria from high to low, respectively. However, these extracts had any effect on tested *Candida* species. The methanol extract of *Ceratonia siliqua* L. showed high effect on *Stapylococcus aureus* ATCC 25923, *Stapylococcus epidermidis* ATCC 12228, *Salmonella typhimirium* ATCC 14028, *Corynebacterium xerosis* ATCC 373, *Pseudomonas aeruginosa* ATCC 35032 and *Micrococcus luteus* ATCC 9341 and the inhibition zones ranged between 13-17 mm. The ethyl acetate extract of *Olea europaea* L. indicated high effect on *Escherichia coli* ATCC 35218, *Enterobacter aerogenes* ATCC 13048, *Salmonella typhimirium* ATCC 14028, *Corynebacterium xerosis* ATCC 373, *Pseudomonas aeruginosa* ATCC 35032, *Streptococcus mutans*, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633 and the inhibition zones ranged between 13-17 mm. However, the methanol extract of *Olea europaea* L. showed high effect on *Stapylococcus aureus* ATCC 25923, *Corynebacterium xerosis* ATCC 373, *Streptococcus mutans* and the inhibition zones ranged between 20-17 mm. The ethyl acetate, acetone and methanol revealed effective secondary metabolites like alkaloids, tannins, and flavonoids against bacteria from plant seeds.

Keywords: *Ceratonia siliqua* L., *Olea europaea* L., antimicrobial effect, agar well diffusion method

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This study was carried out with the opportunities of Microbiology Laboratory of Biology Department, Aydın Adnan Menderes University, Turkey.

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ORAL PRESENTATION

EFFECTS OF THE SEED EXTRACTS OF *Vitis vinifera* L. AND *Gossypium hirsutum* L. ON SOME MICROORGANISMS

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The effects of the seed extracts of *Vitis vinifera* L. and *Gossypium hirsutum* L. against some pathogen microorganisms were investigated in this study. The seeds were removed from fruit and powdered. Fifteen grams of the materials was added separately in 150 mL of ethyl acetate, acetone and methanol. The extraction was carried out for 6 hours using the Soxhlet. Then, the solvent was removed from the active ingredient by evaporation. Under aseptic conditions the extracts were filtered through 0.45µ-pore size diameter filters and stored at 4°C. These extracts were experienced on seventeen bacteria and four yeasts. The agar well diffusion method is used for the antimicrobial effects of extracts. The extracts of the acetone and methanol of *Vitis vinifera* L. were found to be most effective against tested bacteria while the ethyl acetate extract of *Vitis vinifera* L. had moderate effect against tested bacteria. However, the extracts of the ethyl acetate, acetone and methanol of *Gossypium hirsutum* L. showed low effect against tested bacteria and the inhibition zones ranged between 10-13 mm. In addition, whole extracts of *Vitis vinifera* L. and *Gossypium hirsutum* L. had any effect on tested Candida species. The methanol extract of *Vitis vinifera* L. indicated high activity on ten bacteria and the inhibition zones ranged between 15-30 mm. In addition, the acetone extract of *Vitis vinifera* L. demostrated high activity on nine bacteria and the inhibition zones ranged between 15-28 mm. However, the ethyl acetate extract of *Vitis vinifera* L. had moderate effect on four bacteria and the inhibition zones ranged between 12-15 mm. The ethyl acetate, acetone and methanol revealed effective secondary metabolites like alkaloids, tannins, and flavonoids against bacteria from plant seeds.

Key Words: *Vitis vinifera* L., *Gossypium hirsutum* L., antimicrobial activity, agar well diffusion method

Acknowledgements
This study was carried out with the opportunities of Microbiology Laboratory of Biology Department, Aydın Adnan Menderes University, Turkey.

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ORAL PRESENTATION

WORMWOOD POLYPHENOL EXTRACT MEDIATED REMEDIATION OF MITOCHONDRIAL DNA (mtDNA) OXIDATION AND NEUROTOXICITY INDUCED BY MERCURY

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Free radical (ROS) are considered important factors in cancer. Attacks on DNA by ROS, including the hydroxyl radical (_OH), superoxide anion (O_2 .), singlet oxygen, the ferryl or perferryl ion, hydrogen peroxide, and peroxynitrate (OONO_), frequently cause oxidative DNA damage. Recent studies have shown that DNA damage and altered intracellular redox status, such as imbalance of sulfhydryl and redox enzymes, occur after exposure to mercury. Occupational and environmental exposure to chemicals may increase the risk of unrepaired DNA lesions, which could become permanent mutations. Mercury is a ubiquitous and highly toxic environmental pollutant. Polyphenols are a very broad group of chemicals, widely distributed in plant foods, and endowed with antioxidant activity by virtue of their numerous phenol groups. They are widely studied as putative cancer-protective agents, potentially contributing to the cancer preventive properties of fruits and vegetables. In this study, we evaluated the relationship between mercury exposure and mitochondrial DNA (mtDNA) damage, and we evaluated the protective role of polyphenol extract isolated from *Artemisia absinthium* L. plant in imparting protection against mercury mediated oxidative mitochondrial DNA damage of cortex, cerebellum, hippocampus and striatum. Intra-mitochondrial accumulation of 8-hydroxy 2-deoxyguanosine (8-OHdG) was measured as the indictor of mtDNA oxidative damage $HgCl_2$ exposure (5 mg / kg body weight per week) for 10 weeks resulted in dramatically increased formation of 8-OHdG in the mtDNA isolated from all cerebral structures (cortex, cerebellum, hippocampus and striatum). However, a detailed biochemical mechanism by which mercury accelerates mtDNA damage has not yet been identified. Administration of polyphenol extract of *Artemisia absinthium* L. (500mg / L / day/ 10 weeks) reduced formation of 8-OHdG in mtDNA in all cerebral structures including cortex, cerebellum, hippocampus and striatum. These results indicate that treatment with polyphenol extract of *A. absinthium* L. may present a higher capacity to reduce the deleterious effect on the neuronal death and against mercury induced oxidative stress, mtDNA oxidation and neurodegeneration

Keywords: 8-hydroxy 2-deoxyguanosine (8-OHdG); mtDNA oxidation; polyphenol extract; mercury

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ORAL PRESENTATION

THE EFFECT OF SALT AND DROUGHT STRESS ON GERMINATION AND EARLY SEEDLING GROWTH OF *Lavandula stoechas* LINN. SEEDS

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Nowadays, with the increasing interest of use of organic and of herbal, aromatic and medicinal plants (HMAPs) products globally, it is important to incorporate wildering plant species, grown naturally in nature into agriculture production. Collecting medicinal and aromatic plants from nature and serving them on market is an important source of income, especially for rural people. To think that medicinal and aromatic plants will be maintained to be a source of livelihood for rural areas, as these plants are recommended to growers in the evaluation of arid, salty and sloping lands would not be wrong. In this study, seeds were collected from *Lavandula stoechas* plants, naturally growing in Kemer/Antalya Region and their potential tolerance against to salinity and drought was investigated. In order to determine the salinity impact on germination and early seedling growth characteristics, seeds were exposed to at six level NaCl concentrations treatments (0, 50, 100, 150, 200 and 250 mM). To create drought stress, PEG solution was used and seeds were germinated under at six different drought levels (0 -2, -4, -6, -8 and -9.8 MPa). The effects of stress factors were tried to be determined by being used the characters of germination ratio, radicle length and weight, plumule length, and weight. As a result of the studies, *L. stoechas* seeds germinated at 0, 50 and 100 mM NaCl concentrations and drought levels of 0, 2, 4 and 6 MPa. The effects of different salt concentrations on germination ratio, plumule and radicle length and the effects of different drought levels on germination ratio, plumule and radicle length, plumule and radicle weight were found to be significant was to be revealed.

Keywords: *Lavandula stoechas*, salt stress, drought stress, germination, seedling development

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ORAL PRESENTATION

THE MINERAL AND HEAVY METAL CONTENTS OF WILD MEDICINAL PLANT *Taraxacum scaturiginosum* G. E. HAGLUND GROWING IN VAN CITY

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The genus *Taraxacum* belongs to the Asteraceae family and comprises 43 species grown wild in Turkey. *Taraxacum scaturiginosum* is of the commonly consumed for medicinal purposes by inhabitants in Eastern Anatolia. It has been used in traditional Anatolia medicine for liver and gallbladder disorders, hemorrhoids, gout, rheumatic disorders, diuretic, eczema and other skin disorders. Wild plants gathered from nature are cheaper food and important for human health. Thus, in the present study nutritional value and mineral composition of used parts of selected *Taraxacum scaturiginosum* G. E. Haglund Grierson plants were investigated. In laboratory analysis, dry matter, total ash, % N, crude protein, crude fiber and pH were examined as nutritional value. Useful minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) and heavy metals (Cd, Co, Cr and Pb) that hazardous elements for livings were also determined. Results of this research showed that *Taraxacum scaturiginosum* G. E. Haglund contains high value of macro elements such as sodium (0.93 mg/g), potassium (24.48 mg/g), magnesium (3.94 mg/g) and calcium (14.65 mg/g). Also, it has maximum micro-elements such as iron (208.55 mg/kg), manganese (22.62 (mg/kg), copper (29.22 mg/kg and zinc (21.35 mg/g). Therefore, we can conclude that it has high nutritional values from the viewpoint of mineral elements such as potassium, calcium, iron, manganese and zinc.

Keywords: Mineral content, wild plant, *Taraxacum scaturiginosum*, Van

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ORAL PRESENTATION

ASSESSMENT OF WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACTS OF *Pistacia lentiscus* L. LEAVES AND *Quercus ilex* L. BARK IN FULL THICKNESS SKIN EXCISION IN RATS

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Objectives: The objective of our study was to evaluate the wound healing effect of ethanolic extracts of pistachio (*Pistacia lentiscus*) leaves and holm oak (*Quercus ilex*) bark on full-thickness excisions (4 cm^2) in Wistar rats. **Methods:** Forty-two rats were divided into seven equal groups that received different treatments; two groups were treated with *Quercus ilex* bark extract (10% -30%), two other groups with *Pistacia lentiscus* leaves extract (10% -30%), one group received Cicatryl-bio® and considered as a positive control, a group treated with pure Petroleum jelly, and the last was left as an untreated negative control. The evaluation of the excision zones was estimated by a planimetric study using image processing software (ImageJ®) and histological examination of healed skin tissue. **Results**: The results revealed that *Pistacia lentiscus* leaves and *Quercus ilex* bark have to promote wound healing effect which is reflected by an acceleration of wound contraction and a reduction of the epithelialization period. **Conclusion**: *Quercus ilex* bark *and Pistacia lentiscus* leaves ethanolic extract-based ointments exhibited effective wound healing action in Wistar rat's skin excisions. Both preparations showed an accelerated healing process compared to rats from other groups. To the best of the author's knowledge, this is the first study of wound healing effect of pistachio (*Pistacia lentiscus*) leaves and holm oak (*Quercus ilex*) bark.

Keywords: *Pistacia lentiscus, Quercus ilex*, wound healing, rats, skin excision

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ORAL PRESENTATION

ETHNOBOTANICAL STUDY OF MEDICINAL PLANTS IN AIN ANTAR FOREST (NORTH-WESTERN OF ALGERIA)

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Today, despite the development of synthetic chemistry, the use of medicinal plants has retained an important place because of their effectiveness in various therapeutic procedures. Currently, their use is a critical element in human life. Indeed, traditional knowledge is transmitted from generation to generation, thus preserving this knowledge. An ethnobotanical study of medicinal plants was carried out in 2020 in the forest of Ain Antar located in the mountain range of Ouarsenis in the wilaya of Tissemsilt (North-Western of Algeria). This study is considered as a contribution to the study of medicinal plants that are used in traditional herbal medicine by the local population of Boucaid region.

This work was conducted to prepare firstly a floristic inventory of boucaid region while focusing on medicinal plants of this area, to conduct ethnobotanical surveys with its population and also to gather information relating to the traditional phytotherapy of AinAntar (the most used parts, diseases, the different categories of users). For this, a series of ethnobotanical survey was conducted using 320 question cards. The results have identified 44 medicinal species belonging to 28 families. The most important family is *Lamiaceae*. The leaves are the most parts commonly used and the most practiced mode of prepare remedies is the decoction. In addition, among all treated diseases, respiratory disease sand skin conditions are the most frequent. The collection and analysis of data have allowed the establishement of a catalog of medicinal plants and to gather all the information about the therapeutic practices uses by the local population. The results are a very valuable source of information for the study area and to the national medicinal flora. They could also be a database for further research in the field of phytochemistry and pharmacology.

Keywords: Ain Antar, herbal medicine, ethnobotany, traditional medicine.

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ORAL PRESENTATION

PREFORMULATION STUDY OF NANOEMULSION PREPARATION BASED ON ESSENTIAL OILS FOR WOUND HEALING

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Objectives: The wound infection is a problematic issue that alter the healing of the wounds. Therefore, the prevention of bacterial growth and the enhancement of tissue regeneration are the key factors for the improvement of the healing process. Essential oils (EOs) showed strong activity against the growth of bacteria. This study was performed to evaluate the antibacterial effects of essential oils via their incorporation into a nanoemulsion (NE) system. **Methods:** Oregano and Rosemary oils were extracted and analyzed by GC-MS (Agilent Technologies, US) to elucidate the main active substance(s) of the oils. The antibacterial activity of the selected two EOs was assessed and compared through Disc Diffusion method (for inhibition zone) and Broth Microdilution method (for minimum inhibitory concentration) by conducting the oils on one of the most common bacterial strains that are associated with wounds; *Pseudomonas aeruginosa*. The oil with higher antibacterial activity was then incorporated in a nanoemulsion system. Droplet size, zeta potential, and PDI values were then evaluated. **Results:** The main active substance of Oregano and Rosemary oils that is responsible for the antibacterial activity was carvacrol (50.61%) and 1,8-Cineole (39.35%), respectively. The inhibition zone diameter was (10.5 mm) for oregano oil and (10 mm) for rosemary oil, while the MIC for oregano was (0.77 µg/ml) and for rosemary was (3.92 µg/ml). The optimum NE formulation achieved with oregano as an active ingredient and Kolliphor RH® 40 as a surfactant (BASF®, Ludwigshafen, Germany). The droplet size, zeta potential, and PDI results were 16.94 nm, -6.79 mV, 0.208 respectively. **Conclusion:** The obtained results showed that oregano oil is more effective than rosemary oil against the selected bacterium. Therefore, the bacterial growth in the wounds will be inhibited leading to speeding-up the inflammatory phase of the healing process, hence the overall integrity of the skin will be restored faster.

Keywords: Essential oils, wound healing, antibacterial activity, topical formulation, nanoemulsion

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ORAL PRESENTATION

STANDARDIZATION STUDIES ON SILVER LIME (*Tilia tomentosa* MOENCH.) USED AS A TRADITIONAL MEDICINE IN TURKEY

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Tilia species are generally trees that can reach up to 15-40 m height, their flowers are mostly yellowish white colored with nice fragarence. Among them *T. cordata* Miller (small-leaved lime) and *T. platyphyllos* Scop. (large-leaved lime) are registered officinally in many pharmacopoeias as a medicinal drug. However, these species are distributed rare (*T. platyphyllos*) or absent (*T. cordata*) in Turkey. On the other hand, *T. tomentosa* Moench. (syn. *T. argentea* Desf.) is a widespread species in Marmara Region (Turkey). The plant is called as silver lime due to the silvery color of the lower surface of leaves mainly due to dense stellate trichomes accumulation. Silver lime inflorescence (flowers with bracts) contains mucilages (6-8%), flavonoids (1%) and essential oils (0.05%) and exerts sedative, antispasmodic (essential oil), anti-inflammatory (flavonoids), expectorant and emollient (mucilage) effects etc. This study aimed to compare the characteristic specifications of officinal species with *T. tomentosa* collected from various localities in Marmara Region within the scope of preparation of a monograph of "Silver Lime Inflorescence" for Turkish Pharmacopoeia. Accordingly, macroscopic and microscopic examination were first performed. Then marker components were determined by high-performance thin-layer chromatography (HPTLC). Foreign matter, total ash, swelling index and loss in drying tests were done. Lastly, the amounts of tiliroside and isoquercitrin, active marker components, were calculated by a validated high-performance liquid chromatographic (HPLC) method.

Keywords: *Tilia tomentosa* Moench., pharmacopoeial monograph, standardization

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ORAL PRESENTATION

PROSPECTIVE *CRATAEGUS* SPECIES FROM LIBYA AND NORTHERN CYPRUS FOR PRODUCTION OF HERBAL MEDICINE

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Recently, the interest in herbal medicine has been growing worldwide which has raised the international trade of herbal medicine and attracted pharmaceutical companies in commercializing herbal drugs against important health disorders such as heart problems. *Crataegus* species are a topic of concern in treatment of disease related primarily to the cardiovascular system. A number of pharmacologically active metabolites mainly flavonoids are responsible for its therapeutic activity. Today, a number of herbal medicinal preparations of *Crataegus* are manufactured and sold in the pharmacies especially in Europe [1]. Even then only a few species are included in the Pharmacopoeias [2]. In this study major bioactive flavonoids in *Crataegus pallasii* collected from Libya and *Crataegus azarolus* collected from Northern Cyprus are identified and quantified in comparison with Crataegutt® Tropfen (*Crataegus* phytomedicine) using reverse-phase – high-performance liquid chromatography (RP-HPLC-DAD). The method developed is simple, fast, reliable, and sensitive. Prior to HPLC analysis, a novel approach''Indirect-Dispersive Liquid-Liquid Microextraction, (IDLLME)''is proposed to reduce matrix effect. Validation parameters of the method were calculated as follows: LOD ranged from 0.4 to 3.4 mg/g and LOQ from 1.3 to 11.3 mg/g, intraday, and interday precision expressed as %RSD ranged from 1.0 to 2.8 and 1.5 to 4.3, respectively and r^2 values were above 0.9950 for all analytes. The relative recovery of all compounds was more than 98%. Four predominate peaks were identified using certified standards as vitexin 2"-*O*-rhamnoside, rutin, vitexin, and hyperoside, with mass concentration (%w/w) in *C. pallasii* as 4.4, 2.6, 1.4 and 4.8%, in *C. azarolus* as 4.4, 2.9, 1.7 and 4.4% and in Crataegutt® Tropfen as 1.6, 1.0, 0.6 and 0.4% respectively. Thus, the values met the criteria of the United States Pharmacopeia (USP) and the European Pharmacopeia (EP) monographs [3]. Our investigations postulate that *C. pallasii* and *C. azarolus* could be a good source for the production of *Crataegus* phytomedicines.
Keywords: *Crataegus*, vitexin, rutin, hyperoside, vitexin-2-0-rhamnoside, RP-HPLC.

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ORAL PRESENTATION

BIOACTIVE COMPOUNDS, ANTIOXIDANT AND ACE-INHIBITORY ACTIVITIES OF *Hippophae rhamnoides* L. PLANTS AND *IN-VITRO* BIOACCESSIBILITY STUDIES

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The genus *Hippophae rhamnoides* L. (sea buckthorn) belonging to the Elaeagnaceae family (1) is widely grown in Central Asia, China, Siberia, and North and East Anatolia region of Turkey (2). Parts of the sea buckthorn such as fruits, leaves, roots and bark are used for their antihypertensive, antidiabetic, antimicrobial, anticancer, and antioxidant activities as they contain mainly polyphenols and carotenoids (2,3). The objectives of this study were to determine phenolic and carotenoid content, *in-vitro* bioaccessibility, and to measure ACE-inhibitory and antioxidant activities (by DPPH and CUPRAC assays) of the sea buckthorn fruits, leaves and branches obtained from Bayburt, Turkey. According to the results, sea buckthorn leaves (99.15 mgGAE/gDW) contained more phenolics than branch (82.61 mgGAE/gDW) and fruit (17.24 mgGAE/gDW) parts. Similarly, sea buckthorn leaves had the highest amount of flavonoids (141.50 mgRE/gDW) followed by branches (84.39 mgRE/gDW) and fruits (15.74 mgRutin/gDW), respectively ($p<0.05$). As a result, leaves exhibited the highest antioxidant activity in terms of DPPH (191.91 mgTE/g) and CUPRAC (355.41 mgTE/gDW) assays as well as ACE-inhibitory activity (79.13%). The ACE-inhibitory activity of the leaves of was followed by branches (60.12%) and fruits (59.09%) ($p<0.05$). Catechin was the major phenolic compound found in leaves (23.18 mg/g) and branches (7.25 mg/g). Moreover, leaves included EGCG (2.28 mg/g) and caffeine (1.7 mg/g). On the other hand, Sea buckthorn fruits contained total carotenoids (67.28 mgβcarotene/100g DW) and total anthocyanins (4.03 mgCYN-3-GLY/100gDW). Only fruits were exposed to *in-vitro* digestion procedure under simulated gastrointestinal tract conditions since they are edible parts. After *in-vitro* digestion, 12% of total phenolics and trace amounts of total anthocyanins and total flavonoids were recovered. In conclusion, sea buckthorn fruits are a good source of carotenoids. More importantly, sea buckthorn leaves may have the potential to be natural sources of antioxidants and ACE inhibitors and may be employed in dietary supplements.

Keywords: Sea buckthorn, Polyphenols, Carotenoids, Angiotensin converting enzyme inhibition activity, Antioxidant activity, *In-vitro* bioaccessibility

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ORAL PRESENTATION

IN-VITRO ANTIMICROBIAL ACTIVITIES OF SOME BEE PRODUCTS

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Objectives: Bee products, which have benefited from their advantages during the treatment of numerous diseases from the past to the present, are now used in medicine under the name of Apitherapy. Studies on the subject have presented that bee products have antifungal, antibacterial and antiviral properties. As these researches coincide with the trend of avoiding the side effects of drugs and natural nutrition that people are very sensitive to in last decades, interest in clean label products, natural medicines and bee products has increased. **Methods:** This study includes the in vitro antibacterial activity of bee products consisting of 8 various honey and 10 various propolis products were analsed against 7 Gram (-) bacteria and 5 Gram (+) bacteria, yeast, and *Mycobacterium smegmatis*. Drops containing 15% and 30% propolis, spray containing 6% propolis, as well as raw honey mixtures containing 1%, 2% 10% propolis, were analysed by agar well diffusion method after that the diameters of the area of antimicrobial activity were measured and compared. **Results:** The research results pointed out that total phenolic content of the samples ranged between 0.0-108.1 mg GAE/ml and total flavonoid contents were between 0.0-174.5 mg CE/ml. According to the antioxidant capacity (CUPRAC) analysis, the sample's total antioxidant capacity was found to be between 0.0 and 555 mg TE/ml. According to phenolic profiles of the 20 propolis product samples, phenolic compounds from propolis were detected in only 7 samples. Antioxidant capacity and phenolic profile analysis results of only 4 samples are consistent with the amount of propolis indicated on the label. When the results of the study were examined, it was observed that the products in drop form were effective against all Gram (-) and Gram (+) bacterial species analysed. An essential antimicrobial activity was detected chiefly against *Helicobacter pylori* (50 mm), *Bacillus cereus* (15-18 mm), *Staphylococcus aureus* (18-20 mm) and Mycobacterium smegmatis (18-19 mm). When the measurements were interpreted, it was seen that both the spray product and the raw honey mixtures with propolis containing 10% propolis exhibited antimicrobial activity against total 12 various bacteria and yeast species. Besides, inhibition zones ranging from 6 to 45 mm were measured. **Conclusion:** In addition to propolis products, both 4 various manuka honey and Turkish chestnut, astragalus, pine, Hakkari honey were analyzed against ten various microorganisms and the results were compared also. If the data that stands out as a result of the comparisons are listed; against *Staphylococcus aureus*, pine honey is as powerful as manuka honey, and chestnut honey has higher antimicrobial activity against Bacillus cereus, *Citrobacter freundii* and *Escherichia coli* than other samples. Besides, Hakkari region honey has shown antimicrobial activity against two bacterial species and *Candida albicans*. All in all, the adored bee products examined in this study were found to have a high antimicrobial effect.

Keywords: Propolis, Turkish honey, pine honey, antimicrobial, antibacterial, bee products

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ORAL PRESENTATION

INVESTIGATION OF ANTIOXIDANT PROPERTIES OF PROPOLIS PRODUCTS FROM THE WORLD MARKET

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Objectives: The resinous bee product collected by bees from the leaves and buds of trees such as poplar, eucalyptus, alder, pine and chestnut are called propolis. The flavonoids and phenolic acids present in large amounts in propolis play an essential role in its biological activity. The affluence of propolis in terms of these components makes it a powerful antioxidant source. **Methods:** In this research, 50 Anatolian raw propolis samples were investigated based on their wax, total phenolic content, total flavonoid content, and antioxidant capacities. Besides, 130 liquid form propolis products collected from 19 diverse regions were examined based on dry matter, total phenolic, flavonoid content, and antioxidant capacities. Moreover, phenolic profiles of the 20 propolis product samples with antioxidant capacity higher than 100 mg TE/ml were pursued via HPLC-UV and LC-MS/MS. **Results:** The research results pointed out that total phenolic content of the samples ranged between 0.0-108.1 mg GAE/ml and total flavonoid contents were between 0.0-174.5 mg CE/ml. According to the antioxidant capacity (CUPRAC) analysis, the sample's total antioxidant capacity was found to be between 0.0 and 555 mg TE/ml. According to phenolic profiles of the 20 propolis product samples, phenolic compounds from propolis were detected in only 7 samples. Antioxidant capacity and phenolic profile analysis results of only 4 samples are consistent with the amount of propolis indicated on the label. **Conclusion:** It is essential to present propolis, which is one of the bee products whose popularity is increasing day by day as a natural healing source, in its most beneficial form. Hence, it is the most significant step to ensure that consumers are correctly informed about the content of the products. Moreover, a propolis standard including propolis extraction methods should be established to regulate the content of the products and to prevent unethical competition in the market.

Keywords: Anatolian propolis, antioxidant, phenolic, flavonoid

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ORAL PRESENTATION

SIGNIFICANCE OF SEED COAT MORPHOLOGY IN SOME *ISATIS* (BRASSICACEAE) TAXA

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The seed exomorphic characteristics of five taxa (*I. arenaria* Azn., *I. candolleana* Boiss., *I. cappadocica* Desv. subp. macrocarpa (Jaub. & Spach) P.H. Davis, *I. erzurumica* P.H. Davis and *I. floribunda* Boiss. ex. Bornm. belonging to *Isatis* L. were investigated with scanning electron microscopy (SEM). This study presents exomorphic characteristics, including seed colour, shape, winged, measures, seed coat pattern. Three coat patterns (regularly reticulate, irregularly-reticulate, and rugose) were observed, and four shapes (narrowly elliptic, narrowly ovate, oblong and lanceolate) were distinguished. The results showed that the morphological characteristics of seed could contribute as criteria to distinguish taxa.

Keywords: classification, Cruciferae, micromorphology, taxonomy

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ORAL PRESENTATION

EVALUATION OF ANTIBACTERIAL AND WOUND HEALING ACTIVITIES OF THE COMBINATION OF *Foeniculum vulgare*, *Cinnamomum verum*, AND *Syzygium aromaticum* ESSENTIAL OILS IN DIABETIC SEPTIC WOUNDS

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Objective: This study was conducted to assess the antibacterial and wound healing potential of the combination of some essential oils against diabetic wounds associated bacteria. With the increasing of microbial resistance, there is a need to develop a multidisciplinary medicament that has ability to treat the infection and then promotes the healing process in diabetic patients. **Methods:** The checkerboard test was used to assess the interaction between essential oils, then it was tested against control bacteria and clinical isolates. *In vivo* trial was done in infected wounds of alloxan induced diabetic albino rats. The potential of wound healing was assessed by contraction and confirmed by histopathological analysis. *In vivo* antibacterial activity was determined by means of viable counting technique. **Results**: The combination exhibited a synergistic effect against the tested bacteria *in vitro*. Similarly, to the group that treated with Fusiderm ointment, on day (14), the group that contains the essential oils blends showed 100% wound contraction compared to control rats (67.1%). The total bacterial count from the pus on day (4) revealed that the application of the EOs based ointment resulted in a diminishing level of total *S. aureus* count. There was a major reduction from 10^8 CFU/ml to 4×10^4 CFU/ml, while Fusiderm and control groups recorded 10^3 and 5×10^5 CFU/ml respectively. **Conclusion**: The EOs combinations of fennel, cinnamon, and clove had antimicrobial activity *in vitro* and *in vivo* against aerobic and anaerobic bacteria and accelerated the wound healing process.

Keywords: *In vivo* activity, Essential oils, synergism, wound healing, albino rats.

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ORAL PRESENTATION

DETERMINATION OF YIELD AND QUALITY CHARACTERISTICS OF DIFFERENT CORIANDER (*Coriandrum sativum* L.) POPULATIONS IN EASTERN MEDITERRANEAN CONDITIONS

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Objectives: Coriander (*Coriandrum sativum* L.) is an annual, herbaceous culinary plant which originated from the Mediterranean and Middle Eastern regions. The coriander leaves and seeds have usage with many biological properties including antioxidant, anthelminthic, anti-inflammatory, anti-mutagenic activity etc. The plant known as "aş otu" in Turkish colloquial language while "cilantro" in USA. Fresh or dry herb of the plant used after made brine. **Methods:** In the present study, eleven different originated coriander seed were cultivated under East Mediterranean climatic conditions. The plant height (cm), number of branches per plant, fresh herb yield (kg/da), dry herb yield (kg/da), fresh herb essential oil content (%) and essential oil yield (lt/da) were determined. **Results:** Highest fresh herb yield was obtained as 1902.54 kg/da, higherst dry herb yield were found as 279.93 kg/da and highest essential oil yield found as 0.51 lt/da from Altınözü (Hatay) originated plants. **Conclusion:** The populations with higher results were choosen for further investigations.

Keywords: *Coriandrum sativum* L., coriander, Apiaceae, essential oil yield, leaves, herb

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ORAL PRESENTATION

INACTIVATION OF *K. pneumoniae* and *S. aureus* PATHOGENS INOCULATED TO RICE BY *Laurus nobilis* AND *Thymbra spicata* ESSENTIAL OIL

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Laurus nobilis L. known also "bay laurel" is a perennial, tree from family of Lauraceae. Leaves and fruits could use in cosmetic, food and medicinal industries. Turkey is one of the main exporters of bay laurel leaf. *Tyhmbra spicata* L. also known as "spike thyme" is a perennial shrup from Lamiaceae family. *L. nobilis* and *T. spicata* are both important due to their essential oil composition. In the study main components of bay laurel essential oil found as eucalyptol (54.15%), α-terpinyl acetate (16.27%) and sabinene (6.08%) while main components of *T. spicata* were γ-terpinene (23.03%), o-cymene (5.05%) and carvacrol (60.65%). While Minimum Inhibitory Concentrations (MICs) of *L. nobilis* and *T. spicata* on *Klebsiella pneumoniae* were 14.04 mg/mL and 1.2 mg/mL, respectively, The MICs of *L. nobilis* and *T. spicata* on *Staphylococcus aureus* were 14.42 and 1.3 mg/mL, respectively by Spectrophotometric Broth Microdilution. The inhibition zones (IZ) of *L. nobilis* and *T. spicata* were 2.31 mm and 17.16 mm for *K. pneumoniae*, they were 1.5 and 5.01 mm for *S. aureus* by Well Diffusion Method. In the test of inactivation of bacteria inoculated to rice by *L. nobilis* and *T. spicata* EO, *L. nobilis* caused 0.06 log10 CFU/mg reduction on *K. pneumoniae* and 0.07 log10 CFU/mg reduction on *S. aureus* at 150 µL of the the EO. *T. spicata* caused 0.77 log10 CFU/mg reduction on *K. pneumoniae* and 0.62 log10 CFU/mg reduction on *S. aureus* at 150 µL of the the EO. *T. spicata* were more effective on pathogens than *L. nobilis* in this study.

Keywords: Thyme, bay laurel, *Staphylococcus aureus*, *Klebsiella pneumonia*, anti-bacterial

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ORAL PRESENTATION

CHARACTERIZATION AND EVALUATION OF PHENOLIC COMPOUNDS OF BLACKBERRY PULP WILD (*Rubus sp.)* FROM THE DISTRICT OF TINTAY PUNCO VRAEM-HUANCAVELICA- PERU

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The objective of the present investigation was to determine the phenolic compounds in the concentrated pulp and without concentrating in two stages of maturity of the wild fruit *Rubus* spp from the District of Tintay Punco VRAE- Huancavelica -Perú. Branches, flowers, leaves and fruits were collected for the identification of the fruit, carried out according to Cronquist (1981) and The Angiosperm Phylogeny Group (APGIII-2009) in the Biology laboratory of the National Agrarian University of La Molina, his identification was made based on morphological studies: Genus Rubus, Family Rosaceae, of the order Rosales. The identification resulted in the fruit corresponding to the species *Rubus urticifolius* Poir. The proximal analysis of the pulp of ripe fruits (M) were: humidity 79.72% ± 2.49, carbohydrates 12.39% ± 2.02, ash 2.8% ± 0.38, fiber 3.23% ± 0.09, fat 0.015% ± 0.001 and protein 1.84% ± 0.0; in physiological mature (MF) humidity 79.93% ± 1.1, carbohydrates 12.35% ± 1.09, ash 3.55% ± 0.28, fiber 2.5% ± 0.00, fat 0.0% ± 0.00 and protein 3.75% ± 0.00. From where it could be stated with 95% confidence that significant differences were found in ash, fiber and protein $p < 0.05$; In the pulps concentrated at 20º Brix the polyphenols reached 672,000 ± 0.03 and 395,810 ± 0.05 in M and MF respectively, finding significant differences $p < 0.05$. The antioxidant capacity of concentrated pulps was 661.7 ± 3.1 and 666.70 ± 5.6 (mg GAE / 100g) M and MF respectively, there was no correlation of polyphenols and antioxidant capacity. Concluding that the concentrated pulp of the mature fruit of the *R. urticifolios* Poir has a higher polyphenol content than the pulp of the physiological mature fruit.

Keywords: *Rubus*, Blackberry, VRAEM

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ORAL PRESENTATION

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF *Salvia sagittata Ruiz & Pav*

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The objective of the present study was to extract the essential oil, leaves, stems and flowers of *Salvia sagittata Ruiz & Pav;* from the province of Tarma, to determine its physicochemical composition and antibacterial activity. The major components of the oil, such as Caryophylene, were determined in a fresh and dry state with the use of gas chromatography coupled to mass spectrometry. The inhibitory activity was at 25%, 50%, 75%, 100% dilutions, by measuring the diameter of growth inhibition by the diffusion method of disk agar against *Escherichia coli* ATCC 25923 and *Staphylococcus aureus* ATCC 25922. Caryophylene 37.75% and 43.73% were identified in fresh and dry essential oil; Alpha-terpinyt acetate 10.51% and 6.50%; Bicycle (3.1.1) h ept-2-ene, 2.6 dimethyl 1-6-4 10.42% and 0%; Humulene 9.93% and 10.51%; Alfa- Cubebene 5.18% and 5.8%; Trans-alpha Bergamot 0% and 8.68% and others to a lesser extent Linalool 1.15% and 1.19%, Toluene 0.10% and 0.21%, Camphene 0.20% and 0.19%, Copaene 0.18% and 0.14%, Germacrene D 2.39% and 0.84%, respectively. The wet and dry yield was 0.023 ± 0.005 and 0.136 ± 0.012; refractive index 1,460 + 0.0075 and 1,491 + 0.0072; acid number (mg of KOH / 1 g) 0.072 + 0.004 and 0.081 + 0.003; pH 6.533 + 0.0577 and 6.333 + 0.0577, respectively. Better growth inhibition of *Escherichia coli* and *Staphylococcus aureus* was obtained at 100% concentration of essential oil in the fresh and dry state, being able to inhibit 32.94%, in the minimum inhibitory concentration (MIC) against E. coli and S. aureus it had a value of 50% or 22,025 mg / ml in the fresh and dry state and for both microorganisms, the concentration of the lowest degree of absorbance fluctuation with a straight-line graph tendency. respectively; in conclusion, the different types of monoterpenes are the predominant components of the essential oil and have inhibitory properties against these pathogenic bacteria.

Keywords: *Salvia sagittata* Ruiz & Pav, antibacterial activity, caryophyllene

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ORAL PRESENTATION

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF PACHA MUÑA ESSENTIAL OIL (*Hedeoma mandoniana* Wedd) EXTRACTED BY SUPERCRITICAL FLUIDS

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The objective of this research was to extract the essential oil from the leaves of the pacha muña (*Hedeoma mandoniana* Wedd), coming from the province of Tarma, by means of the supercritical fluid method from the dry plant, its physicochemical composition was determined, chemistry and antibacterial activity. A yield of 2.2% ± 0.04, density 0.976 ± 0.003 and refractive index 1.498 ± 0.055 were obtained. 24 components were identified that were analyzed by Gas Chromatography coupled to Mass Spectrometry (GC / MS), the majority component being the Pulegone oil in 60.54%, and in a lesser percentage the 1-0cten-3-yl-acetate 12.39 %, D-Limonene 4.28%, Linalool 3.52%, 1-Butanol 2-methyl-acetate 2.85%, alpha-Pinene 2.80% among others. Antimicrobial activity was evaluated by means of the agar diffusion method (Kirby Bauer method) against Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923), demonstrating that the essential oil of *Hedeoma mandoniana* Wedd has a high percentage of inhibition against the Staphylococcus aureus with an inhibition halo of 17.08 ± 0.51mm and an inhibition percentage of 121.05 ± 3.64%, and is intermediate compared to Escherichia coli with an inhibition halo of 16.43 ± 1.91mm and an inhibition percentage of 53.22 ± 6.19%. In addition, the essential oil of pacha muña medicinal plant has different types of terpenes, terpenes with alcohol groups, monoterpenes, which have inhibitory properties against these pathogenic bacteria.

Keywords: *Hedeoma mandoniana* Wedd, antibacterial activity, oil essential, Pulegone, minimum inhibitory concentration, aromatic plants.

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ORAL PRESENTATION

DEVELOPMENT OF NANOFORMULATION CONTAINING WHALE HERB OIL

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Whale herb (*Cordia verbenaceae*) is a shrub plant native to the Atlantic Forest region that has anti-inflammatory, healing, analgesic, antifungal, diuretic, laxative and muscle relaxant properties. Its leaves have a group of chemical compounds called monoterpenes, triterpenes, flavonoids and fatty acids. The delivery of natural products in nanostructured formulations can enhance the permeation and penetration power of these compounds, optimizing the therapeutic action. The present study aims to develop a nanoformulation containing *Cordia verbenaceae* oil. The oil was extracted by hydrodistillation. The development of formulations (A, B and C) occurred by the phase inversion method using the components hydrogenated castor oil, sorbitan monolaurate, pure water, polymer and whale oil. The macroscopic stability of the formulation was evaluated after 24h of preparation, observing the presence or not of signs of instability, such as precipitation and phase separation. The characterization was carried out regarding the average diameter, polydispersity index (PI) and zeta potential. Whale herb oil was solubilized in hydrogenated castor oil, PEG and DMSO in formulations A, B and C, respectively. Visually formulations A and C incorporated 100% of the oil and proved to be stable. However, formulation B did not incorporate all the oil, causing separation of the nanoemulsion in two phases. The average diameter of formulation A was 181.6 ± 1.48. The IP value of the formulation was less than 0.3 (0.298), characterizing it as monodispersed. The zeta potential was -44.2 ± 1.12. Formulation A was selected in the study because it is stable and within the parameters of characterization of nanoemulsion, and may be a good alternative for enhancing the therapeutic effects of whale herb oil.

Keywords: Oil, herb, nanoemulsion.

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ORAL PRESENTATION

STUDY AND RATIONAL USE MEDICINAL PLANTS IN CONDITIONS OF APSHERON

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Azerbaijan, Baku, Mardakan settle., S. Yesenin str.89, Az 1044

Despite the ever-expanding range of synthetic drugs, herbal products not only have not lost their importance, but, on the contrary, their use is increasing every year. Synthesis of many natural substances produced by plants has not yet been mastered. Cardiac glycosides do not have equivalent synthetic substitutes to this day. A significant amount of drugs is still made up of plants. The study and rational use of natural herbal medicinal resources, the study of the geographical distribution and their reserves, the identification of substitutes for scarce plants and new medicinal plants in various republics, territories and regions, including Azerbaijan, is of great importance.

In this regard, the Institute of Dendrology Azerbaijan National Academy of Sciences is studying bioecological features, medicinal features of trees, shrubs and herbaceous plants in an open area, in greenhouses and their rational use in scientific and in folk medicine in conditions of Apsheron. In the scientific research work in the open area and in the Dendrary greenhouses, various species of the following genera of medicinal trees, shrubs and herbaceous plants were studied: *Elaeagnus* L., *Morus* L., *Zizyphus* Mill., *Punica* L., *Citrus* L., *Laurus* L., *Olea* L., *Acacia* L., *Ruta* L., *Syringa* L., *Juniperus* L., *Artemisia* L., *Rosa* L., *Rosmarinus* L., *Matricaria* L., *Eucalyptus* L., *Cupressus* L., *Pinus* L., *Berberis* L., *Vinca* L., *Buxus* L., *Magnolia* L., *Datura* L., *Laurocerasus* Roem., *Viola* L., *Veronica* L., *Aloe* L., *Euonymus* L., *Nerium* L., *Quercus* L., *Pistacia* L., *Feijoa* Berg., *Calendula* L. and etc.

As a result of scientific research work, it was found that the study species of medicinal plants are perspective, have many medicinal features, adapted well in conditions of Apsheron and recommended their rational use in scientific and in folk medicine of Azerbaijan.

Keywords: Medicinal plants, rational use, herbal plants, folk medicine

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ORAL PRESENTATION

RESEARCH ON CANCER PREVENTION CAPACITIES OF OLIVE STONES AS A POTANTIAL CANDIDATE FOR MATRIX METALLOPROTEINASE INHIBITORS

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Olive tree *(Olea europaea* L., fam: *Oleaceae*), a well-known medicinal plant used traditionally for medicinal purposes because of its 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, and aromatherapy. Although 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 and rich source of cellulase, hemicellulose, lignin and polyphenols. Thus, this research aimed to investigate cancer prevention and matrix metalloproteinases (MMPs) inhibitor activities of green olive stones could be assumed as the first report for the literature. The MMPs are represented by a family of proteolytic enzymes, and increased MMP-2 and MMP-9 activities are determined in cancer cells and tissues. Green olives, known as 'KilisYağlık', were collected from Kilis province, crude extracts were prepared and then fractioned in this research. The anticancer effect of the stone extracts was tested on human colorectal carcinoma (HT-29, ATCC® HTB-38) and gastric carcinoma SNU-5, ATCC® CRL-5973) cell lines with a focus on matrix metalloproteinase (MMP) activity and MMP-linked pathways. Cell viability was determined using MTT and LDH leakage assays as previously described [1,2,3] and cell invasion assay was conducted through the BD Matrigel invasion chambers [2,3]. In the presence of increased concentration of olive stones extract MMP-2 and MMP-9 activities were shown to be decreased in a concentration dependent manner. In conclusion, the findings herein suggest that stones of green olive tree include anti-MMP bioactive constituents, which might inhibit MMP activity and suppress MMP-linked pathways. Indeed, effective anti- MMP properties may possibly be due to of the olive stones' rich secondary metabolite content. It is worth to note that, the results of our screening on the olive stones-extracts could provide valuable scientific evidence for cancer researches however detailed further analyses are necessary to discover plant-based pharmaceutics.

Keywords: Green olive, KilisYağlık, olive stones, MMP, cancer prevention, pathway

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ORAL PRESENTATION

ANALYSIS OF BIOACTIVE COMPOUNDS OF CULTIVATED GARLIC (*Allium sativum* L.) AND ONION (*Allium cepa* L.) IN ALGERIA

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In all times mankind has used several species of the genus *Allium* as food, spice, or herbal remedy. Some of these species have been cultivated, such as garlic (*Allium sativum*) or onion (*Allium cepa*). Today, their value for human health care is one of the most important aims of research, and up to now many applications of *Allium* species are known for the use of phyto-pharmaceutical preparations. Therefore the present study aimed to determine the phytochemical profile of cultivated varieties of garlic (*Allium sativum*), and red onion (*Allium cepa*) in Algeria, quantitatively (Total phenolic contents, Total flavonoids contents, Condensed and Hydrolysable tannins contents) and qualitatively (Phytochemical screening), to characterize the phenolic compounds by HPLC method and to evaluate the antioxidant properties by measuring radical scavenging activity using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

The best extract yield recorded by the maceration method was for garlic in comparison to red onion, with the values of 62.87±0.001% and 57.38±0.33% respectively. The determination of total phenolic compounds in methanol extract of garlic and onion using Folin Ciocalteu reagent gave an amount of 45±0.33 mg GAE /100g; 86±0.33mg GAE /100g of dry matter respectively. Flavonoids content in garlic and red onion, were 34.62±0.066mg QE /100 g, 43.1±0.001mg QE /100g, of dry matter respectively. Condensed and hydrolysable tannins analysis gave a value of 6.8±0.33 mg CE /100g, 4.4±0.001 mg CE/100g and 0.04±0.33 mg TAE /100g 0.22±0.066 mg TAE /100g of dry matter respectively. The phytochemical screening of above extracts revealed the presence of flavonoids, tannins, anthocyanins, steroids, terpenoids, cardiac glycosides and mucilage. Chromatographic analysis of the samples showed that five phytochemical molecules were identified in onion methanolic extract: (Gallic acid, Quercitin, Rutin, Hyperoside and Karempferol) and one molecule in garlic methanolic extract: (Gallic acid).

The antioxidant activity of the methanol extract of our plants gave IC 50 values; IC50= 919.87 µg/ml, 420.9µg/ml, for garlic and onion, respectively. This research has shown that our plants are rich in phenolic compounds such as flavonoids and tannins, and possessed a good antioxidant activity.

Keywords: *Allium sativum* L., *Allium cepa* L., phytochemistry, HPLC, DPPH, Algeria.

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ORAL PRESENTATION

IN VITRO AGE DEPENDENT CELL-MEDIATED RESPONSES TO ANTIMICROBIAL PLANT EXTRACTS IN FREE RANGE MANGALITZA PIGS

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Objective: Mangalitza pig bread is raised for tasty lean meat (Radovic et al., 2019), mainly on low input farms. The microbial aggression outcome highly depends on pig's immunity, and medicinal plants, available on the pastures or in the fodder pigs get, could act as immunity enhancers, strengthening the pigs' resistance to diseases. **Methods:** The research was carried out on extensively raised Mangalitza suckling, weaned piglets and sows (n=10 for each group). Blood was sampled during the official campaign for brucellosis testing, with regard to ethical and animal welfare provisions, and subjected to blast transformation test. For that, the blood was mixed 1:4 with RPMI1640 (Sigma Aldrich, USA), divided in 200µl aliquots in 96 well-plates and supplemented with alcoholic plant extracts (*Calendula officinalis, Thymus vulgaris, Allium sativum, Coriandrum sativum, Cucurbita maxima*), 1.5 µl/well. The plates were incubated at 37ºC for 48 h, residual glucose was quantified spectrophotometrically (SUMAL PE2, Karl Zeiss, Jena) and glucose consumption was calculated (%). The groups were compared by Student's t test for statistical significance of the results. **Results:** The results indicated statistically significant differences between the young age groups, suckling and weaned piglets (p=0.017 to 0.000016) for all plants except marigold, for weaned piglets and sows (p=0.0001-0.0359) for all plant extract, while for suckling piglets – sows p=0.0035 and p=0.0461 were recorded for thyme and garlic, respectively. **Conclusion / Discussion:** The plant extracts used to know for biological effects impacted based on age of the pigs and plant family, proving their immune stimulating capacity.

Keywords: Mangalitza, low input, adaptive immune response, plant extracts

Reference:
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ORAL PRESENTATION

IN VITRO ADAPTIVE CELLULAR RESPONSE TO ALCOHOLIC PLANT EXTRACTS IN *Brucella ovis* INFECTED RAMS

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Objective: *Brucella ovis* infection still represents a threat by causing reproductive disorders and high economic impact in numerous sheep breeding countries (Lopez-Santiago et al., 2019). We hypothesized that serologically positive rams will have an enhanced reaction to immune stimulating plant extracts, due to their reactive status to the intracellular pathogen. **Methods:** Blood was sampled on heparin from three groups of rams, with positive, doubtful and negative complement fixation test (CFT) results. An *in vitro* blast transformation test was carried out using alcoholic extracts of *Taraxacum officinale, Achillea millefolium, Salvia officinalis, Arctium lappa, Equisetum palustre, Avena sativa, Hypericum perforatum*. Glucose concentration measurements (orto-toluidin method) were performed after 63 hours of incubation at 37ºC and stimulation indices were calculated. The statistical significance of the differences between the groups was interpreted by Student-s t test. **Results:** *Brucella ovis* infection non-significantly increased the spontaneous blast transformation (% - 35.42, 25.45 and 31.03 in positive, doubtful and negative groups, respectively). The highest responses were obtained for *Taraxacum officinale* (38.47%) and *Equisetum palustre* (37.4%). The differences in plant induced blast transformation indices were not supported statistically. **Conclusion:** The extent of the cell-mediated responses to plant extracts in chronically stimulated animals was lower than expected and the plants envisaged as coming from the pasture failed to exert a strong immune stimulating effect.

Keywords: Rams, brucellosis, CFT, medicinal plants, immunity

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ORAL PRESENTATION

AGRO-MORPHOLOGICAL AND YIELD PROPERTIES OF DIFFERENT FENUGREEK GENOTYPES AND CULTIVARS UNDER IRRIGATED AND DRY LAND CONDITIONS

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Fenugreek (*Trigonella foenum-graecum* L.) is one of the most important medicinal and aromatic plants used in the world through human history. It is cultivated widely in arid and semi-arid zones as irrigated or dry land conditions. It also adapts easily different soil properties. This study was conducted to determine the morphological and yield properties of 18 fenugreek genotypes and 3 cultivars under irrigated and dry land conditions. Randomized complete block trial design was used with three replicates in Bolu Abant İzzet Baysal University application and research area. Plant height (cm), branch number, first pod height (cm), pod number, biological yield (kg/da), 1000 seed weight (g), seed yield (kg/da) and harvest index (%) were determined as 38.42-68.03, 32.57-55.57 cm, 2.36-4.10, 2.0-3.50 number, 17.17-30.33, 3.30-24.80 cm, 8.67-20.0, 4.93-10.77 number, 191.26-713.48, 118.92-432.80 kg/da, 10.22-17.92, 11.22-20.72 g, 37.17-142.02, 31.14-141.37 kg/da and 16.87-28.22%, 19.60-32.05% in irrigated and dry land conditions, respectively. Generally, the highest values were found in irrigated land conditions in terms of examined properties, except 1000 seed weight and harvest index. As a result of the study, PI 568215 and PI 617076 genotypes can be selected to obtain the highest seed yield and biological yield in irrigated and dry land conditions.

Keywords: Fenugreek, irrigated and dry land, cultivation, medicinal and aromatic plant

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ORAL PRESENTATION

VARIATION IN SOME QUALITY PROPERTIES OF DIFFERENT BASIL GENOTYPES

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Ocimum basilicum L. (sweet basil) is an essential oil producing crop used in culinary and fragrance applications. The essential oils of sweet basil are used for the treatment of dry mouth and dental complaints, diarrhea and chronic dysentery, and respiratory disorders, and are also effective in the treatment of fungal diseases and stomach discomfort, in addition to having influential antitussive, diuretic, anthelminthic, tranquilizer and expectorant roles in medical applications. Present investigation comprised of thirty-seven genotypes of sweet basil was undertaken to characterize the genotypes based on chemical variation of leaves and stems like essential oil yield, mineral content, and protein content. Leaves and stems, previously air-dried at room temperature, were hydro-distilled for three hours. Results of this study revealed significant variations (0.11-1.67%) in essential oil content. Ames 32312 genotype was found superior in case of essential oil amounts as compared with other genotypes. The highest PO_4^{-3}, Cl^- and SO_4^{-2} contents were obtained in Ames 29184, Midnight, PI 296390 genotypes which were significantly different compared to the other genotypes. Overall, in Moonlight (%18.26) genotype exhibited the highest protein content.

Keywords: Basil, essential oil, protein, anion and cation

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ORAL PRESENTATION

YIELD, YIELD COMPONENTS AND SOME QUALITY PROPERTIES OF FENUGREEK CULTIVAR AND GENOTYPES

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Fenugreek (*Trigonella foenum-graecum*) belongs to fabaceae family. It is evaluated as multipurpose and commercially important spice plant. It has been used in alternative medicine through human history. Fenugreek has lots of health benefits and some of them are listed as diabetes control, decreasing blood sugar levels, cholesterol levels, inflammation, loss of appetite. This research was carried out to determine the yield and some quality properties of one cultivar (Güraslan) and 2 fenugreek lines (line 1 and line 2) in 5 different sowing times (October, November, December, March and April) under Ordu ecological conditions during 2013-2014 years. Experiments were designed and applied in split block design with three replications. 11 properties were examined. Plant height, pod number per plant, seed number per pod, pod length, branche number per plant, 1000 seed weight, biological yield, seed yield, harvest index, crude protein content, crude oil rate was determined as 30.13-63.90 cm, 3.0-11.33 number, 21.33-68.33 number, 10.50-14.0 cm, 1,0-4,33 number, 17-20.82 g, 161.48-378.44 kg/da, 45.64-86.98 kg/da, 19.91-43.08%, 4.64-5.32% and 2.26-4.93%, respectively. Significant differences were determined among the fenugreek cultivar and lines except crude protein content and crude oil rate. While the highest seed yield and crude oil rate were found in line 1 in second sown date (October), crude protein content was obtained in Gürarslan cultivar in December and line 1 genotype in April. Generally, line 1 can be selected for crude protein content and crude oil in fifth sown date (April) and line 2 can be chosen for seed yield second sown date (November).

Key Words: Fenugreek, yield characters, genotype, Bolu

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ORAL PRESENTATION

THE CHEMICAL CONTENTS OF *Salvia nemorosa* L. GROWING AROUND VAN LAKE

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The genus Salvia is the most important and the largest genus of Lamiaceae family with over 1000 species. Many species of the genus *Salvia* (Lamiaceae) are to be found in East Anatolia. Nutritional value and mineral compositions of wild edible plants have been investigated for food security and human health. It is though that wild edible plants gathered from nature are cheaper food and important for human health. Although they are cheap and available in the near, their mineral composition and nutritional value are important for public health. Thus, in the present study nutritional value and mineral composition of used parts of selected *Salvia nemorosa* L was investigated. In laboratory analysis, dry matter, total ash, % N, crude protein, crude fiber and pH were examined as nutritional value. Useful minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) and heavy metals (Cd, Co, Cr and Pb) that hazardous elements for livings were also determined. Results of this research showed that Salvia *nemorosa* contains high value of macro elements such as sodium (0.84 mg/g), potassium (15.20 mg/g), magnesium (3.26 mg/g) and calcium (11.72 mg/g). Also, it has maximum micro-elements such as iron (322.72 mg/kg), manganese (55.46 (mg/kg), copper (28.53 mg/kg and zinc (15.73 mg/g). Therefore, we can conclude that it has high nutritional values from the viewpoint of mineral elements such as potassium, calcium, iron, manganese and zinc.

Key words: Nutrient content, wild plant, *Salvia nemorosa* L, Van

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ORAL PRESENTATION

GARLIC (*Allium sativum* L.) ESSENTIAL OIL: CHEMICAL COMPOSITION AND ITS POTENTIAL EFFECT IN FOOD AND MEDICAL APPLICATIONS

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Abstract

Essential oils have been used across many civilizations for a variety of purposes, not only in the manufacture of perfumes and cosmetics, but also for medical purposes to "heal body and soul". At present, they are viewed with great interest which makes them a potential source of bioactive natural molecules. These are significantly affected by the extraction method applied. *Allium sativum* essential oil is rich in several sulfur-compounds mostly diallyl dislfude, diallyl sulfide, diallyl trisulfide, allylmethyl trisulfide and diallyl tetrasulfide. It has been shown to be a prophylactic and therapeutic medical agent and its use for these purposes is widespread and growing. This review provides a description of the garlic oil extracting methods, its chemical composition, and its food and medical applications.

Keywords: Garlic, *Allium sativum,* volatile compounds, chemical composition, food, medical applications

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POSTER PRESENTATION

ESSENTIAL OIL BEARING PLANTS OF NIGERIA

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Abstract

The essential oil-bearing plants of Nigeria are vast and have numerous medicinal values. Many of these medicinal values are known by the local herbalists, traditional healers, plants scientists. However, not much is known of the constituents of these medicinal plants. Many Nigerians (especially those in rural areas) are dependent on crude herbal remedies or traditional medicines. This work presents some of the aromatic plants, their uses as well as the essential oil compositions. Several articles and publications were studied and put into this review article to bring the essential oil constituents of these medicinal plants into light. This study was conducted using several mediums such as botanical, pharmaceutical and related journals, and websites of Pubmed, Sciencedirect, Scopus, Springer, Elsevier and Research Gate, etc.

Several families of plants were reviewed in the articles studied; however, the family Asteraceae had the most appearance. Some of them endemic plants such as *Tithonia diversifolia, Ageratum conyzoides, Chromolena odorata* belong to the family Asteraceae. Leaves dominated the part of the plant part used while the entire part was the least utilized part. The main methods of preparation are decoction and infusion. In summary, this paper examines already existing articles on aromatic plants of Nigeria, presents the uses of some of these plants as earlier reported [1,2]. It depicts the dependence of indigenes (especially those of rural communities) on these medicinal plants and helps to provide valid information on the constituents responsible for some of the health benefits. Further studies remain to be carried out to discover more essential oil constituents of aromatic plants of Nigeria and how they contribute to the medicinal uses of these plants.

Keywords: Essential oil, Nigeria, aromatic plants, traditional medicine, decoctions.

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POSTER PRESENTATION

REVIEW: ANTIBACTERIAL EFFECT OF BERGAMOT OIL

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In early 1900's, food-borne deaths were mostly caused by infectious diseases of pathogenic microorganisms. It is also known that elderly people and children, can be classified as risk groups, are more sensitive to diseases caused by pathogenic microorganisms. To eliminate pathogen microorganisms, heat treatments and additives are used. However, natural and less-process-needed technics are researched because of consumer demand on minimally processed and natural foods, and aromatic oils are natural candidates, thanks to their functional properties. Oils obtained from aromatic plants can be classified as fixed oils and essential oils. Fixed oils are generally obtained from the oily seeds of the plants by the press method. Fixed oils commonly are not highly functional. However, they are more suitable for general consumption. Essential oils can be obtained from various parts of plants like flowers and leaves. Essential oils have characteristic odor and aromatherapy properties. The antibacterial compounds generally contain hydroxyl groups in their structures. They may interfere with the enzyme systems and may affect the enzymatic reactions of pathogenic bacteria. It may also inhibit enzyme synthesis at the ribosomal level. They may also affect the structure of the membrane and as a result of weakening of the phospholipid layer formed by the fat and protein structure in the cell membrane; they may cause disruptions in the selective permeable structure. Bergamot oil is one of the essential oils that can be used in the industry due to its antibacterial properties of terpenes such as limonene and linalool. In addition, essential and fixed vegetable oils generally have a strong aroma, so these oils are limited to commercial use in foods. However, bergamot oil can be used more comfortably in processed foods from citrus fruits. In this review, antibacterial effects of bergamot oil were studied.

Keywords: Functional food, natural antimicrobials, essential oils, bergamot oil, antibacterial effect.

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POSTER PRESENTATION

ANTIOXIDATIVE POTENTIAL OF THE ETHANOLIC EXTRACT OF *Chenopodium botrys* L.

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The genus *Chenopodium* is a genus that contains annual or perennial herbaceous flowering plant species distributed in almost all parts of the world and contains more than 200 species. The species belonging to the genus *Chenopodium* are used in the treatment of various diseases including chest complaints, cough, abdominal pain, lung obstruction and nerve disorders. *Chenopodium botrys* species are used in the treatment of colds and asthma and there are also studies about the analgesic, anthelmintic and headache relieving effects of their leaves. In this study, the antioxidative potential of the ethanolic extract of *Chenopodium botrys* L. was evaluated. Evaluation of the antioxidative potential of ethanolic extract of *C. botrys* was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. According to the results obtained, the IC_{50} value of the ethanolic extract of *C. botrys* is 0.88 ± 0.01 mg/ml. In our future studies, extracts prepared from the same plant using different solvents and their fractions will be evaluated in terms of radical scavenging.

Keywords: Kızılbacak, DPPH, Turkey.

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POSTER PRESENTATION

CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM LEAVES OF *Lippia citriodora* GROWING IN TURKEY

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Lippia citriodora H.B.K. (syn. *Lippia triphylla* (L'Her.) Kuntze*; Aloysia triphylla* (L'Her.) Britton), mostly known as lemon verbena, is a perennial flowering plant of the verbena family (Verbenaceae). The plant is most valued for its strong, sweet, lemon-scented leaves that produce an intensified aroma when crushed. Thus, much of the plant uses, particularly in traditional remedies and/or as food additives, is due to the characteristic flavour and fragrance of the plant essential oil. In the study, we aimed to characterize chemical composition of essential oil of *L. citriodora*. The essential oil from leaves of *L. citriodora* was extracted by hydrodistillation system using a Clevenger apparatus. The essential oil composition of dried leaf samples were determined using Gas Chromatography Mass Spectrometry (GC–MS). Thirty-five essential oil components, which constitute 93.28% of the total essential oil yield, were detected in the essential oil of *L. citrodora*. Limonene (24.64%) was the principal component of the essential oil which, together with the citral isomers, E-citral (23.1%) and Z-citral (18.63%), make the oil obtained from this species with characteristic flavor and fragrance. Other major compounds found in essential oils of lemon verbena were *trans*-caryophyllene (6.05%), caryophyllene oxide (6.25%), junipene (6.12%), neryl acetate (4.98%) and α-curcumene (3.51%) which constitute 93.28% of the total essential oil yield.

Keywords: Chemical composition, *Lippia citriodora*, limonene, GC-MS.

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POSTER PRESENTATION

ISOLATION AND IDENTIFICATION OF BENEFICIAL BACTERIAL ENDOPHYTES ISOLATED FROM *Laurus nobilis* L. GROWING IN HATAY PROVINCE OF TURKEY

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Essential oils have been used in many fields scientifically and commercially for many years. The antimicrobial activities of some plants containing essential oil have been demonstrated in several studies. Laurel (*Laurus nobilis* L.) is an evergreen plant in the form of trees or large shrubs belonging to the Lauraceae family which is widely grown naturally along the Mediterranean, Black Sea and Aegean coasts. Microbial communities with multi-functional plant growth promoting traits within healthy medicinal plant are important for phytoremediation, plant nutrition, health and metabolism. In this study, the diversity and beneficial characteristics of endophytic microorganisms have been studied in medicinal plant *Laurus nobilis*. Total of 32 endophytic bacterial isolates, having with possible plant growth promoting traits (PGPB), were isolated from roots and shoots of *Laurus nobilis* collected from different districts of Hatay province of Turkey and identified by MALDI-TOF. According to identification results, these endophytic bacteria mainly belonged to different species of the genera *Bacillus* (31.25%), *Pseudomonas* (28.1%), *Acinetobacter* (12.5%), *Staphylococcus* (12.5%), *Wautersiella* (3.1%), *Brevundimonas* (3.1%), *Lactobacillus* (3.1%), *Micrococcus* (3.1%) and *Enterococcus* (3.1%). Although further studies are needed, these endophytes might have great potential in the field of biocontrol and plant growth promoting for sustainable agricultural practices.

Keywords: *Laurus nobilis,* PGPB, MALDI-TOF.

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POSTER PRESENTATION

SYNTHESIS AND SCREENING OF ANTIBACTERIAL AND ANTIFUNGAL EFFECTS OF MANNICH BASES DERIVED FROM KOJIC ACID

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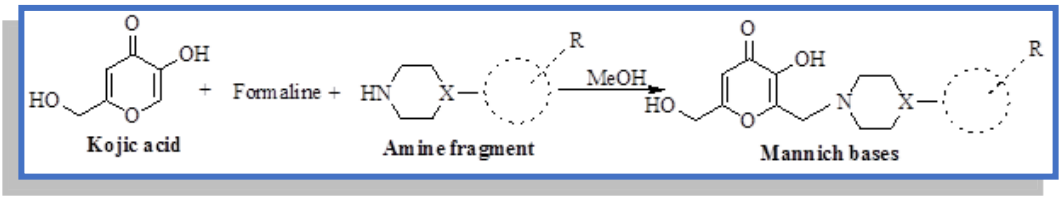
Since the introduction of the first chemotherapeutics into the medicine, there has been an increasing "race" between scientists trying to create new drugs against pathogenic microorganisms. Despite the production of several novel antibiotics, the problem of resistance caused by excessive and uncontrolled intake, has still been a major concern. Dermatophytic infections caused by dermatophytes including *Trichophyton*, *Microsporum* and *Epidermophyton* have increasing incidence and severity especially in patients with impaired immunity (1). Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is one of the top-ten causes of death worldwide (2). Additionally, *M. avium* is an opportunistic pathogen causing pulmonary diseases (3). As long as clinical management of patients with *M. tuberculosis* and *M. avium* infections is difficult, it is necessary to search for new antimycobacterial agents. Kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one), which is a natural fungal metabolite, have drawn much attention due to various bioactivities. Also, its function as a tyrosinase inhibitor is utilized in the food industry as an additive to prevent the browning of vegetables and in cosmetic area to prevent/treat hyperpigmentation disorders (4).



Herein, a series of Mannich bases of kojic acid were synthesized and a wide search programme was used for screening bioactivities as antibacterial activity (*H. influenza*, *P. aeroginosa*, *A. baumannii*, *S. pneumoniae*, *S. pyogenes*, *S. aureus*, *S.* epidermitis *M. tuberculosis*, *M. avium*) and antifungal activity (*Candida albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *Microsporum gypseum*, *Trichophyton mentagrophytes var. erinacei*, *Epidermophyton floccosum*) by using method described previously (5). Cytotoxicity of the compounds on MRC-5 and He-La cell lines was determined and expressed as maximum non-toxic concentration. The compounds displayed antimicrobial activity towards all of the standard strains of the tested bacteria and fungi at MIC value ranges of 8-256 and 4-64µg/ml, respectively. All compounds were to be shown bioactive under non toxic concentration (≥128 - ≥256 µg ml^{-1}).

Keywords: Kojic acid, mannich bases, antityrosinase, anticancer
Acknowledgement
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POSTER PRESENTATION

EVALUATION OF ENZYME INHIBITORY ACTIVITIES OF SOME *POLYGONUM* L. SPECIES GROWING IN TURKEY

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The present study aimed to determine the *in vitro* anti-cholinesterase, anti-collagenase, anti-elastase, and anti-tyrosinase activities of some *Polygonum* species growing in Turkey. The genus *Polygonum* (Polygonaceae) is represented with 43 taxa, 8 of which are endemic, in Turkey. Wound-healing, anti-inflammatory, antibacterial, antiviral, antioxidant, immunomodulatory, anti-aging, and estrogenic activity studies on different species have been carried out by *in vitro/in vivo/in silico* methods. Bioactive components such as resveratrol in *Polygonum* species are known to be effective in neurodegenerative diseases and used in cosmetics. The samples of *P. aviculare* L., *P. cognatum* Meissn., *P. patulum* Bieb. subsp. *patulum,* and *P. setosum* Jacq. subsp. *setosum,* were collected from Konya in May, 2020. The ethanol extracts prepared from the aerial parts and roots of the *Polygonum* species. The extracts were tested for their acetylcholinesterase (AChE), butrylcholinesterase (BChE), elastase, collagenase, and tyrosinase (TYR) inhibitory activities using ELISA microplate reader at different concentrations. Our outcomes indicated that the root extracts of *Polygonum* species have moderate AChE, TYR, and potent BChE inhibitory activities. Collagenase was inhibited most effectively by *P. cognatum* root extract followed by *P. aviculare* root extract, which also had the highest elastase inhibition at 333 µg/mL concentration. In conclusion, especially the root extracts of the *Polygonum* species that we tested have promising enzyme inhibitory effects related to cosmetics and neurodegenerative diseases and deserve further investigation to identify effective natural compounds.

Keywords: *Polygonum*, cholinesterase, elastase, collagenase, tyrosinase, enzyme inhibition

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POSTER PRESENTATION

RESEARCH ON TYROSINASE INHIBITORY EFFECTS OF THREE *HAPLOPHYLLUM* A. JUSS. SPECIES (RUTACEAE) GROWING IN TURKEY

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The aim of the study is to determine the inhibitory effects on tyrosinase (TYR) activity by the ethanol (EtOH) extracts obtained from the root, stem and flower of *Haplophyllum sahinii* Tugay & Ulukuş (HS), the aerial parts of *H. armenum* Spach, (HA) and *H. ptilostylum* Spach (HP). The genus *Haplophyllum* species belonging to the family of Rutaceae, known as 'Citrus' in Turkey, are important medicinal and aromatic plants. The *Haplopyhllum* genus has a total of 18 taxa, of which 10 are endemic to Turkey. *Haplopyhllum* species known as 'Sedo' in Turkey contains alkaloids, lignans, coumarins and flavanoids. HS, HA and HP were collected from Konya, Artvin and Şanlıurfa provinces in Turkey, in May, respectively. The plants were identified by Prof. Dr. Osman TUGAY and Dr. Deniz ULUKUŞ from the Selçuk University. The plants are deposited at the KONYA Herbarium, Faculty of Sciences, Selçuk University (Konya, Turkey). The ethanol extracts prepared from different parts of three *Haplophyllum* species. The extracts were tested for their TYR inhibitory activities using ELISA microplate reader at 200 $\mu g/mL^{-1}$. Among them, extracts, which are the extract of the flower of HS (34.41 % ± 0.83) exhibited the highest inhibition against TYR. The extracts of the aerial parts of HA, HP and the root of HS have weak inhibition against TYR. In conclusion, especially the flower of the HS have promising effects as whitening agents for cosmetics products. To the best of our knowledge, we report the first study on tyrosinase inhibitory effective of HS, HA and HP.

Keywords: Rutaceae, *Haplophyllum,* tyrosinase, enzym inhibition, Turkey

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POSTER PRESENTATION

ESSENTIAL OILS BEARING PLANTS OF CAMEROON

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In Cameroon, several plants are used both for their medicinal properties and as food. Many of them contain essential oils as one of their secondary metabolites. Although traditional healers rarely, if ever, use essential oils as the basis for their treatments, it is thought that the latter would be found in their products in view of the preparation processes used. And therefore, several activities attributed to their drugs are linked to the presence of essential oils. Many studies have revealed the presence of essential oils in several plants found in Cameroon, and validated their traditional use. The purpose of this study is to identify these plants on the basis of studies carried out in order to make a review which will serve as support for future studies. To carry out this work, we consulted the websites of research journals, namely Sciencedirect, Pubmed, Springer and Researchgate. We have identified fifty plants that have been the subject of proven studies and scientific publication. They are mainly distributed in the families of Annonaceae (09: 18%), Rutaceae (09: 18%), and Lamiaceae (08: 16%). We believe that an important work remains to be done in the search for new essential oils in Cameroon, because the country is endowed with a very great botanical diversity.

Keywords: Essential oils, medicinal plants, traditional medicine, Cameroon.

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POSTER PRESENTATION

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL OF *Pistacia lentiscus* FROM ALGERIA

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Pistacia lentiscus is a medicinal plant known for its wide use in traditional medicine especially by people in rural areas. The essential oil of this plant is characterized by pharmacological properties used for therapeutic purposes. The search for natural antioxidants remains an interesting avenue to investigate in order to overcome the excessive use of harmful synthetic antioxidants.

The aim of this work is to study the chemical composition of the essential oil of *Piatcia lentiscus* and the measurement of its antioxidant activity. The essential oil of the aerial part of lentisk harvested in Algeria was extracted by steam distillation. The chemical composition of the extracted oil was analyzed by GC-MS using a Trace GC Ultra instrument coupled with a DSQ II mass spectrometer. The in-vitro antioxidant activity of *Pistacia* essential oil was evaluated by the DPPH radical scavenging test and the ferric reducing power (FRAP). These reactions were monitored by UV-Visible spectrophotometry by measuring the antioxidant capacity in Trolox equivalent (TEAC), which allows comparison of the oil's reactivity with Trolox, the hydrophilic equivalent of vitamin E.

The GC-MS analysis proves that the essential oil is rich in hydrocarbon monoterpenes whose major compound is α-pinene, followed by β-pinene. This oil is also characterized by the presence of oxygenated monoterpenes in moderate amounts (terpineol, bornyl acetate, terpine-4-ol...).The result conducted on the evaluation of the antioxidant activity of this oil reported a moderate activity with FRAP test (TEAC=124.5 µg/ml) and a low antiradical activity with DPPH one (TEAC=4.30µg/ml). These redox properties of *Pistacia* essential oil may be due to the presence of oxygenated terpenes. The FRAP test recorded a better antioxidant effect of the oil compared to DPPH one. This indicates that *Pistacia* essential oil could be used for pharmaceutical interest.

Keywords: *Pistacia lenticus*, essential oil, antioxidant activity, GC-MS, FRAP, DPPH.

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POSTER PRESENTATION

AROMATIC PLANTS AND THEIR BIOLOGICALLY ACTIVE COMPOUNDS - A REVIEW -

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Aromatic plants consist of plenty biologically active compounds which have been found to own many properties such as antimicrobial, antioxidants and anti-inflammatory ones. In this paper are acknowledged some of the essential functionally bioactive compounds and their possible usage in different fields. The aromatic plants extracts can become the new generation substances for both human or animal nutrition and health. The objective of this review is to provide an outline of the literature enclosing the aromatic plants bioactive compound and the usage of them for different purposes.

Keywords: Aromatic plants, bioactive compounds

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POSTER PRESENTATION

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTITUMORAL ACTIVITY OF *Syringa vulgaris* L. ETHANOLIC EXTRACT

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Syringa vulgaris L. (common lilac) is one of the most popular ornamental plant, but also a promising not comprehensively studied source of bioactive compounds with therapeutic potential [1].
Objective: Our study was designed to characterize the chemical composition and to assess the antioxidant and antiproliferative properties of an ethanolic extract obtained from *Syringa vulgaris* L. flowers. **Methods.** The chemical profile of the ethanolic extract was investigated using chromatographic (HPLC, GC-MS) and spectral (UV-Vis, FT-IR and fluorescence spectroscopy) methods. The protective effect of the ethanolic extract against free radicals was evaluated *in vitro* (DPPH, FRAP, CUPRAC, inhibition of β-carotene bleaching), while the cytotoxicity and antiproliferative potential of this extract were tested on human keratinocyte (HaCaT) and murine melanoma (B16F10) cell lines using CCK8 and MTT assays. **Results.** By GC-MS analysis, fatty acids and terpenic compounds, such as lilac alcohols were identified. The HPLC analysis demonstrated the presence of polyphenols, like flavonoids and polyphenolic acids (e.g. rosmarinic acid and trans-p-coumaric acid). Preliminary FT-IR and fluorescence spectroscopic methods indicated compounds with blue fluorescence that could belong to the polyphenols' group. As for the medicinal potential, *Syringa vulgaris* ethanolic extract displayed the ability to efficiently protect against ROS. Furthermore, CCK8 and MTT tests pointed out a relevant antiproliferative potential expressed in a dose-dependent manner towards the tumoral line. **Conclusion.** The study showed for the *Syringa vulgaris* extract the presence of several polyphenols with valuable antioxidant and antitumoral potential.

Keywords: *Syringa vulgaris* L., ethanolic extract, chemical composition, antioxidant, antiproliferative.

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POSTER PRESENTATION

SEMISYNTHETIC AND BIOLOGICAL ACTIVITY OF IMINONARIGIN DERIVATIVES

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A series of 4-iminonaringin **2-6** have been prepared in 91-96% overall yields from a condensation reaction between naringin and primary amines. The results spectroscopic UV, IR, NMR^{1}H and ^{13}C allowed us to confirm the structures of all products. In order to better value our synthesized molecules, biological tests (antioxidants, antifungals and antibacterials) were carried out, and the results showed that these derivatives exhibited an antioxidant activity with respect to the greater DPPH than that of naringin. and naringenine. The antifungal tests using *Candida albicans* strains are negative, as regards to the antibacterial effect, the results allowed us to affirm that the synthesized products have a weak inhibitory activity with respect to *Escherichia coli* and *Staphylococcus aereus*. And negative for *Enterococcus feacalis* and *Pseudoumonas aeroginosa*.

Keywords*:* N- 4-iminonaringins, Naringin, Imine, semisynthetic, biological activity

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POSTER PRESENTATION

THE CHEMICAL AND BIOLOGICAL PROFILES OF TWO *Thymus vulgaris* L. (LAMIACEAE) COMMERCIAL ESSENTIAL OILS

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Essential oil obtained from *Thymus vulgaris* L./ Lamiaceaea (common thyme) is among the most valuable plant- derived products in terms of therapeutically properties [1]. **The Purpose of the Research:** This study was aimed to evaluate the chemical composition as well as the antimicrobial properties and the cytotoxic potential of two commercial essential oils obtained from the aerial parts of *Thymus vulgaris* L. **Methods**. The chemical composition was determined using gas chromatography–mass spectrometry (GC-MS). The antimicrobial potential was initially investigated using the agar well diffusion assay, followed by MIC and MBC values determination by the broth microdilution method. The *in vitro* antibacterial efficacy was further characterized by time-kill kinetics assay and antibiofilm formation, as well as in combination with two antibiotics, whilst 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and cell morphology and attachment level assessment were carried out to establish the *in vitro* toxicity on human fibroblasts (cell line HFL-1). In addition, the antioxidant potential was evaluated using several *in vitro* models (DPPH, FRAP, CUPRAC, inhibition of β-carotene bleaching). **Results**: GC-MS analysis indicated thymol (36.08% and 46.885%) and p-cymene (35.054% and 20.258%) as the major compounds and pointed out both qualitative and quantitative differences between the two tested essential oils. Certain compounds such as carvacrol and methyl ethers of thymol were detected in only one of the tested essential oils. Also, other organic components like camphene, myrcene, 1,8- cineole, camphor, 4-terpineol were found in a higher concentration. The results suggested also strong and broad bactericidal properties towards both planktonic biofilms forming bacteria and synergistic activity for ampicillin combination. The established MICs values did not significantly impact the cell viability, morphology and attachment levels. **Conclusion**. The tested commercial essential oils displayed antimicrobial and antimicrobial properties comparable to those reported for standardized products.

Keywords: *Thymus vulgaris* L., essential oils, chemical composition, antibacterial, cytotoxicity

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POSTER PRESENTATION

CAROTENOIDS, TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY OF *Taxus baccata* FRUITS

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Taxus baccata (European yew) is a tree from *Taxaceae* family, widespread in Europe and Asia. Although it is considered a toxic plant, *Taxus baccata* contains in its shoots many alkaloids, Taxol (generic name Paclitaxel) being one of the most valuable due to its anticancer properties noticed especially in the treatment of ovarian and breast cancer. The extract of *Taxus baccata*'s leaves can be used internally for asthmatic bronchitis and indigestion treatments, and externally in the rheumatism treatment, as infusion. The only part of this plant which is not poisonous is represented by the fruits (aryls) which are red, gelatinous, have approximately 10 mm in diameter, and contain only one seed. These are rich in carotenoids, especially the ones with retro structure, but also in water-soluble antioxidants from the class of polyphenols [1,2]. **Objective:** The aim of this research was to bring new data's regarding the carotenoids, phenolic composition and the antioxidant activity of the active compounds of *T. baccata* aryl. **Methods**: The total carotenoid content was evaluated spectrophotometrically and the pigments were separated using different chromatographic methods (TLC, HPLC). Total polyphenols content was determined using Folin Ciocalteu method. Total antioxidant activity was evaluated using a colorimetric assay kit (Elabscience). **Results**: The main carotenoids identified were: rhodoxanthin, lycopene, zeaxanthin and β-carotene, with an average total carotenoid content of 3.378±0.005 mg/100 g FW aryl. The fruit extracts contain important amounts of polyphenols (233.91±22.364 mg GAE/100 g FW aryl) and showed relevant antioxidant activity. **Conclusion**: The results suggest that *T. baccata* fruits are valuable sources of carotenoids and polyphenols with relevant antioxidant activities.

Keywords: *Taxus baccata*, carotenoids, polyphenols, antioxidants
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POSTER PRESENTATION

THE *IN VITRO* ANTIMICROBIAL EFFICACY OF HERBAL PRODUCTS COMBINATIONS AGAINST BACTERIA ISOLATED FROM BOVINE MASTITIS

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Bovine mastitis represents of one of the most important pathologies that require antimicrobial therapy and given the emergence of the multidrug resistant strains, plant derived products are proposed as an alternative to the classical antibacterial agents. **Objective:** This study was aimed to evaluate the *in vitro* efficacy of herbal derived products combinations towards the most frequently isolated bacteria isolated from bovine mastitis. **Methods**: Both essential oil and ethanolic extracts obtained from thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), lemon balm (*Melissa officinalis* L.), rosemary (*Rosmarinus officinalis* L.), and sweet basil (*Ocimum basilicum* L.) were tested individually and in combinations towards clinical strains of *Staphylococcus aureus* (n=5) and *Escherichia coli* (n=5). The *in vitro* antimicrobial activity was investigated using several methods, namely the agar well diffusion assay and the broth microdilution method to characterize and establish the antimicrobial potential and MIC and MBC values, respectively. In addition, time-kill kinetics assay and antibiofilm formation test were carried out considering mastitis associated pathogenesis. **Results**: The preliminary evaluation confirmed broad spectrum antimicrobial properties in case of the essential oils compared to the ethanolic extracts. The most active products were represented by the essential oils obtained from *Melissa officinalis* and *Thymus vulgaris.* Synergistic activity was noticed in case of *Melissa officinalis* combinations. **Conclusion**: The tested herbal products are considered for further *in vitro* and *in vivo* studies to evaluate clinical efficacy and safety.

Keywords: Essential oils, ethanolic extracts, bacteria, bovine mastitis

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POSTER PRESENTATION

CHEMICAL FINGERPRINTING AND EVALUATION OF BIOACTIVE PROPERTIES OF TWO EDIBLE HALOPHYTHES: *Limonium effusum* (BOISS.) O. KUNTZE AND *Limonium sinuatum* (L.) MILLER

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The genus *Limonium* Mill., (Plumbaginaceae), is represented by 22 taxa in Turkey, of which two halophytic species, *L. effusum* (Boiss.) O. Kuntze (Kaya marulu) (LE) and *L. sinuatum* (L.) Miller (Deniz out, deniz marulu, herdemtaze, limon out, sahil karanfili) (LS) are edible. Both species grow wildly in seashores, sand dunes or rocky coasts. *L. effusum*, which is endemic to Turkey, also grows in salty inlands of Aegean Region of Turkey. Having in mind the stimulation of synthesis and accumulation of polyphenols and possible high antioxidant capacity of halophytes due to their resistance to salt stress, in this study, total phenolic content of methanolic extracts and their hexane, dichloromethane, ethyl acetate and water fractions prepared from aerial parts were determined. Phenolic compounds of phenolic rich ethyl acetate fractions were qualified and quantified by a UHPLC–ESI–MS/MS analysis. In addition, *in vitro* antioxidant, antimicrobial and neuroprotective activities of extracts and fractions were evaluated. The total phenolic content resulted about 2-fold higher in ethlyacetate fraction of LE (561.82±5.81mg GAE/g extract) than in that of LS (273.91±1.09mg GAE/g extract). According to UHPLC–ESI–MS/MS analysis tannic acid was the most abundant phenolic acid in both species, however an apparent difference in its quantitative content was found (71,439.56±3643.3 µg/g extract in LE and 105,453.5±5328.1 µg/g extract in LS), whereas hyperoside was the most abundant flavonoid found 14,006.90±686.1 µg/g extract in LE and 1,708.51±83.6 µg/g extract in LS. The antioxidant capacity of the extracts was evaluated by DPPH and TAC assays and a stronger activity in ethylacetate fractions were highlighted. Methanol extracts and their fractions of both species are more active against Gram positive bacteria than Gram negatives and showed considerable growth inhibitions against tested fungi. Promising acetylcholinesterase and butyrylcholinesterase activities were observed for water fraction of LS and hexane fractions of both species, respectively.

Keywords: Antioxidant, antimicrobial, neuroprotective, *Limonium,* halophytes

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POSTER PRESENTATION

ASSESSMENT OF *IN VITRO* CYTOTOXICITY OF LEAF EXTRACT AND FRACTIONS OF *Cotinus coggygria* SCOP. ON HUMAN NON-MELANOMA SKIN CANCER CELLS

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Non-melanoma skin cancer, including basal cell carcinoma and squamous cell carcinoma, is the most common type of cancer in Caucasian population. The prevalence of skin cancer has been increasing over the past years. *Cotinus coggygria* Scop. is a medicinal plant species widely used in the Balkan traditional folk medicine mainly to treat lesions of the skin and mucosal tissues. Our previous research detected anticancer activity of the herb on human breast, ovarian and cervical cancer cell lines [1, 2]. The aim of the present study was to evaluate the cytotoxic potential of crude leaf aqueous ethanolic extract from Bulgarian *C. coggygria* and its chloroformic and aqueous fractions on human squamous cell carcinoma cell line A431 and to compare it to the effect on viability of normal skin cell line BJ. Cytotoxicity effects were assessed through MTT cell viability assay and Trypan blue test after 24 h exposure in parallel with observation of cell morphology under light microscope. The obtained results showed selectively higher rate of inhibition of A431 epidermoid carcinoma cells viability in comparison to BJ normal dermal cells as the strongest cytotoxic potential against A431 cells was registered for the chloroformic fraction of the extract. Alterations in cell morphology of A431 cells were also observed. Future studies will be focused on the more detailed investigations of anti-non-melanoma activities of *C. coggygria* substances.

Keywords: *Cotinus coggygria* Scop., cytotoxic potential, A431 epidermoid carcinoma cell line, BJ normal dermal cell line

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POSTER PRESENTATION

GROWTH ENVIRONMENT AND ORGAN SPECIFIC VARIATION IN *IN-VITRO* CYTOPROTECTIVE ACTIVITIES OF *Picea mariana* IN PC12 CELLS EXPOSED TO GLUCOSE TOXICITY: A PLANT USED FOR TREATMENT OF DIABETES SYMPTOMS BY THE CREE OF EEYOU ISTCHEE (QUEBEC, CANADA)

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Background: The Cree of Eeyou Istchee (James Bay area of northern Quebec) suffer from a high rate of diabetes and its complications partly due to the introduction of the western lifestyle within their culture. As part of a search for alternative medicine based on traditional practice, this project evaluates the biological activity of *Picea mariana* (Mill.) Britton, Sterns & Poggenb. needle, bark, and cone, in preventing glucose toxicity to PC12-AC cells in vitro (a diabetic neuropathy model) and whether habitat and growth environment influence this activity. **Methods:** Three different organs (needle, bark, and cone) of *P. mariana* were collected at different geographical locations and ecological conditions and their 80% ethanolic extracts were prepared. Extracts were then tested for their ability to protect PC12-AC cells from hyperglycemic challenge at physiologically relevant concentrations of 0.25, 0.5, 1.0 and 2.0 µg/mL. Folin-Ciocalteu method was used to determine the total phenolic content of *P. mariana* extracts. **Results:** All extracts were well-tolerated in vitro exhibiting LD50 of 25 µg/mL or higher. Extracts from all tested organs showed a cytoprotective concentration-dependent response. Furthermore, the cytoprotective activity was habitat- and growth environment-dependent with plants grown in bog or forest habitats in coastal or inland environments exhibiting different cytoprotective efficacies. These differences in activity correlated with total phenolic content but not with antioxidant activity. In addition, this paper provides the first complete Ultra-Performance Liquid Chromatography-quadrupole time-of-flight (UPLC-QTOF) mass spectrometry analysis of *Picea mariana*'s bark, needles and cones. Conclusions: Together, these results provide further understanding of the cytoprotective activity of Canadian boreal forest plants identified by the Cree healers of Eeyou Istchee in a cell model of diabetic neuropathy. Their activity is relevant to diabetic peripheral neuropathic complications and shows that their properties can be optimized by harvesting in optimal growth environments.

Keywords: DPPH, glucose toxicity, PC12-AC, phenolics, stilbenes, traditional medicine.

FULL PAPERS

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FULL TEXT – ORAL PRESENTATION

A STUDY ON THE CULTIVATION AND ADAPTATION OF *STEVIA REBAUDIANA* BERTONI PLANT TO ANTALYA CONDITIONS

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Abstract

Objectives: *Stevia rebaudiana* Bertoni (stevia, sweetleaf, sugarleaf) is the homeland of South America, was discovered in 1887 by a South American natural scientist, Antonio Bertoni. *S. rebaudiana* is member of Asteraceae family which likes moist conditions, temperatures mean 25°C and grows up to 60-90cm high. The plant, detected 79 species in North America is stated to be found more than 200 species in South America. The main components of stevia extract is stevioside, rebaudioside-A and steviol molecules. The stevia plant is known to have been used as a source of sugar by the locals for centuries. Dried form of stevia leaves is 10-15 times sweeter than sugar and the glycoside extract obtained by purification from the stevia plant is 200-300 times sweeter than refined sugar. The existence of some research, showing that non-calorie stevia plant does not increase blood sugar levels, has an effect on increasing insulin sensitivity and even insulin secretion, lend support its use in the treatment of diabetes. With this research, it was aimed to determine the favourable genotype or genotypes and the appropriate sowing frequency by investigating the adaptation of *Stevia rebaudiana*, *Stevia rebaudiana* Candy and *Stevia rebaudiana* Organic genotypes, imported as an introduction material, for Antalya plain conditions. **Methods:** Compared with the varieties used in the research, *Stevia rebaudiana* Candy genotype comes to the fore in terms of single plant dry leaf weight and rebaudioside-A/stevioside ratio which is an important quality criterion. While there was no significant difference in rebaudiosideA/stevioside ratios from three different plant densities (30x30cm, 40x40cm and 50x50cm) applied in the experiment, single plant/herb, fresh leaf and dry leaf weights were found high in 50x50cm application. **Results:** Considering the number of plants in the unit area, the yield per decare is higher with the application of 30x30cm, but as the plant density increases, the risk of fungal disease increases depending on the temperature and humidity. **Conclusion:** When the features which are examined are taken into consideration; Plant height, number of side branches, single plant dry leaf weight values vary depending on year, application and genotype. The values of single plant fresh herb weight and single plant age leaf weight vary depending on application and year. Rebaudioside-A content varies depending on the genotype, stevioside content and rebaudioside-A/stevioside ratio.

Keywords: *Stevia rebaudiana*, stevia, sweetleaf, sugarleaf, medicinal and aromatic plants

1. Introduction

Especially in the last two decades, medicinal and aromatic plants are among the top product groups that are increasing in importance and current in the world. Medicinal and aromatic plants (MAPs), which have entered the terminology as a general term that include not only in the plants used in treatment, but also the plants used in the food, spices, cosmetics and paint industries grow naturally in almost every region of our country and 90 % of them are collected from the nature and offered to the market.

Turkey has great richness and diversity due to its taking place in the intersection three plants geography region Europe-Siberia, the Mediterranean and the Iran-Turan. Our country, in the subtropical climate zone, is suitable for the cultivation of many medicinal and aromatic plants by virtue of its richness of microclimate and besides has convenient conditions for the cultivation of many introduction materials too.

Stevia rebaudiana (sugar herb) plant, perennial indigenous to South America, was discovered in 1887 by the South American naturalist Antonio Bertoni. The plant, which belongs to the Asteraceae family, grows in a humid environment at a mean temperature of 25 ^{o}C and grows up to 60-90 cm in length. It is stated that there are 79 species, detected in North America and 220-230 species in South America.

The best prominent feature of stevia extract, derived from stevia plant is that it does not contain calories, fat, saccharin and toxic substances as a natural sweetener and diet food. The fact that The use easily and safely of stevia-induced glycosides in bakery foods that are boiled like jam pudding in all hot and cold beverages or baked at high temperatures up to 300 ^{o}C in the oven makes this plant an important alternative among other sweeteners for diabetes or blood pressure patients.

With this study, the seeds which belong to 3 genotypes of stevia species (*Stevia rebaudiana*, *Stevia rebaudiana* Candy, *Stevia rebaudiana* Organic) were provided from an international seed sales company and their adaptation to Antalya plain conditions was investigated.

2. Material and Methods

In the study, 3 genotypes were used, namely *Stevia rebaudiana* c.s, *Stevia rebaudiana* Candy and *Stevia rebaudiana* Organic, which belong to the species *Stevia rebaudiana*. In the first year, the seeds used as the starting material were germinated under greenhouse conditions and the seedlings obtained were transplanted to the soil under greenhouse conditions and rootstock parcels were established. In April, the second year, seedlings were propagated with shoot cuttings in the greenhouse and with these seedlings produced in May, a trial was established in the field conditions of the Bati Akdeniz Agricultural Research Institute Aksu campus. The stable manure was applied to the soil before the seedlings were transplanted to the field.

Ridges that seedlings were planted into were prepared and mulching was made using a durable ground cover for weed control. Seedlings were planted into the ridges prepared at 30x30 cm, 40x40 cm and 50x50 cm (between-row spacings x within-row spacings), and a trial pattern was established in three repetitive randomized blocks design. The plots are composed of 2 lines of 3 m length. Drip irrigation was done according to the needs of the plant.

The method information of agronomic measurements and content analysis performed in the study are given below.

i. Seed germination: In greenhouse conditions, a seed bed was prepared with 70 % peat and 30 % perlite mixture. The seeds, sown in the form of sprinkle sowing were covered with a 0.5 cm thick peat. The germination of the seeds was ensured in the fogging conditions, which were adjusted to fogger 1 minute every 20 minutes.

ii. Germination Percentage: In the seeds harvested in the first year were calculated according to the formula; (number of germinated seeds/total number of seeds) x 100.

iii. Number of elapsed days till from germination to seedling: The period from germination stage (emergence of cotyledon leaves) until a 4-leaf seedling is obtained seedling is determined as the number of days.

iv. Number of elapsed days from germination stage to flowering: The period from germination satage to 50 % flowering is determined as the number of days.

v. Number of elapsed days from germination stage to seed setting: The period from germination stage to seed setting is determined as the number of days.

vi. Plant length (cm): The length of 5 plants randomly selected from each plot from the soil surface to the plant end point was measured and averaged.

vii. Number of branches: The branches of 5 plants randomly selected from each plot were counted and averaged.

viii. Herba (above-ground parts) single plant weight (g): Five plants selected from each plot are single plant values, obtained by cutting 10 cm above the soil surface and weighing them.

ix. Wet and dry folium (leaf) weight (g): After each plot was harvested and the leaves were separated from their branches, fresh leaf weight was determined by being weighed. Dry leaf weight was found by being weighed after these leaves were dried for 48 hours at 40 ˚C in the drying oven.

x. Extraction of glycosides: For the extraction of glycosides from stevia leaves was taken 1 g sample from dried and grinded bulk plant leaf samples, and 100 ml of 70 % ethanol solution was added into it and kept in a water bath set at 70 °C for 30 minutes. The extract taken from the water bath was allowed to cool, 10 ml was taken from the cooled part and filtered using a 0.45 µm syringe filter. 10 µl of filtered extract was used in HPLC analysis (Kolb *et al*, 2001).

xi. HPLC analysis: Analysis of glycosides by HPLC was made by isocratic solution method. For this purpose, acetonitrile: water (80:20 v/v, pH: 5) was used as the mobile phase. The analysis was performed at 200 nm using Nucleodur Hilic column (250 mm, 5 µm particle diameter) at room temperature and the flow rate was set to 2 ml/min. Quantification was made using the external standard method (Kolb *et al*, 2001).

3. Results and Discussion

3.1. Results

The germination rate of seeds, purchased in the first year of the project is 11 % for *Stevia rebaudiana*, 35 % for *Stevia rebaudiana* Candy and 12 % for *Stevia rebaudiana* Organic. The germination rates of the seeds, obtained from the parcels were repeated in 2016 with the seeds, received in 2015 and the germination rates were determined as 17.3 %, 8.3 % and 13.3

% for *Stevia rebaudiana*, *Stevia rebaudiana* Candy and *Stevia rebaudiana* Organic, respectively.

The mean times from germinated seeds until 4-leaf seedlings is obtained were determined 97 days for *Stevia rebaudiana*, 89 days for *Stevia rebaudiana* Candy and 93 days for *Stevia rebaudiana* Organic.

The period from germination stage of seeds to 50 % flowering were determined as 125 days for *Stevia rebaudiana*, 113 days for *Stevia rebaudiana* Candy and 117 days for *Stevia rebaudiana* Organic.

The period from germination stage to seed setting is determined as 148 days for *Stevia rebaudiana*, 145 days for *Stevia rebaudiana* Candy and 147 days for *Stevia rebaudiana* Organic.

Plant length measurements were taken from the first plants (rootstock plots) obtained from seed in the first year of the project (2012) and they were taken from the plants, in trial plots in 2015 and 2016 years. The first year's measurement values are 63.4 cm for *Stevia rebaudiana*, 66.0 cm for *Stevia rebaudiana* Candy and 54 cm for *Stevia rebaudiana* Organic. Data for 2015 and 2016 are summarized in Table 1. Data for number of plant samples branches taken from trial parcels are summarized in Table 2.

In the first year of the experiment, it couldn't be obtained data at the parcel level from the plants but single plant weights were measured. These values are determined as 107.23 g for *Stevia rebaudiana*, 49.80 g for *Stevia rebaudiana* Candy and 84.42 g for *Stevia rebaudiana* Organic. The single plant herba data for 2015 and 2016 are summarized in Table 3.

In 2014, the first year of the experiment, it couldn't be obtained data at the parcel level from the plants, but weights of single plant dry leaves were measured. These values are determined as 13.17 g for *Stevia rebaudiana*, 6.65 g for *Stevia rebaudiana* Candy and 10.14 g for *Stevia rebaudiana* Organic. Parcel single plant fresh leaf weigts for 2015 and 2016 are summarized in Table 4, parcel single plant dry leaf weights are summarized in Table 5.

In 2014, the first year of the experiment, it couldn't be obtained data at the parcel level from the plants, but the glycoside content of the bulk samples taken from each genotype was examined as three repeat. The glycoside data for 2014 are summarized in Table 6, rebaudioside-A values in 2015 and 2016 are in Table 7, stevioside values are in Table 8 and rebaudioside-A/stevioside ratios are summarized in Table 9.

3.2. Discussion

Our study on the adaptation of stevia plant to Antalya plain conditions was carried out between 2012 and 2016. The data obtained according to the randomized blocks test pattern were interpreted below using the appropriate analysis method.

The low germination rate of stevia seeds has been reported by many researchers (Goettemoeller and Ching (1999), Shock (1982), Carneiro (1997). That the plant's growing area was limited due to the weak germination rate was also reported by Goettemoeller and Ching (1999). With the increase in demand for the plant in recent years, its production with shoot-cutting has become widespread and than it is also performed in vitro seedling propagation. During our research, it was observed that stevia, a short-day plant began to flower as of September depending on the day length and flowering continued as long as the air temperature was suitable, so after a while, mature seeds, maturing seeds and flowers were found on the plant at the same time.

While a perennial plant, stevia, is trying to mature its seeds from all flowers, along with the fall of temperatures it goes into dormant period and not all of the seeds can mature due to

the this changing climate conditions. Therefore, while seed harvesting is made, immature seeds mix into also mature seeds and this situation is thought to decrease the rate of seed germination, Figure 1.

Goettemoeller and Ching (1999) grouped stevia seeds as black and brown, they reported that black seeds are heavier and their germination rates are higher. Reporting that, Shock (1982) long summer days affected positively leaf weight and leaf stevioside content but seed production was difficult under these conditions.

It was observed that in stevia, the tropical climate plant, the duration from seed germination to obtaining 4-6 leaf seedlings, starting flowering and keeping seeds varied depending on the temperature and day length. Carneiro (2007) reported that flowering in plants started as depending on photoperiod, and that this critical day length interacted with genotype and many environmental factors such as, nutrient intake, heat and water stress.

According to the data obtained from the 53 trial plots, evaluated, the plant height parcel mean ranges from 83.7 cm to 148.4 cm. In Table 1, plant lenght means, obtained from varieties are given by years. According to these values, coefficient of variation value of the related feature was found to be 9.32. The overall mean of plant lenght, belonging to two years is 113.5 cm, the mean of the first year is 105.9 cm, and the mean of the second year is 121.0 cm. The means belonging to three different plant densities (30x30 cm, 40x40 cm and 50x50 cm) applied in the experiment, are 105.6 cm, 116.9 cm and 117.9 cm respectively. Means plant lenght of the genotypes are 110.6 cm for *Stevia rebaudiana*, 120.2 cm for *Stevia rebaudiana* Candy and 109.7 cm for *Stevia rebaudiana* Organic. Duncan grouping which is belonging to year, application and genotypes is given in Table 10, 11 and 12.

In plant height, the difference between years and among applications was found statistically significant at the level of 1 % and the difference among cultivars at the level of 5 %. Application*Genotype interaction was found significant at the 5 % level. Belonging to interaction Duncan groupings are given in Table 13. It has been reported that while plant height of stevia is given as 60-90 cm in natural spreading areas, its plant lenght reaches up to 120 cm when it is cultured, (https://uses.plantnet-project.org).

According to the data, obtained from the trial parcels, evaluated, the number of branches ranges from 5.3 to 50.0. The data, obtained from the trial parcels are summarized in Table 2. According to these values, belonging to the feature, coefficient of variation was found to be 31.53. The overall mean of sub-branches number, belonging to two years is 15.6, the mean for the first year is 16.7, and the mean for the second year is 22.4. The means belonging to three different plant densities (30x30 cm, 40x40 cm and 50x50 cm) applied in the experiment, are 18.9, 19.0, 14.8 respectively. Means sub-branches number of the genotypes are 13.2 for *Stevia rebaudiana*, 24.4 cm for *Stevia rebaudiana* Candy and 15.1 for *Stevia rebaudiana* Organic. Duncan groupings which are belonging to year, application and genotypes are given in Table 10, 11 and 12.

The difference between years and among genotypes was found statistically significant at the level of 1 %, and the difference among applications statistically significant at the level of 5 %. Year*Application, Application*Genotype and Year*Genotype interactions were found statistically significant at the level of 5 %. Duncan groupings which are belonging to interactions are given in Table 14, 15 and 16 respectively.

According to the data obtained from the evaluated trial parcels, the mean fresh herb weight ranges from 390 g to 2383 g. Data obtained from trial parcels are given in Table 3. Coefficient of variation of the related feature was found to be 32.95. The overall mean of parcel single plant herb weight for two years is 1118.7 g the mean for the first year is 977.9 g, and the

mean for the second year is 1259.5 g. The means belonging to three different plant densities (30x30 cm, 40x40 cm and 50x50 cm) applied in the experiment, are 797.7 g, 1153.7 g, 1404.9 g respectively. The parcel single plant herb weight means of genotypes are 1211.8 g for *Stevia rebaudiana*, 1169.9 g for *Stevia rebaudiana* Candy and 974.4 g for *Stevia rebaudiana* Organic. Duncan groupings which are belonging to year, application and genotypes are given are given in Table 10, 11 and 12.

The difference between years and among applications was found statistically significant at the level of 1 %, as a result of the studies, in which they investigated the harvest effects on leaf and glycoside yields Moraes *et al* (2013) reported that the fresh herb yield was 3495.09 kg/da (629.18 g/plant) for a single harvest application in plant density (30x60 cm and 5555 plants/da) under Mississippi conditions.

Kumar *et al* (2014) reported that the plant spacing in 30x30 cm yielded in terms of total biomass 41.2 % more fresh herb in the first year and 42.8 % in the second year compared to the 60x45 cm row spacing.

According to the data obtained from the evaluated trial plots, single plant fresh leaf weight parcel mean values range from 216.8 g to 897.0 g. Data obtained from trial parcels are given in Table 4. Coefficient of variation value of the related feature was found to be 27.15. The overall mean of parcel single plant fresh leaf weight, belonging to two years is 456.2 g, the mean of the first year is 361.7 g and the mean of the second year is 550.7 g. The means belonging to three different applications (30x30 cm, 40x40 cm and 50x50 cm) are 374.0 g, 473.3 g and 521.4 g respectively. The parcel single plant wet leaf weight means, belonging to genotypes are 476.8 g for *Stevia rebaudiana*, 455.2 g for *Stevia rebaudiana* Candy and 436.8 g for *Stevia rebaudiana* Organic. Duncan groupings which are belonging to year, application and genotypes are given in Table 10, 11 and 12.

The difference between years and among applications was found statistically significant at the level of 1 %. Brandle and Rosa (1992) reported in their study that the yield they obtained planting spacing 30x30 cm (within-row x between-row) as 3000 kg/ha (270 g/plant).

According to the data obtained from the evaluated trial plots, single plant dry leaf parcel mean values range from 60.0 g to 290.6 g. Data obtained from trial parcels are given in Table 5. Coefficient of variation value of the related feature was found to be 23.82.

The overall mean of parcel single plant dry leaf weight, belonging to two years is 138.7 g, the mean of the first year is 102.9 g and the mean of the second year is 174.6 g. The means belonging to three different applications (30x30 cm, 40x40 cm and 50x50 cm) are 118.2 g, 141.2 g and 156.9 g respectively. The parcel single plant dry leaf weight means, belonging to genotypes are 143.1 g for *Stevia rebaudiana* 145.3 g for *Stevia rebaudiana* Candy and 127.8 g for *Stevia rebaudiana* Organic. Duncan groupings which are belonging to year, application and genotypes are given in Table 10, 11 and 12.

The difference between years and among applications was found statistically significant at the level of 1 %, and the difference among genotypes statistically significant at the level of 5 %. Application*Genotype interactions were found statistically significant at the level of 5 %. Duncan groupings which are belonging to interactions are given in Table 17.

The data, obtained from the trial parcels in 2015 and 2016 are summarized for the amounts of rebaudioside-A in Table 7. According to these values, the parcel means, belonging to rebaudioside-A amounts range from 24.29 mg/kg to 64.57 mg/kg. Coefficient of variation value of the related feature was found to be 17.32.

The overall mean of rebaudioside-A amounts, belonging to two years is 43.9 mg/kg, the mean of the first year is 42.9 mg/kg and the mean of the second year is 44.9 mg/kg. The means

belonging to three different applications (30x30 cm, 40x40 cm and 50x50 cm) are 44.3 mg/kg, 44.9 mg/kg and 42.4 mg/kg respectively. The parcel rebaudioside-A amounts belonging to genotypes are 42.8 mg/kg for *Stevia rebaudiana*, 47.8 mg/kg for *Stevia rebaudiana* Candy and 41.1 mg/kg for *Stevia rebaudiana* Organic. Duncan groupings which are belonging to year, application and genotypes are given in Table 10, 11 and 12.

The difference among genotypes was found statistically significant at the level of 5 %. Year*Application and Application*Genotype interactions were also found significant at the 5 % level. Duncan groupings which are belonging to interactions are given in Table 18 and Table 19 below.

The data, obtained from the trial parcels in 2015 and 2016 are summarized for the amounts of stevioside in Table 8. According to these values, the parcel means, belonging to stevioside amounts range from 34.075 mg/kg to 78.095 mg/kg. Coefficient of variation value of the related feature was found to be 12.35.

The overall mean of stevioside amounts, belonging to two years is 54.8 mg/kg, the mean of the first year is 45.0 mg/kg and the mean of the second year is 64.6 mg/kg. The means belonging to three different applications (30x30 cm, 40x40 cm and 50x50 cm) are 55.4 mg/kg, 54.7 mg/kg and 54.4 mg/kg respectively. The parcel Stevioside amounts belonging to Genotypes are 55.3 mg/kg for *Stevia rebaudiana*, 52.5 mg/kg for *Stevia rebaudiana* Candy and 56.6 mg/kg for *Stevia rebaudiana* Organic. Duncan groupings which are belonging to year, application and genotypes are given in Table 10, 11 and 12.

The difference between years and among genotypes was found statistically significant at the level of 1 %. Application*Genotype interactions were found significant at the 5 % level. Duncan groupings which are belonging to interactions are given in Table 20, 21 and 22.

Kumar et al. (2014) reported that the amount of steviol glycosides did not change depending on the plant material and plant spacing, but there was a higher amount of steviol glycosides at 30x30 cm plant spacing compared to 60x45 cm. Serfaty *et al* (2013) reported in their study in Israel that they found the stevioside yield as 23.5, 28.0 and 30.9 g/m^2 at plant densities of 6, 8, 10 plants/m^2, respectively.

The data, obtained from the trial parcels in 2015 and 2016 are summarized for rebaudioside-A/stevioside ratio in Table 9. According to these values, the parcel means, belonging to rebaudioside-A/stevioside ratio range from 0.45 to 1.53. Coefficient of variation value of the related feature was found to be 19.87.

The overall mean of to rebaudioside-A/stevioside ratio, belonging to two years is 0.84, the mean of the first year is 0.98 and the mean of the second year is 0.71. The means belonging to three different applications (30x30 cm, 40x40 cm and 50x50 cm) are 0.83, 0.88 and 0.82 respectively. The parcel rebaudioside-A/stevioside ratio belonging to genotypes are 0.81for *Stevia rebaudiana*, 0.96 for *Stevia rebaudiana* Candy and 0.76 for *Stevia rebaudiana* Organic. Duncan groupings which are belonging to year, application and genotypes are given in Table 10, Table 11 and Table 12.

The difference between years and among genotypes was found statistically significant at the level of 1 %. Year*Application interactions were found significant at the 5 % level and Application*Genotype interactions were found significant at the 1 % level. Duncan groupings which are belonging to interactions are given in Table 23 and 24.

Montoro *et al* (2013) reported that the main components of steviol glycosides in stevia leaf are stevioside at the ratio of 6-10 % and rebaudioside-A glycosides at the ratio of 2-4 %, while other glycosides are found at the ratio of 1-2 %. The 0.4 % w/v stevioside solution tastes 300 times sweeter than sugar, but the compound has an astringent taste. Rebaudioside-A is the

glycoside with the highest rate of steviol glycosides once after stevioside, and it feels 250-450 times sweeter than sugar. It is also more suitable for use in food and beverages as a sweetener because of not leaving with no bitter aftertaste. For these reasons, it is preferred that the amount of rebaudioside-A is high and the amount of stevioside is low (Kinghorn, 2004).

Table 1. Plant length mean values

Block	*Stevia rebaudiana* (cm)		*Stevia rebaudiana* Candy (cm)		*Stevia rebaudiana* Organic (cm)	
	2015	2016	2015	2016	2015	2016
30 x 30 cm	93.5	105.9	102.5	117.1	99.7	114.7
40 x 40 cm	99.2	117.1	119.4	141.9	105.1	118.5
50 x 50 cm	119.4	128.3	113.1	126.7	101.1	119.0
Mean	104.0	117.1	111.7	128.6	102.0	117.4

Table 2. The mean values of the number of branches

Block	*Stevia rebaudiana*		*Stevia rebaudiana* Candy		*Stevia rebaudiana* Organic	
	2015	2016	2015	2016	2015	2016
30 x 30 cm	8,4	19,1	16,3	36,9	9,9	22,8
40 x 40 cm	10,2	11,6	16,5	40,1	13,5	21,9
50 x 50 cm	13,7	15,8	16,7	19,9	8,7	13,9
Mean	10,8	15,5	16,5	32,3	10,7	19,5

Table 3. The mean fresh herb weights

Block	*Stevia rebaudiana* (g)		*Stevia rebaudiana* Candy (g)		*Stevia rebaudiana* Organic (g)	
	2015	2016	2015	2016	2015	2016
30 x 30 cm	733.0	763.2	698.3	1069.4	647.7	874.5
40 x 40 cm	1219.7	1158.3	1069.7	1513.6	846.1	1114.6
50 x 50 cm	1423.7	1972.6	1143.3	1526.2	1020.0	1343.6
Mean	1125.5	1298.0	970.4	1369.7	837.9	1110.9

Table 4. The mean single plant fresh leaf weights

Block	*Stevia rebaudiana* (g)		*Stevia rebaudiana* Candy (g)		*Stevia rebaudiana* Organic (g)	
	2015	2016	2015	2016	2015	2016
30 x 30 cm	299.5	437.3	271.7	503.3	257.6	474.7
40 x 40 cm	423.3	494.3	384.9	628.0	349.8	559.7
50 x 50 cm	490.1	716.3	368.7	574.3	410.0	568.7
Mean	404.3	549.3	341.8	568.5	339.1	534.4

Table 5. The mean single plant dry leaf weights

Block	*Stevia rebaudiana* (g)		*Stevia rebaudiana* Candy (g)		*Stevia rebaudiana* Organic (g)	
	2015	2016	2015	2016	2015	2016
30 x 30 cm	88.9	142.8	83.7	164.5	74.0	155.2
40 x 40 cm	108.9	155.5	106.0	222.6	94.4	159.8
50 x 50 cm	144.5	218.1	112.0	183.0	113.7	169.9
Mean	114.1	172.1	100.6	190.0	94.0	161.6

Table 6. Content analysis at the genotype level in 2014

Genotypes	Rebaudioside-A (mg/kg)	Stevioside (mg/kg)	Rebaudioside-A/Stevioside
Stevia rebaudiana	34.960	29.055	1.20
Stevia rebaudiana Candy	38.134	20.916	1.82
Stevia rebaudiana Organic	52.351	25.306	2.07

Table 7. Belonging to parcels, the amounts of rebaudioside-A (mg/kg)

RebaudiosideA	*Stevia rebaudiana*		*Stevia rebaudiana* Candy		*Stevia rebaudiana Organic*	
Blok	2015	2016	2015	2016	2015	2016
30 x 30 cm	33.580	41.361	47.398	56.589	43.893	43.226
40 x 40 cm	41.669	53.181	45.934	50.618	38.425	39.615
50 x 50 cm	47.122	40.164	43.372	42.881	44.348	36.486
Mean	40.790	44.902	45.568	50.029	42.222	39.776

Table 8. Belonging to parcels, the amounts of stevioside (mg/kg)

Stevioside	*Stevia rebaudiana*		*Stevia rebaudiana* Candy		*Stevia rebaudiana Organic*	
Block	2015	2016	2015	2016	2015	2016
30 x 30 cm	55.909	64.125	45.515	53.857	47.659	65.409
40 x 40 cm	40.478	69.532	36.212	67.184	43.369	71.146
50 x 50 cm	40.776	60.911	46.928	65.479	48.153	63.994
Mean	45.721	64.856	42.885	62.173	46.394	66.850

Table 9. Belonging to parcels rebaudioside-A/stevioside ratio

Rebaudioside-A/Stevioside	*Stevia rebaudiana*		*Stevia rebaudiana* Candy		*Stevia rebaudiana* Organic	
Block	2015	2016	2015	2016	2015	2016
30 x 30 cm	0,60	0,65	1,06	1,05	0,94	0,66
40 x 40 cm	1,04	0,77	1,27	0,76	0,89	0,56
50 x 50 cm	1,16	0,65	0,93	0,66	0,94	0,58
Mean	0,93	0,69	1,09	0,83	0,92	0,60

Table 10. Belonging to years Duncan grouping

Year	Plant Height (cm)	Number of Side Branches	Fresh Herb Weight (g)	Fresh Leaf Weight (g)	Dry Leaf Weight (g)	Rebaudioside -A amounts (mg/kg)	Stevioside amounts (mg/kg)	Rebaudioside-A/ stevioside
1 2015	105.358 B	12.619 B	906.00 B	356.79 B	96.638 B	42.696 A	45.162 B	0.97538 A
2 2016	121.000 A	22.452 A	1259.54 A	550.74 A	174.600 A	44.902 A	55.602 A	0.70519 B

Table 11. Table 10 Belonging to applications Duncan grouping

Applications	Plant Height (cm)	Number of Side Branches	Fresh Herb Weight (g)	Fresh Leaf Weight (g)	Dry Leaf Weight (g)	Rebaudioside-A amounts (mg/kg)	Stevioside amounts (mg/kg)	Rebaudioside-A/ stevioside
1 30x30 cm	105.556 B	18.917 A	786.1 B	374.02 B	118.2 B	44.341 A	51.715 A	0.82722 A
2 40x40 cm	116.839 A	18.978 A	1153.9 A	473.33 A	134.47 B	44.907 A	49.398 A	0.88389 A
3 50x50 cm	117.835 A	14.835 B	1332.0 A	523.19 A	157.58 A	42.118 A	50.319 A	0.80000 A

Table 12. Belonging to genotypes Duncan grouping

Genotypes	Plant Height (cm)	Number of Side Branches	Fresh Herb Weight (g)	Fresh Leaf Weight (g)	Dry Leaf Weight (g)	Rebaudioside -A amounts (mg/kg)	Stevioside amounts (mg/kg)	Rebaudioside-A/ stevioside

SR	110.018 B	13.094 B	1185.3 A	476.04 A	143.04 AB	42.595 AB	56.142 A	0.79118 B
SRC	120.100 A	24.428 A	1130.0 A	455.16 A	145.31 A	47.799 A	52.529 A	0.95778 A
SRO	109.678 B	15.111 B	948.5 A	436.73 A	121.09 B	40.999 B	43.085 B	0.76167 B

Table13. Belonging to plant length, Application*Genotype interaction Duncan grouping

Applications	Genotypes	Mean Square	
40 x 40 cm	SRC	130.63333	A
50 x 50 cm	SR	122.43333	AB
50 x 50 cm	SRC	119.86667	ABC
40 x 40 cm	SRO	111.76667	ABC
50 x 50 cm	SRO	110.06667	ABC
30 x 30 cm	SRC	109.80000	ABC
40 x 40 cm	SR	108.11667	BC
30 x 30 cm	SRO	107.20000	BC
30 x 30 cm	SR	99.66667	C

Table 14. Belonging to number of side branches Year*Application interaction Duncan grouping

Year	Applications	Mean Square	
2	30 x 30 cm	26.288889	A
2	40 x 40 cm	24.555556	A
2	50 x 50 cm	16.511111	B
1	40 x 40 cm	13.400000	B
1	50 x 50 cm	12.511111	B
1	30 x 30 cm	11.544444	B

Table 15. Belonging to number of side branches Application*Genotype interaction Duncan grouping

Applications	Genotypes	Mean Square	
40 x 40 cm	SRC	28.333333	A
30 x 30 cm	SRC	26.633333	AB
50 x 50 cm	SRC	18.316667	ABC
40 x 40 cm	SRO	17.700000	BC
30 x 30 cm	SRO	16.366667	C
50 x 50 cm	SR	13.950000	C
30 x 30 cm	SR	13.750000	C
50 x 50 cm	SRO	11.266667	C
40 x 40 cm	SR	10.900000	C

Table 16. Belonging to number of side branches Year*Genotype interaction Duncan grouping

Year	Genotypes	Mean Square	
2	SRC	32.311111	A
2	SRO	19.533333	B
1	SRC	16.544444	BC
2	SR	15.511111	BC
1	SRO	10.688889	C
1	SR	10.222222	C

Table 17. Belonging to dry leaf weight Application*Genotype interaction Duncan grouping

Applications	Genotypes	Mean Square	
50 x 50 cm	SR	182.55000	A
40 x 40 cm	SRC	164.30000	AB
50 x 50 cm	SRC	147.48333	AB

Applications	Genotypes	Mean Square	
50 x 50 cm	SRO	141.76667	AB
40 x 40 cm	SR	132.20556	AB
30 x 30 cm	SRC	124.14167	AB
30 x 30 cm	SR	115.84771	B
30 x 30 cm	SRO	114.60000	B
40 x 40 cm	SRO	106.90000	B

Table 18. Belonging to the amounts of rebaudioside-A Year*Application interaction Duncan grouping

Year	Applications	Mean Square	
2	40 x 40 cm	47804.556	A
2	30 x 30 cm	47058.667	A
1	50 x 50 cm	45322.667	AB
1	40 x 40 cm	42009.333	AB
1	30 x 30 cm	41623.556	AB
2	50 x 50 cm	39843.778	B

Table 19. Belonging to The amounts of rebaudioside-A Application*Genotype interaction Duncan grouping

Applications	Genotypes	Mean Square	
30 x 30 cm	SRC	51993.333	A
40 x 40 cm	SRC	48275.833	AB
40 x 40 cm	SR	47425.167	AB
50 x 50 cm	SR	44206.000	AB
30 x 30 cm	SRO	43559.333	AB
50 x 50 cm	SRC	43126.500	AB
50 x 50 cm	SRO	40417.167	AB
40 x 40 cm	SRO	39019.833	AB
30 x 30 cm	SR	37470.667	B

Table 20. Belonging to The amounts of stevioside Year*Application interaction Duncan grouping

Year	Applications	Mean Square	
2	40 x 40 cm	58776.889	A
2	50 x 50 cm	54292.000	AB
2	30 x 30 cm	53735.778	ABC
1	30 x 30 cm	49694.333	BC
1	50 x 50 cm	45043.778	CD
1	40 x 40 cm	40019.667	D

Table 21. Belonging to the amounts of stevioside Year*Genotype interaction Duncan grouping

Year	Genotypes	Mean Square	
2	SR	64855.889	A
2	SRC	62173.222	A
1	SRO	46393.556	B
1	SR	45479.333	B
1	SRC	42884.889	B
2	SRO	39775.556	B

Table 22. Belonging to the amounts of stevioside Application*Genotype interaction Duncan grouping

Applications	Genotypes	Mean Square	
30 x 30 cm	SR	60016.833	A
50 x 50 cm	SRC	56203.667	AB
40 x 40 cm	SR	55005.333	AB
40 x 40 cm	SRC	51697.833	ABC
50 x 50 cm	SR	50480.667	ABC
30 x 30 cm	SRC	49685.667	ABC

Applications	Genotypes	Mean Square	
30 x 30 cm	SRO	45442.667	BC
50 x 50 cm	SRO	42319.333	C
40 x 40 cm	SRO	41491.667	C

Table 23. Belonging to rebaudioside-A/stevioside ratio Year*Application interaction Duncan grouping

Year	Applications	Mean Square	
1	40 x 40 cm	1.0688889	A
1	50 x 50 cm	1.0144444	AB
1	30 x 30 cm	0.8677778	ABC
2	30 x 30 cm	0.7866667	BCD
2	40 x 40 cm	0.6988889	CD
2	50 x 50 cm	0.6300000	D

Table 24. Belonging to rebaudioside-A/stevioside ratio Application*Genotype interaction Duncan grouping

Applications	Genotypes	Mean Square	
30 x 30 cm	SRC	1.0583333	A
40 x 40 cm	SRC	1.0183333	AB
50 x 50 cm	SR	0.9100000	ABC
40 x 40 cm	SR	0.9066667	ABC
30 x 30 cm	SRO	0.7983333	ABC
50 x 50 cm	SRC	0.7966667	ABC
50 x 50 cm	SRO	0.7600000	ABC
40 x 40 cm	SRO	0.7266667	BC
30 x 30 cm	SRO	0.6250000	C

Figure 1. Simultaneous view mature seed, ripening seed and flower on the stevia plant

4. Conclusion

Considering the results of the statistical analysis and the data obtained from the trial we established with the genotypes of *Stevia rebaudiana*, *Stevia rebaudiana* Candy and *Stevia rebaudiana* Organic in 3 repetitive randomized blocks trial pattern in Antalya plain conditions: On the basis of years, the difference between plant height, number of side branches, single plant fresh herb weight, single plant wet leaf weight, single plant dry leaf weight was found to be significant.

Three different plant densities were tested in the experiment. When evaluating the data, obtained as a result of applying the spacings between rows and within rows as 30x30 cm, 40x40 cm and 50x50 cm, the difference among the means was found to be statistically significant in terms of plant height, number of side branches, single plant fresh herb weight, single plant wet leaf weight, single plant dry leaf weight.

Even tough single plant herb weight, fresh and dry leaf weights were found to be high in 50x50 cm application, considering the number of plants per unit area, the yield per decare is higher with 30x30 cm application. However, planting with this plant density is likely to break out fungal diseases in cases where air circulation is not sufficient. In the light of all these data, when deciding on plant density, it should be made decision by considering environmental factors.

When the genotypes, used in the study were compared to among each other, there was no statistically significant difference in terms of single plant fresh herb weight and single plant wet leaf weight, while the difference of among plant height, number of side branches and single plant dry leaf weight were found to be statistically significant.

When these data are evaluated all in all, the *Stevia rebaudiana* Candy genotype stands out statistically. On the basis of the examined features; plant height, number of side branches, single plant dry leaf weight values vary depending on the year, application and variety. Single plant fresh herb weight and single plant wet leaf weight values vary depending on the year and application.

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FULL TEXT – ORAL PRESENTATION

MEDICINAL AND AROMATIC PLANTS AS A SOURCE OF ANTIOXIDANT DURING COVID-19 PERIOD

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Abstract

Today, increased consumption of ready-made food, crowded city life and stressful living conditions cause the formation of free radicals in our body. Antioxidants appear as valuable compounds that can reduce or eliminate the harmful effects of these free radicals. Antioxidants are generally classified in two groups as natural and artificial. Vitamins (C, E and A vitamins), phenolic compounds (flavonoids), terpenoids (carotenoids) and sulphur containing (allicin) compounds, can be counted as the leading of natural antioxidants. Today, natural and synthetically derived antioxidants are used to preserve the freshness of foods for a long time, or in other words to extend their shelf life and preserve their quality. However, even though synthetic antioxidants are affordable over time, the emergence of negative health aspects made natural antioxidants to be preferred. Medicinal and aromatic plants, like many vegetables and fruits, are also powerful sources of antioxidants. The best examples of these herbs include rosemary, thyme, sage and turmeric. In this review, medicinal and aromatic plants that can be used as natural antioxidants will be mentioned.

Keywords: Vitamin C, flavonoids, carotenoids, polyphenols, α-tocopherol.

Introduction

Increasing of air pollution, synthetic food additives, alcohol and cigarette consumption, unconscious nutrition, stressful and inactive living conditions cause synthesis of free radicals in our body. Free radicals especially attack the cell and immune system (Alaca Güre ve Arabacı, 2005). In addition, it damages cells and prevents them from fighting cancer, accelerates the aging process and causes memory loss (Raghavan, S., 2007). The roles of antioxidants include protecting cells against the toxic effects of free radicals and contributing to disease prevention. Antioxidants are valuable compounds that show a protective effect by neutralizing free radicals, which are toxic by-products of normal cell metabolism (Pham-Huy et al., 2008; Sen et al., 2010). While living organisms are healthy, antioxidants and free radicals are in balance. However, when free radicals increase, sensitivity occurs against many disorders, from gastrointestinal diseases to infertility, from cardiovascular diseases to respiratory and excretory system disorders (Karabulut ve Gülay, 2016).

Synthetic antioxidants such as BHT (buthyl hydroxytoluene), BHA (buthyl hydroxyanisol), TBHQ (tertiary butyl hydroxyquinone), PG (propyl gallate) are used in the industry to extend the shelf life of foods (Çoban ve Patır, 2010). The unfavorable conditions caused by increasing free radicals in living organisms have brought the use of medicinal and aromatic plants as natural antioxidant sources. Medicinal and aromatic plants used as spice give the flavor, taste, odor and color to the foods, due to

the phenolic compounds they contain. Apart from this, they extend the shelf life of foods and prevent the negative effects of free radicals on living organisms that consume these foods.

Plants belonging to the Lamiaceae family are widely used as natural antioxidant sources. Especially rosemary has a wide usage area in Europe and America (Peter, 2004). Rosmarinic acid was the main phenolic compound of the family (Shan et. Al. 2005). The phenolic compounds can be used as antioxidant agents for humans or animals. (Kamdem et al.,2012). In this family lots of plants contain phenolic compounds such as; rosemary, sage, oregano, thymus, majorana, sweet balm and basil. Other medicinal and aromatic plants with high antioxidant content are turmeric, clove, chilli pepper, cinnamon, ginger, garlic, fenugreek, sumach, parsley, black seed, bay, fennel, coriander, pimento and nut meg.

Vitamins (C, E and A vitamins), phenolic compounds (flavonoids), terpenoids (carotenoids), alkaloids (caffeine), and sulphur containing compounds (allicin), can be counted as the leading of natural antioxidants.

Vitamin C: Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. Citrus fruits, green vegetables, red peppers and tomatoes, parsley and rosehip are plants rich in vitamin C.

Vitamin E: Vitamin E is an oil-soluble and has a high antioxidant potential, is available in different forms. The form with the highest bioactivity for the human body is α-tocopherol (Pham-Huy, 2008). It is found in vegetable oils such as wheat germ oil, soy, sunflower and corn oil, and nuts such as almonds, hazelnuts and peanuts.

Carotenoids: Yellow, red and orange color pigments are the most common after chlorophyll in nature. After taken into the body, they turn into vitamin A if needed.

beta-carotene: It is a precursor substance that is stored in the body in the form of provitamin and turns into vitamin A when needed. It is also found in fruits, grains, oils, carrots and spinach.

Lycopene: It is a bright red carotenoid pigment and is a powerful antioxidant found in tomatoes and other red fruits.

Flavonoids: Flavonoids are polyphenolic substances which are present in most plants in seeds, fruit peel, bark, and flowers (Miller,1996). More than 4000 flavonoids have been identified according to chemical structure and classified as flavanols, flavanones, flavones, iso-flavones, catechins, anthocyanins, proanthocyanidins (Pham-Huy, 2008).

In addition to many vegetables and fruits that are natural sources of antioxidants, there are also powerful sources of antioxidants in medicinal and aromatic plants. Examples of some medicinal and aromatic plants belonging to the family of Lamiaceae that have antioxidant effects.

1. ROSEMARY

***Rosmarinus officinalis* L. (Lamiaceae):** Rosemary is an evergreen plant in the form of a bush with dark green leaves. It is a plant that is widely cultivated and has a wide range of uses (Al-Sereiti,et.al., 1999). Rosemary is used fresh, dried or as an essential oil and its essential oil has antioxidant properties because of containing flavones, steroids, diterpenes and triterpenes. Rosemary includes caffeic acid, rosmarinic acid, ursolic acid, carnosic acid, and carnosol as the main active component (Ngo et.al.,2011). Carnosol and carnosic acid are also primarily responsible for its antioxidant effect (Tural and Turhan, 2017). Carnosic acid and carnosol account for over 90 % of its antioxidant activity in rosemary leaves (Oliveira, 2016).

2. SAGE

***Salvia* spp. (Lamiaceae):** Sage is one of the most widely used and cultivated medicinal and aromatic plant of the Lamiaceae family. Salvia extracts constitute a rich source of polyphenols and could be used as powerful herbal antioxidants (Farhat et al., 2013). Salvia genus has many phenolic compounds belonging mainly to the phenolic acids, phenolic glycosides, phenolic diterpenes, flavonoids, anthocyanins and coumarins (Cuvelier et al., 1994; Lu and Foo, 2002). Tepe (2008) reported that the new potential antioxidant substances of Salvia are rosmarinic acid derivatives, sagecoumarin and sagerinic acid.

3. THYME, OREGANO

There are many species known as thyme. Some of them are *Origanum, Thymus, Coridothymus, Satureja,* and *Thymbra.* Their common feature is that they contained high amounts of essential oils and the main component of the essential oil was carvacrol and / or thymol. Liolios et al., (2009) and Puertas-Mejia et al. (2002) also reported that mixture of thymol and carvacrol has powerful antioxidant effect than that of only thymol and carvacrol due to a synergistic effect.

4. SWEET BALM

***Melissa officinalis* L. (Lamiaceae):** Lemon balm belongs to the Lamiaceae family and is a perennial plant which is widespread in the Mediterranean countries and the Southern Alps. *Melissa officinalis* contains flavonoids (quercitrin, rhamnocitrin, luteolin), polyphenolic compounds (rosmarinic acid, caffeic acid, and protocatechuic acid), monoterpenoid aldehyde, monoterpene glycosides, triterpenes (ursolic and oleanolic acids), sesquiterpenes, tannins, and essential oils (citral) (Miraj et al.,2017). Popova et al. (2016) reported that Lemon balm has 10 times stronger antioxidant effects than the vitamin C and vitamin B.

5. SWEET BASIL

***Ocimum basilicum* L. (Lamiaceae):** Sweet basil (*Ocimum basilicum* L.) is an important spice and essential oil plant that belongs to the Lamiaceae family and comprises annual and perennial herbs. Basil leaves are rich in phenolic acids such as rosmarinic, chicoric, caffeic, and caftaric; (Flanigan et al., 2014), flavonol (quercetin, kaempferol), glycosides and anthocyanins (Złotek et al., 2016, Ghasemzadeh et al.,2016). Another important component of basil leaves and flowers is essential oil, its main constituents are linalool, methyl chavicol, 1,8-cineole, eugenol, methyl eugenol, methyl isoeugenol, thymol, methyl cinnamate, citral, and camphor (Skrypnik 2019)

Some other spice herbs with powerful antioxidant effects such as turmeric, clove, chilli pepper, cinnamon, ginger, garlic, sumach, parsley, black seed, bay leaf, tea, fennel, coriander, pimento and nut meg.

1. CLOVE

***Syzygium aromaticum* (Myrtaceae):** The essential oil of clove extracted from the dry flower bud of the clove tree, has an antioxidant activity due to the presence of eugenol and other phenolic compounds (Radünza et al., 2019).

2. GİNGER

***Zingiber officinale* (Zingiberaceae):** Ginger is a perennial herb with yellowish brown color, fleshy, thick and misshapen rhizomes (Baydar, 2019). It is commonly use in food as spice and the main components of ginger are 6-gingerol, 6-shogaol, 8-gingerol and10- gingerol and these constituents had exhibited strong antioxidative activity. (Maizura et. Al. 2011).

3. TURMERIC

***Curcuma longa* (Zingiberaceae):** Turmeric is a perennial herbaceous plant with yellow flowers, large and sharp leaves. It is an important tropical spice mainly for its colour, aroma and antioxidant property (Ishita et al., 2004; Sharma et al., 2005; Cousins et al., 2007). Used in curry making. Turmeric rhizomes contain the yellow color substance which name is curcumin.

4. CINNAMON

***Cinnamomum* spp. (Lauraceae):** Cinnamon is an evergreen perennial aromatic tree from the Lauraceae family. From the bark of these plants, branches and young shoots, after the cork and parenchyma tissue are stripped, the inner bark is dried according to the technique, and cinnamon is obtained by grinding. The cinnamon contains a number of antioxidative components such as vanillic, caffeic, gallic and ferulic acids. Eugenol which is found in clove and cinnamon essential oils is an effective antioxidant compound.

5. RED PEPPER

***Capsicum* spp. (Solanaceae):** The fully ripe fruits are dried in accordance with the technique, after the stems are removed, the ground red pepper, annealed with water, grinded in different sizes or crushed, mixed with edible vegetable oil and cooking salt is called red pepper. Red color is one of the most

important quality parameters of red pepper which is due to the carotenoids, i.e., capsanthin and capsorubin; and violaxanthin; capsanthin-5,6 epoxide, zeaxanthin, lutein, β-cryptoxanthin, and β-carotene providing the yellow-orange color. Red pepper also has provitamin A, vitamin C and polyphenols (Deng et. al., 2018).

6. TEA

***Camellia* spp. (Theaceae):** Tea is a cultivated plant which grows in tropical and subtropical humid climates, especially mountainous regions. Tea refers to the products of the leaves, leaf buds, and internodes of the *Camellia sinensis* or *Camellia assamica* plants. It is prepared by fermentation, heating, drying and mixing of tea leaves with other fruits or herbs. It is a beverage obtained by boiling or brewing tea leaves processed in this way. It is a natural source of theine, caffeine, theophylline and antioxidants. Flavonoids (and their fraction-catechins) are the responsible for basic phenolic compounds in green tea antioxidant activities, such as neutralization of free radicals that are formed in the process of metabolism (Tunçel et. al., 2016)

Recent studies show that medicinal and aromatic plants have antioxidant effects. Many researchers have also reported that medicinal and aromatic herbs can be used in many industrial fields such as food and cosmetics instead of synthetic antioxidants. Shobana and Aklihender Naidu (2000) reported that the relative antioxidant activities decreased in the order of clove, cinnamon, pepper, ginger, garlic, mint and onion. Hinneburg et al (2006) studied with basil, laurel, parsley, juniper, aniseed, fennel, cumin, cardamom, and ginger extracts, and also reported that basil and laurel extracts could be an alternative to the synthetic substances as food components with their antioxidant activity. Özcan and Aslan (2011) reported that cinnamon oil is the best in preventing oxidation of crude oil followed by clove and rosemary oil. Ahmed and Ayar (1993) investigated the antioxidant effects of some medicinal and aromatic plants grown in Turkey and they determined that the rosemary plant had the strongest antioxidant effect, followed by sage, sumac and thyme plants, respectively.

Conclusion

Due to reasons such as metropolitans, air pollution, malnutrition and stressful living conditions, the antioxidant balance in the human body may be disrupted in favor of free radicals. This unbalanced decreases the body's immunity and causes it to be vulnerable to fighting diseases. However, in today's pandemic (covid-19) conditions, our strongest weapon is a strong immune system. Medicinal and aromatic herbs as antioxidant sources to make this system powerful. In addition, the antioxidant effects of these plants are used in the protection of various food products. Vegetable and animal products contain unsaturated fatty acids. These fatty acids are sensitive to oxidation. Oxidation causes loss of nutritional value and degradation in food products. In order to produce healthy and stable food, synthetic or natural antioxidants are needed to prevent free radicals caused by oxidation. The fact that they are natural and effective as synthetics is preferred by consumers. Therefore; antioxidants should be used as protective in industry such as food and cosmetics. Also; medicinal and aromatic herbs with antioxidant effects should be preferred in our daily lives as spice to our meals and salads, and as tea between meals.

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FULL TEXT – ORAL PRESENTATION

COMPUTATIONAL BOTANY - A REVIEW

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Objectives: In this paper are summarized some of the publications about automatic plans identification regarding introductory concepts and some examples of using them. We all know that technology is a part of human being due to its diversity and people have started using it as a more viable resource in different areas. One of the areas is botany, that includes vital sources of living for human life such as plants and ecology of the Earth mainly depends upon them. The world bears thousands of plants species, some of them close to extinction (Fig. 1) and others that are harmful to human (Fig 2). Regarding the problem about endangered species, nowadays collection, conservation and protection of them have become extremely critical[1-7].

Fig. 1 Endangered species[8]

Fig. 2 Harmful species[9]

Moreover than 480 million species worldwide have been stored in museum as herbarium (Fig. 3), all the information were manually research and conserved that mean time consuming, needs for funds and destruction of ecosystem. But these things have changed due to technological evolution, meaning that it has been used different techniques such as photos, database, computer vision programs, that help protecting biodiversity and contribute to automatic plants species identification.

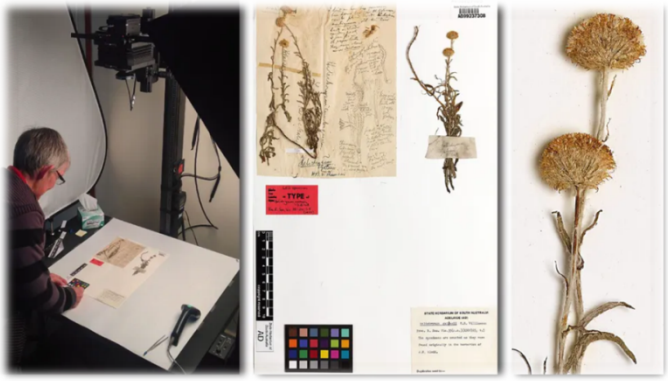

Fig. 3 Herbarium[10]

Using automatic identification of plants is a very useful for general public who don't have expertision in botany field. Identification of a plants requires recognizing it by one or more characteriristc such as reproductive parts, leaveas, bark, fruit and linked that recognition with a name, either common or scientific name. It is very importnat to have a accurate identification of a plants, because it can be helpfful in knowing how it grows as well as how to care and protect it from pests and diseases[11,12].**Methods:** For this review paper it has been used different literature papers about botany and how the digitalized techniques have been implemented in this area.. **Results:** Some of approach for species identification it can be image-based methods. That means a user can take a photo with his mobile device of a plant in the field and analyse in with an installed recognition application to identify the species. Images bades methos can be categoriesd in 2 parts:- phtotos and scans. The difference between this 2 parts is that images from photos are made in a natural backgroung. Next figures present a flow diagram of identification process(Fig 4) and some of the fundamental steps in image-bades identification (Fig.5). Is important to mention thata all of the automatic planst idetification programs, need an expert knowledged at at base of the program and manually insertion of information[12-14]. Apllication of automatic identification have been used in identification the stages of mango in order to know which magoes are mature and suitable for consumption. Mango is an economically important well know fruit crop. In industry it is very essential to identify the different maturity stage of mangoes, especially during export and imports, since those maturity stages indicate the self life.For human experts it is very difficult to classify thousands of mangoes manually and for these reasons automated system of mangoes identification is necesaary, so that the industry can be able to process a huge nuumber of mangoes within very short time and with reduced cost. In the Fig. 6 we can see different maturity stages of Himsagor mangoes and studies conclued that stage -1 is suitable for harvesting, while stage 6 is suitable fo consumption[15-17]. The Fig. 7 present a print screen of a front end search window from a deploy computer databases for medicinal plants, that helps general public to know information regard different plants with online a simple search using only common name of medicinal plants [2, 18]. In the Fig.8 it can be seen the differenec of description of flowers between a human eyes of a botanist and a computer vision program. It can truthly see that using a computer vision give a more accurate identification of a plant[13].

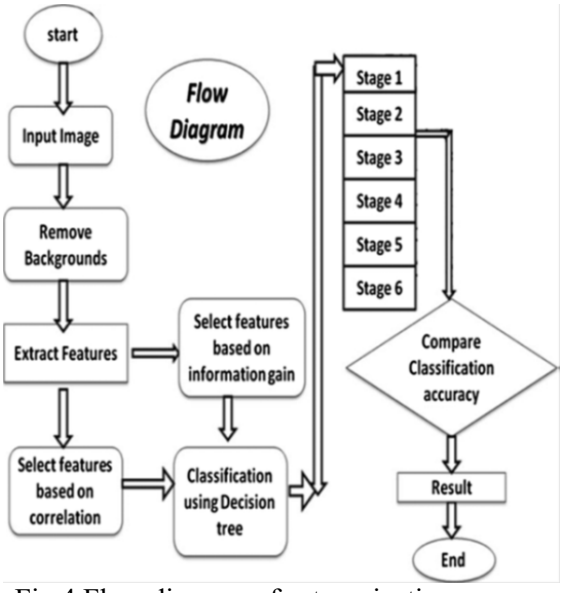

Fig.4 Flow diagram of categorization process

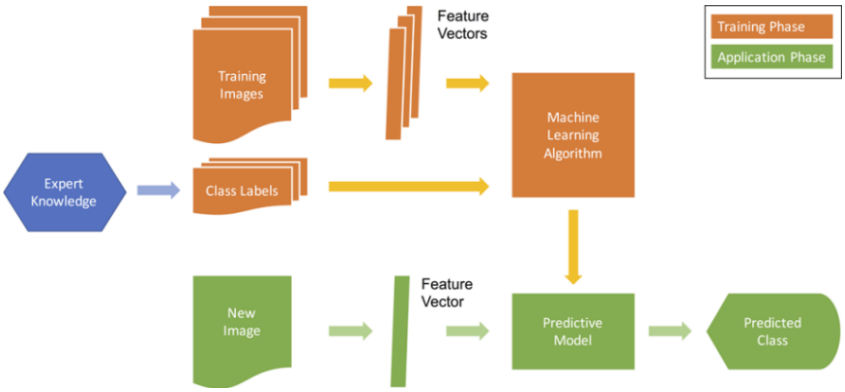

Fig. 5 Fundamental steps of supervised machine learning for image-based species identification

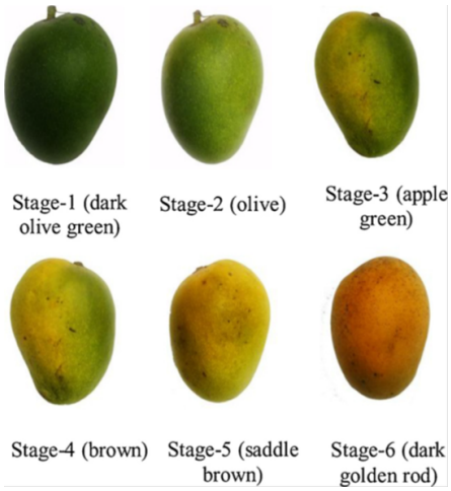

Fig. 6 Different maturity stages of Himsagor mangoes

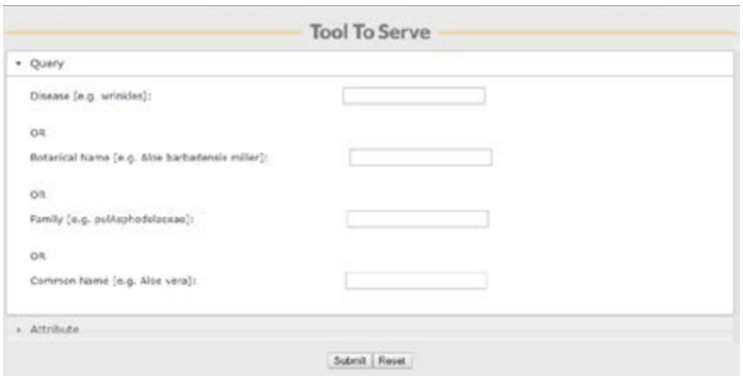

Fig. 7 The front end search window having four basic search section comprising Disease, Botanical name, Family and Common name that allow the user to make an input of interest

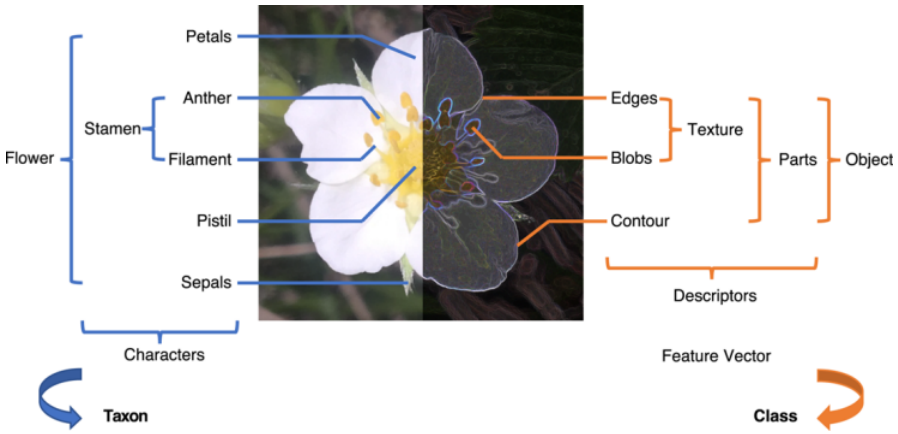

Fig. 8 Botanists' (left) versus computer vision (right) description of flowers

Conclusion: It seems that automatic plants species identification is a non-destructive method to collect, identify and conserve, it takes less time, being more effective, rapid and more accurate, and it can be used by general public with no studies in the botany filed.

Key words: technology, botany, review

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FULL TEXT – POSTER PRESENTATION

AROMATIC PLANTS AND THEIR BIOLOGICALLY ACTIVE COMPOUNDS - A REVIEW –

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Objectives: We all heard about herbs and spices, such as rosemary, oregano, peppermint, that have been used since antiquity as folk medicine and as preservatives in foods, but did we know that these are called aromatic plants? We can find them all over the world, either in the wild or cultivated, originally from the Mediterranean area. The literature provides us evidences of the preservative and medicinal proprieties of the aromatic plants since approximately 5000BC in the Middle East. These proprieties are enhancing the aroma and flavor of foods[1,2,3]. Aromatic plants consist of plenty biologically active compounds, such as polyphenols, flavonoids, alkaloids, polypeptides[4,5,6]. have been found to own many properties such as antimicrobial, antioxidant, anti-inflammatory and antiseptic ones[3-8]. These active compounds have the potential to become new generation substances for human and animal nutrition and health. We should not forget about the odorous, volatile, hydrophobic compounds from aromatic plants, known as essential oils **Methods:** For this review paper it has been used different literature papers about aromatic plants in order to provide an enclosing outline of the aromatic plants bioactive compound and the usage of them for different purposes. **Results:** It has been proved that the bioactive components in the aromatic plants possess the ability to protect the body from damage caused by free radicals induced oxidative stress[2,9]. In Figure 1 it can be seen the uses of the extracts from aromatic plants.[10]

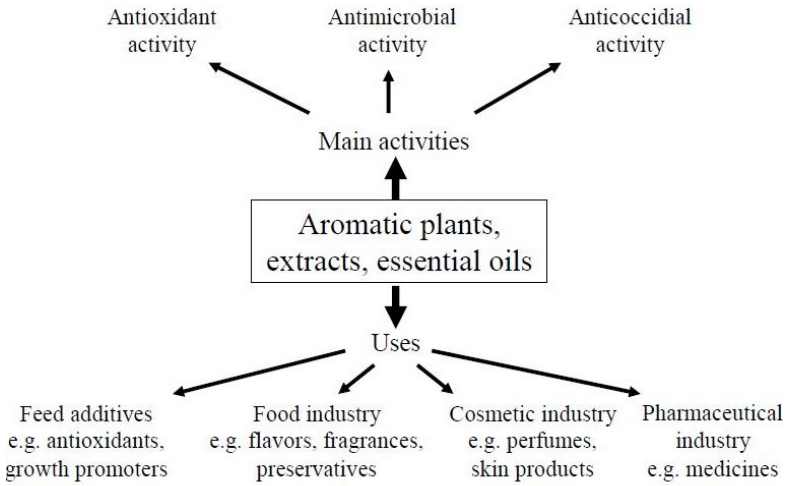

Figure 1. Uses of aromatic plants

After studying different papers, about the antimicrobial activity of the biologically compounds, all the results were that some compounds were demonstrating high antimicrobial activity due to studies aganis Gram negative bacteria[11,12]. It seems that the food industry has been using this compounds as biopreservatives to prevent food spoilage and to increase the shelf life of products.[12].

After many years of studies it been shown that the biologically coumpounds from aromatric plants are linked to the beneficial health functionality of the some phenolic antioxidants for the reason that they contribute to the delay of many oxidative-stress related diseases, such as cardiovascular diseases, cancer, diabetes and Alzheimer's[13,14]. **Conclusion:** Aromatic plants contains a variety of biologically compounds, which have a lot of possible applications in different industries. This short paper was just a review of the literature regarding aromatic plants and also an introduction from the next papers with the same subject. The discussion about this subject will be developed on further papers, regarding specific activities, aromatic plants and biological compounds.

Keywords: aromatic plants, bioactive compounds

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FULL TEXT – ORAL PRESENTATION

THE EFFECT of PHOSPHORUS and ZINC FERTILIZATION on LYCORINE and GALANTAMINE ACCUMULATION in GIANT SNOWDROP

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Abstract

Giant snowdrop (*Galanthus elwesii* Hook) is an important bulbous species that contains the acetylcholinesterase inhibitor galantamine and lycorine alkaloids with anticancer activity in all plant parts. This research was carried out to determine the effect of phosphorus (0, 30, 60, and 120 kg ha^{-1} P_2O_5) and zinc (0, 25, 50, and 100 kg ha^{-1} $ZnSO_4$) applications on lycorine and galantamine accumulation in *G. elwesii* in two cultivation seasons. Aerial and underground parts of the plant were sampled at the stages of flowering and fruit ripening, and the quantities of galantamine and lycorine were determined with Reversed Phase-High Performance Liquid Chromatography (RP-HPLC). The highest quantity of lycorine (26.90 µg/g) was determined in underground organs at flowering stage, while the highest quantity of galantamine (69.66 µg/g) was found also in underground plant parts at fruit ripening stage. These findings provide the first comprehensive galantamine and lycorine alkaloids data of giant snowdrop with regard to soil P and Zn availability.

Keywords: Alkaloids, Amaryllidaceae, *Galanthus elwesii* Hook, RP-HPLC

1. Introduction

Giant snowdrop is one of the most important species in the Amaryllidaceae family (Baktır, 1996). Species in the Amaryllidaceae family include nivalin, galantamine, tazettine and lycorine, whose numbers extend to 150, which are called Amaryllidaceae alkaloids. These alkaloids have high biological activity and are known to have antiviral, antioxidant, anti-microbial, anti-leukemia and immune system boosting effects according to their structure. In particular, galantamine alkaloid is used in polio, Alzheimer's disease and muscle-vessel diseases (Ago et al., 2011). In plants such as snowdrops, the quantity of secondary metabolites synthesized in different harvest times (flowering period and fruit ripening period) as well as synthesized from underground (root and bulbs) and aerial (leaves and flowers) parts of them, the quantity of components and antioxidants capacity changes. For this reason, significant variations can occur in the underground (root and bulb) and aerial (leaves and flowers) parts of plants and at different times (flowering period and fruit maturation period). On the other hand, secondary metabolites are known to be synthesized often in specialized cells, during certain periods of plants and especially in stress conditions. Plants may encounter many biological and non-biological stress factors simultaneously or at different times during their lives. Factors that cause stress in the plant may be from living beings, such as pathogen microorganisms and pests, and can also be sourced from the lifeless origin such as salinity, drought, low and high temperatures, radiation and lack or excess of nutrients (Yaşar, 2003; Kalefetoğlu and Ekmekçi, 2005). The lack or excess of one nutrient can show a positive or negative effect on the utility and toxicity of another nutrient, and this can cause a stress effect

in terms of plant development. For this reason, the effects of enhancing doses of macro and micronutrient treatments on plant development of some medicinal and aromatic plants and especially on secondary metabolites have progressively become important. In this context, due to the interactions between them, phosphorus and zinc are among the main nutrients which should be remembered.

Although there have been some studies on Amaryllidaceae alkaloids in the literature, we encountered no studies on the effect of fertilizer applications on alkaloid ratio and components, especially in giant snowdrops. Therefore, the effects of phosphorus and zinc administration on galantamine and lycorine alkaloids in Giant Snowdrop (*Galanthus elwesii* Hook) were determined in this study.

2. Materials and Methods

2.1. Plant Material

The study was conducted in Suluova District of Amasya province for two years in the 2017-2018 and 2018-2019 production seasons. Giant Snowdrop (Galanthus elwesii Hook) species with bulb diameter greater than 4 cm were used in the research. The research was established in Randomized Plots Design with 3 replications. Phosphorus doses (0, 3, 6 and 12 kg/da P_2O_5) were included in the main parcels and zinc doses (0, 2.5, 5 and 10 kg/da $ZnSO_4$) were included in the sub-parcels. In both years of the study, the harvest in the flowering period and the harvest of underground and aerial organs were performed during the fruit set period.

2.2. Preparation of Plant Samples Before Extraction

After being collected, the plant samples were washed with tap water. Snowdrop bulbs were dried on a blotter for two weeks in a clean room. After drying, plant samples were labeled and taken into storage boxes. In our study, underground (root, bulb) and aerial (flower, fruit) parts of snowdrops were examined.

2.3. Alkaloid Extraction of Plant Samples

In the study, the samples were weighed to be 5 g of each on a sensitive scale and transferred into glass jars with lids and 200 ml of methanol was added. The mixtures obtained were then macerated with the help of the shaker for 3 days (72 hours). The plant suspensions obtained at the end of this period were filtered through a filter paper, and the liquid part was removed, then the pulp was stored. The obtained liquid extracts were processed in the rotary evaporator and the sonar methanol was removed from the environment. Methanol is acidified with 10% CH_3COOH crude extract obtained after evaporation with a rotary evaporator device (pH 2-3). The extract was treated with chloroform, and the removal of substances such as oils, waxes, etc., except for alkaloids, was realized. The remaining extract was alkalinized with 25% NH_3 until the pH became 8-9, allowing the alkaloids in the extract to become free, and the resulting phase was extracted with chloroform.

2.4. Preparation of The Galantamine Standard

For this purpose, 2 mg pure galantamine was dissolved in 5 ml methanol in a volumetric flask and the resulting solution was filtered using 13mm Sem Concept Syringe and 0.45 µ filter paper.

2.5. Preparation of The Measure Curve of Galantamine Standard

Six dilutions of 62.5 µg/ml, 31.25 µg/ml, 15.63 µg/ml, 7.81 µg/ml, 3.91 µg/ml, 1.95 µg/ml were prepared from the standard galantamine solution, and field values were measured 3 times by applying 20 µl of each. In line with the results, the measurement curve of galantamine was prepared in the form of the field read against the applied amount.

2.6. Preparation of The Lycorine Solution Standard

As described above, a sample of 2 mg pure lycorine was dissolved in 5 ml methanol in a volumetric flask, and the solution Sem Concept Syringe was filtered (13 mm, 0.45 µ filter).

2.7. Preparation of The Measure Curve of Lycorin Standard

Six dilutions of 62.5 µg/ml, 31.25 µg/ml, 15.63 µg/ml, 7.81 µg/ml, 3.91 µg/ml, 1.95 µg/ml were prepared from the standard lycorine solution, and 20 µl of each dilution were applied 3 times, so the field values were measured. In line with the results, a measurement curve showing the area read against the applied quantity was prepared.

2.8. Determination of Alkaloid components with HPLC

HPLC working condition and gradient elution program were used for the quantitative determination of alkaloid compounds from extracted plant samples. High pressure liquid chromatography (HPLC) analysis requirements are as follows; device: Shimadzu Prominence Modular LC20A HPLC, column furnace: CTO-10AS VP, column: Intersil ODS 3, 5um 4, 6x250 mm, mobile phase: 95% TFA-water / 5% acetonitrile, detector: SPD-M2OA.

2.9. Statistical Analysis

The variance analysis of the obtained data was done using JUMP statistical package software according to the Randomized Plots Design. The statistical significance between average differences was determined using the Duncan test.

3. Results and Discussion

3.1. Galantamine Quantity

The galantamine quantities of aerial and underground organs were given as µg/g for the flowering period in Tables 1 and 2, respectively, also for the ripening period in Tables 3 and 4.

Table 1. The quantity of galantamine (µg/g) of underground organs harvested during the flowering period in Giant Snowdrop which different doses of phosphorus and zinc were applied to

Fosfor Dozları (kg/da)	1st Year					2nd Year					Phosphorus Two Years Avg.
	Zinc Doses (kg/da)					Zinc Doses (kg/da)					
	0	**2.5**	**5**	**10**	**Ort.**	**0**	**2.5**	**5**	**10**	**Ort.**	
0	0.64	4.06	26.58	4.29	8.89	8.53	4.73	3.63	33.23	12.53	10.71
3	2.18	2.99	8.53	1.07	3.69	7.79	8.32	4.78	4.60	6.37	5.03
6	3.58	2.13	2.90	2.49	2.77	25.73	6.90	18.16	7.07	14.46	8.62
12	1.25	**0.46**	2.39	7.53	2.90	32.61	3.64	**68.69**	3.01	26.98	14.94
Phosphorus Avg.	1.91	2.41	10.10	3.84	4.56	18.66	5.89	23.81	11.97	15.08	
Phosphorus Two Years Avg.	10.28	4.15	7.99	7.91							

Table 2. The quantity of galantamine (µg/g) of the aerial organs harvested during the flowering period in the Giant Snowdrop which different doses of phosphorus and zinc were applied to

Phosphorus Doses (kg/da)	1st Year					2nd Year					Phosphorus Two Years Avg.
	Zinc Doses (kg/da)					Zinc Doses (kg/da)					
	0	2.5	5	10	Ort.	0	2.5	5	10	Ort.	
0	8.27	6.12	5.96	6.69	6.76	5.72	9.59	9.15	10.89	8.83	7.79
3	2.26	1.73	**18.78**	8.20	7.74	8.21	2.80	0.82	6.94	4.68	6.21
6	3.56	3.51	12.44	12.19	7.92	7.35	7.85	4.64	1.21	5.26	6.59
12	2.77	4.10	7.69	3.03	4.39	3.11	**1.05**	8.74	8.44	5.33	4.86
Phosphorus Avg.	4.21	3.86	11.21	7.52	6.70	6.09	5.32	5.83	6.87	6.02	6.03
Phosphorus Two Years Avg.	5.15	4.59	8.52	7.19							

Table 3. The quantity of galantamine (µg/g) of underground organs harvested during the ripening period of the Giant Snowdrop which different doses of phosphorus and zinc are applied to

Phosphorus Doses (kg/da)	1st Year					2nd Year					Phosphorus Two Years Avg.
	Zinc Doses (kg/da)					Zinc Doses (kg/da)					
	0	2.5	5	10	Avg.	0	2.5	5	10	Avg.	
0	7.74	14.52	1.87	17.73	10.46	21.30	5.48	5.65	4.77	9.3	9.88
3	5.00	9.58	**69.66**	0.99	21.30	3.16	2.48	7.69	5.94	4.81	13.06
6	**0.69**	18.30	11.15	1.46	7.90	10.20	1.91	1.57	6.30	4.99	6.44
12	1.82	3.82	3.87	2.94	3.11	-	3.33	2.32	10.54	4.04	3.58
Phosphorus Avg.	3.81	11.55	21.63	5.78	10.69	8.66	2.46	3.72	4.25	5.79	
Phosphorus Two Years Avg.	6.23	7.42	12.77	6.33							

Table 4. The quantity of galantamine (µg/g) of aerial organs harvested during the ripening period of the Giant Snowdrop which different doses of phosphorus and zinc are applied to

Phosphorus Doses (kg/da)	1st Year					2nd Year					Phosphorus Two Years Avg.
	Zinc Doses (kg/da)					Zinc Doses (kg/da)					
	0	2.5	5	10	Avg.	0	2.5	5	10	Avg.	
0	0.43	5.72	0.54	7.04	3.43	7.94	4.19	36.78	9.13	14.51	8.97
3	1.19	0.50	0.36	1.58	0.90	11.94	1.20	7.63	6.92	6.92	3.91
6	0.38	0.11	0.24	**0.07**	0.20	11.36	12.91	11.62	2.12	9.50	4.85
12	0.11	0.11	4.79	0.17	1.29	7.43	1.56	**18.18**	-	6.79	4.89
Phosphorus Avg.	0.52	1.61	1.48	2.21	1.45	9.66	4.96	18.55	4.54	9.43	
Phosphorus Two Years Avg.	5.09	3.28	10.01	3.37							

As can be observed from Table 1, Table 2, Table 3 and Table 4, the highest galantamine quantity in the Giant Snowdrop, which different doses of phosphorus and zinc were applied to, was found to be 69.66 µg/g in underground organs during the ripening period. The lowest quantity of galantamine was found to be 0.07 µg / g in the aerial organs of the plant during the fruit ripening period.

According to previous studies, Emir et al. (2013) reported that the galantamine ratio in *Galanthus woronowii* was 0.003-0.506%. Georgieva et al. (2007) reported that galantamine content in flower bulbs collected from eighteen Bulgarian origin populations of *L. aestivum* and in extracts from shoots from 8 different populations grown in *in vitro* ranged between 28 and 2104 µg/g dry weight in bulbs and 454 µg/g dry weight in shoots. Petruczynik et al. (2013) reported that the highest quantity of galantamine was 2.3524 mg/g in the roots of *L. aestivum* and 1.6611 mg/g in its leaves. Kaya et al. (2014) determined the galantamine content in the aerial parts and bulbs of *G. elwesii* plant collected from the village of Cimi (Antalya) to be 0.346% and 0.042%, respectively. The aerial parts of *G. elwesii* plant collected from Ibradi (Antalya) were found to contain 0.287% galantamine, while the bulbs were found to contain 0.095% of this alkaloid. Galantamine was not detected in *G. elwesii* samples collected from Kayrak village (Mersin). Our study is in line with previous studies. Alkaloids in the plant can vary according to plant organ, harvest time, the geographical area where it grows, cultural processes and environmental factors.

3.2 Lycorine quantity

In Table 5 and 6, the lycorine amounts of underground and aerial parts in flowering periods were given as µg/g respectively, also Table 7 shows the values belonging to the ripening period.

Table 5. The quantity of Lycorine (µg/g) of underground organs harvested during the flowering period of Giant Snowdrop which different doses of phosphorus and zinc were applied to

Phosphorus Doses (kg/da)	1st Year					2nd Year					Phosphorus Two Years Avg.
	Zinc Doses (kg/da)					Zinc Doses (kg/da)					
	0	**2.5**	**5**	**10**	**Ort.**	**0**	**2.5**	**5**	**10**	**Ort.**	
0	-	0.04	0.33	0.14	0.12	9.78	16.94	2.50	5.66	8.72	4.42
3	-	0.04	0.05	-	0.02	9.84	11.94	16.92	9.94	12.16	6.09
6	0.22	-	0.05	0.13	0.10	14.94	1.36	22.55	16.62	13.86	6.99
12	0.04	**0.01**	-	0.18	0.05	**26.90**	16.05	4.61	0.42	11.99	6.02
Phosphorus Avg.	0.06	0.02	0.10	0.11	0.07B	15.36	11.57	11.64	8.16	11.68A	
Phosphorus Two Years Avg.	7.71	5.79	5.87	4.13							

Table 6. The quantity of Lycorine (µg/g) of aerial organs harvested during the flowering period in Giant Snowdrop which different doses of phosphorus and zinc were applied to

Phosphorus Doses (kg/da)	1st Year					2nd Year					Phosphorus Two Years Avg.
	Zinc Doses (kg/da)					Zinc Doses (kg/da)					
	0	2.5	5	10	Ort.	0	2.5	5	10	Ort.	
0	1.45	-	0.09	-	0.38	1.73	**6.48**	1.55	3.31	3.26	2.19
3	0.003	-	-	0.31	0.07	1.63	0.42	1.43	3.08	1.64	1.35
6	0.01	-	-	4.40	1.10	1.79	0.83	1.51	1.77	1.47	5.91
12	0.006	0.009	**0.001**	0.01	0.006	0.43	0.87	-	0.23	0.38	0.58
Phosphorus Avg.	0.36	0.002	0.022	1.18	0.39B	1.39	2.15	1.12	2.09	1.69A	
Phosphorus Two Years Avg.	0.88	1.07	0.57	1.63							

Table 7. The quantity of Lycorine (µg/g) of underground organs harvested during the ripening period in Giant Snowdrop which different doses of phosphorus and zinc were applied to

Phosphorus Doses (kg/da)	1st Year					2nd Year					Phosphorus Two Years Avg.
	Zinc Doses (kg/da)					Zinc Doses (kg/da)					
	0	2.5	5	10	Ort.	0	2.5	5	10	Ort.	
0	1.93	0.005	-	**0.002**	0.48	3.95	**20.91**	0.60	13.36	9.70	5.09
3	0.09	-	0.02	0.04	0.03	3.89	3.51	8.39	4.44	5.05	2.55
6	0.02	0.26	-	0.03	0.07	6.65	1.25	1.74	-	2.41	1.24
12	-	0.09	-	1.08	0.29	2.21	5.62	-	0.59	2.10	1.19
Phosphorus Avg.	0.51	0.08	0.005	0.28	0.22B	4.17	7.82	2.68	4.59	4.81A	
Phosphorus Two Years Avg.	2.34	3.95	1.34	2.44							

As can be observed from Table 5, Table 6, Table 7, the highest content of lycorine in the Giant Snowdrop which the different doses of phosphorus and zinc were applied to was found to be 26.90 µg/g in underground parts during the flowering period of the plant. The lowest content of lycorine was found to be 0.001 µg/g in the aerial organs during the flowering period of the plant. For the fruit ripening and bloom period, the year effect was statistically significant, while the effect of phosphorous and zinc fertilizers and their interaction were statistically insignificant.

Kaya et al. (2014) studied to determine the lycorine content in the aerial parts and bulbs of *G. elwesii* plant collected from Cimi village (Antalya), and found the lycorine only in the *G. elwesii* bulbs collected from Ibradi (Antalya) and Kayrak village (Mersin), at the rate of 0.005% and 0.015%, respectively. Muhtar and Şener (1996) investigated the lycorine content in the bulbs of the *Narcissus tazetta, Leucojum aestivum, Galanthus elwesii* and *Galanthus ikariae*, which are exported as wildflower bulbs in Turkey, by HPLC method, and found that the bulbs of *Narcissus tazetta* (0.089%) had more lycorine in their bulbs than others. Akbulut (2004) investigated the presence of galantamine and lycorine for drog samples prepared separately during flowering and fruitful vegetation periods of the *Galanthus plicatus* (Bieb) subsp.

byzantinus (Baker) D.A. Webb plant, which grew wild in the vicinity of Bolu-Abant Lake forest house, with two different methods including High Pressure Liquid Chromatography (HPLC) and the spectrophotometry combined with I.T.K. The results showed that galantamine and lycorine were not found in the plant in quantifiable quantities. Li et al. (2012) developed a high-performance liquid chromatographic method with reverse phase to determine the three alkaloids of *Lycoris radiata*. It was 3,375 mg/L for galantamine, 0.475 mg/L for lycoramine and 0.495 mg/L for lycorine. The method was applied for the detection of three alkaloid content in different parts of *Lycoris radiata*. As a result, it has shown that the content varies significantly in different plant parts. In the leaves, the content of lycorine was found to be the highest and the contents of galantamine were found to be the lowest. There are points where our work resembles and differs from previous studies. This is because secondary metabolites can vary depending on harvest time, environmental factors, and sampled part of the plant.

4. Conclusion

In our study, the highest quantity of galantamine in Giant snowdrops was found to be 69.66 µg/g in underground organs during the fruit ripening period of the plant. The lowest quantity of galantamine was found to be 0.07 µg/g in the aerial organs of the plant during the ripening period of the fruit. The highest quantity of lycorine was found to be 26.90 µg/g in underground organs during the flowering period of the plant. Also, the lowest quantity of lycorine was found to be 0.001 µg/g in the aerial organs during the flowering period of the plant. When we evaluate our study based on the harvested plant parts, it is clear that the quantity of galantamine and lycorine is the highest in the underground organs. When Giant snowdrop is evaluated according to different harvest times, it is observed that the quantity of lycorine is the highest during the flowering period, and the quantity of galantamine is the highest during the ripening period of the fruit.

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[12]

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FULL TEXT – ORAL PRESENTATION

SIGNIFICANCE OF SEED COAT MORPHOLOGY IN SOME *ISATIS* (BRASSICACEAE) TAXA

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Abstract
The seed exomorphic characteristics of five taxa (*I. arenaria* Azn., *I. candolleana* Boiss., *I. cappadocica* Desv. subp. *macrocarpa* (Jaub. & Spach) P.H. Davis, *I. erzurumica* P.H. Davis and *I. floribunda* Boiss. ex. Bornm. belonging to *Isatis* L. were investigated with scanning electron microscopy (SEM). This study presents exomorphic characteristics, including seed colour, shape, winged, measures, seed coat pattern. Three coat patterns (regularly reticulate, irregularly-reticulate, and rugose) were observed, and four shapes (narrowly elliptic, narrowly ovate, oblong and lanceolate) were distinguished. The results showed that the morphological characteristics of seed could contribute as criteria to distinguish taxa.

Keywords: Classification, Cruciferae, micromorphology, taxonomy

1. Introduction

Turkey is one of the richest countries in the world in terms of *Brasseicacea* species with 571 species. At the first volume of the Flora of Turkey, the number of *Brassicaceea* genus and species was indicated to be 86 and 464, respectively (Davis, 1965). These numbers increased to 88 genus and 526 species in the tenth volume (Davis et al., 1988), and to 91 genus and 555 species in the eleventh volume (Güner et al., 2000). In the second supplement of Flora of Turkey, the total number of the species increased to 538 with the addition of one genus, 28 species, 15 subspecies, and two varieties (Al-Shehbaz et al., 2007). *Isatis* includes about 79 species (Al-Shehbaz et al., 2006) and the genus is distributed primarily in the northern hemisphere, especially in the Irano-Turanian region, where nearly 90% of its species grow (Appel and Al-Shehbaz, 2003; Davis, 1965). *Isatis* species sometimes show a morphologically highly polymorphic pattern (Davis, 1965; Hedge, 1968; Jafri, 1973).

Seed surfaces, color, and size are valuable characteristics for distinguishing taxa in Brassicaceae (Vaughan and Whitehouse, 1971; Barthlott, 1981; Koul et al., 2000; Karaismailoğlu, 2019). Therefore the main objective of this study is to evaluate the taxonomic significance of the seed coat pattern as seen by SEM in some taxa of the *Isatis*.

2. Material and Methods

The study material comprised samples of ripe seeds from four taxa belonging to *Isatis* collected from natural habitats in Turkey between 2015 and 2016. The samples were stored at Konya Selçuk University Faculty of Science Herbarium (KNYA). The locations and collector registration numbers of the taxa studied are provided in Table 1; the seed micromorphology characteristics are provided in Table 2. Twenty seeds from each taxon were examined in our study.

Samples examined in our analyses using scanning electron microscopy (SEM) were first passed through a series of 70, 80, 96, and 100% alcohol for 20 min each. The surfaces were then observed and photographed with the ZEISS EVO LS-10 scanning electron microscope at 30x, 1000x, and 2000x magnification in high vacuum mode.

The seed micromorphology terminology used was according to Stearn (1992), Koul et al. (2000), and Zeng et al. (2004).

Table 1. Localities of the studied taxa

TAXA	LOCALITY
I. arenaria	C4 Konya: Karapınar, Karaören Köyü, tarla kenarı, 1250 m, 17.06.2015, *E. Şirin 566 & M. Şirin* (KNYA)
I. candolleana	A8 Bayburt: Yeşilyurt, Karpuz Yaylası, step, 700 m, 12.06.2016, *E. Şirin 646 & M. Şirin* (KNYA)
I. cappadocica subsp. *macrocarpa*	C4 Konya: Hadim-Taşkent arası, step, 1500 m, 12.05.2015, *E. Şirin 535 & M. Şirin* (KNYA)
I. erzurumica	A8 Erzurum: Horasan-Hasankale arası, molozlu yamaç, 1700 m, 13.08.2015, *E. Şirin 589 & M. Şirin* (KNYA)
I. floribunda	B5 Aksaray: Regülatör mevkii, step, 1100 m, 29.06.2016, *E. Şirin 660 & M. Şirin* (KNYA)

3. Results And Discussion

The micrographs from the seeds of the four taxa studied are shown in Fig. 1 and the macro- and micromorphological properties of the seeds are provided in Table 2.

I. arenaria: Seed brown, narrowly elliptic, unwinged, glabrous, 5.41–5.76 x 1.81–3.01 mm and the seed coat pattern is regularly reticulate (Table 2, Fig. 1).

I. candolleana: Seed dark brown to black, narrowly ovate, unwinged, glabrous, 2.91–3.22 x 1.21–1.43 mm and the seed coat pattern is irregularly reticulate (Table 2, Fig. 1).

I. cappadocica subsp. *macrocarpa*: Seed dark brown to black, oblong, unwinged, glabrous, 6.42–6.72 x 3.02–3.71 mm and the seed coat pattern is irregularly reticulate (Table 2, Fig. 1).

I. erzurumica: Seed brown, lanceolate, unwinged, glabrous, 3.12–3.32 x 1.01–1.32 mm and the seed coat pattern is irregularly reticulate (Table 2, Fig. 1).

I. floribunda: Seed dark brown to black, narrowly elliptic, unwinged, glabrous, 3.42–3.82 x 0.81–1.02 mm and the seed coat pattern is rugose (Table 2, Fig. 1).

Table 2. Micro- and macromorphological features of studied taxa

TAXA	COLOUR	SHAPE	LENGTH (mm)	WIDTH (mm)	ORNAMENTATION
I. arenaria	Brown	Narrowly elliptic	5.41–5.76	1.81–3.01	Regularly reticulate
I. candolleana	Dark brown to black	Narrowly ovate	2.91–3.22	1.21–1.43	Irregularly reticulate
I.cappadocica subsp. macrocarpa	Dark brown to black	Oblong	6.42–6.72	3.02–3.71	Irregularly reticulate
I. erzurumica	Brown	Lanceolate	3.12–3.32	1.01–1.32	Irregularly reticulate
I. floribunda	Dark brown to black	Narrowly elliptic	3.42–3.82	0.81–1.02	Rugose

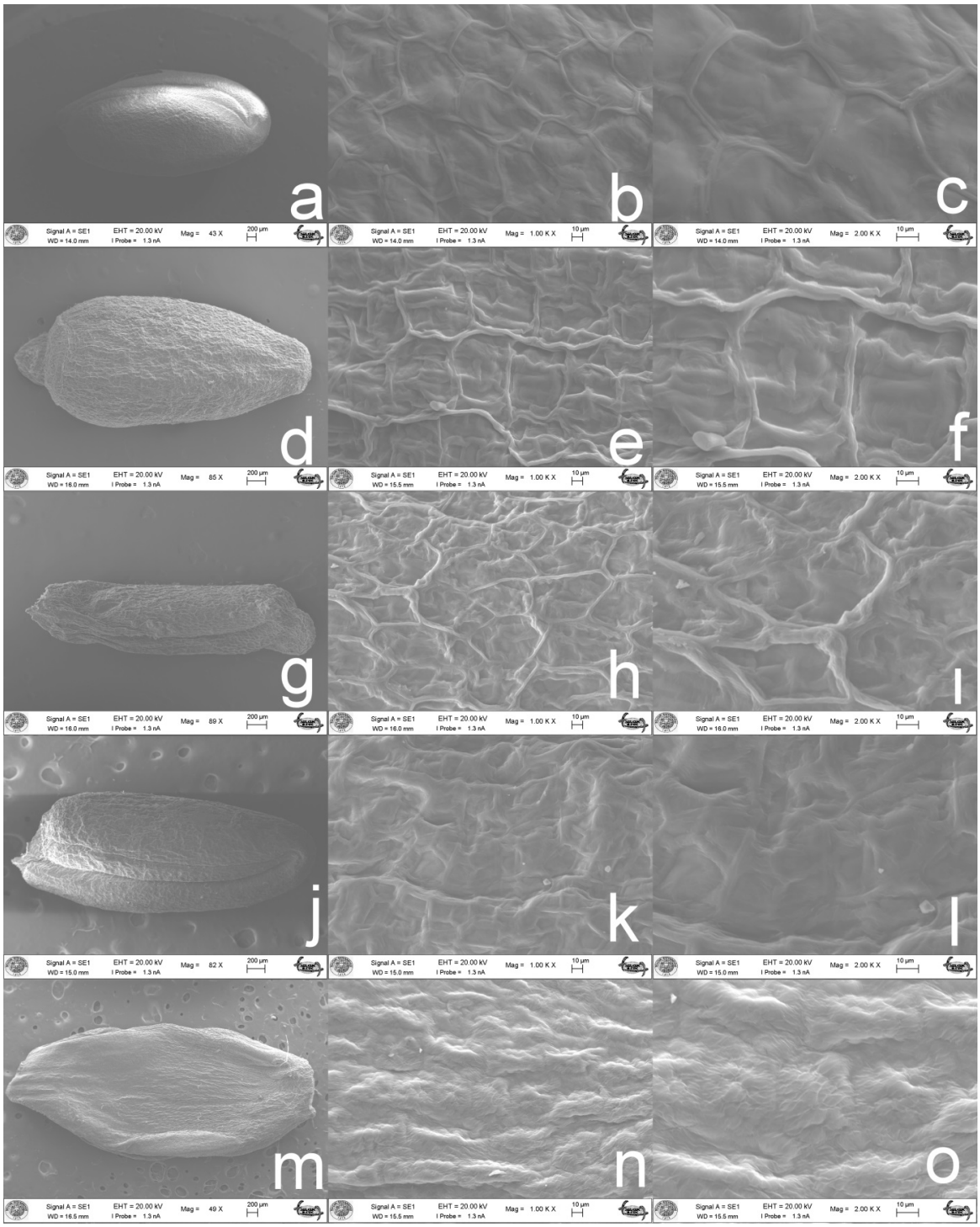

Figure 1. SEM micrographs of seeds of studied taxa: *I. arenaria* (a–c), *I. candolleana* (d–f), *I. cappadocica* subsp. *macrocarpa* (g–i), *I. erzurumica* (j–l) and *I. floribunda* (m–o).

SEM studies showed that seed, fruit, and leaf surface model characteristics are useful for describing different families and genera (Kumar et al., 2012; Shavvon et al., 2012; Akçin et al., 2013).

Although Karaismailoğlu (2019) defined the seed shapes of *I. arenaria* and *I. erzurumica* as ellipticus-anguste and ovatus, respectively, it is more convenient to describe *I. arenaria* to be narrowly elliptic and *I. erzurumica* to be lanceolate according to Stearn's (1992) system. This characteristic cannot be considered distinctive because the seeds of all taxa studied were wingless. Some other species within the same family that have a wingless seed structure are *Nasturtium officinale* R.Br., *Rorippa islandica* (Oeder) Borbás, *Brassica napus* L., *and Iberis linifolia* L. (Vaughan and Whitehouse, 1971).
I. cappadocica subsp. *macrocarpa* has relatively larger seeds, while *I. candolleana* has relatively smaller seeds than those of other *Isatis* species. Karaismailoğlu (2019) reported the seed coat patterns of *I. arenaria* to be rugose and *I. erzurumica* to be smooth. However, it is more appropriate to interpret *I. arenaria* as regularly reticulate due to its regular web-like structure on the seed surface and *I. erzurumica* as irregularly reticulate due to the irregular web-like structure on the seed surface.

Color characteristics cannot be considered distinctive because the color of the seeds from all studied taxa brown or dark brown to black. Bona (2013) has observed a similar seed color in *Lepidium* L. species that are not related to those of *Isatis*. The seed surface of all taxa studied was glabrous. Similarly, Ghaempanah et al. (2013) have reported a glabrous structure in *Erysimum* L. species that are not related to those of *Isatis*.

4. Conclusion

The studied characteristics were not distinctive for *Isatis* in genus level but the shape and size of the seeds and that seed coat patterns can be used as distinctive characteristics among the studied taxa. In future seed morphology studies about the rest of the *Isatis* species will contribute to the interpretation of relationships of the taxa.

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FULL TEXT – ORAL PRESENTATION

VARIATION IN SOME QUALITY PROPERTIES OF DIFFERENT BASIL GENOTYPES

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Abstract

Ocimum basilicum L. (sweet basil) is an essential oil producing crop used in culinary and fragrance applications. The essential oils of sweet basil are used for the treatment of dry mouth and dental complaints, diarrhea and chronic dysentery, and respiratory disorders, and are also effective in the treatment of fungal diseases and stomach discomfort, in addition to having influential antitussive, diuretic, anthelminthic, tranquilizer and expectorant roles in medical applications. Present investigation comprised of thirty-seven genotypes of sweet basil was undertaken to characterize the genotypes based on chemical variation of leaves and stems like essential oil yield, mineral content, and protein content. Leaves and stems, previously air-dried at room temperature, were hydro-distilled for three hours. Results of this study revealed significant variations (0.11-1.67%) in essential oil content. Ames 32312 genotype was found superior in case of essential oil amounts as compared with other genotypes. Moonlight (%18.26) genotype exhibited the highest protein content. Most of the highest mineral content was found from PI358466 genotype.

KeyWords: Basil, essential oil, protein, anion and cation.

1. Introduction

Basil (*Ocimum basilicum* L.) is the most common species in Lamiaceae family. Ocimum genus includes about 150 species and within the species *Ocimum basilicum* L. (Pushpangadan and Bradu, 1995).

There are a number of varieties that differ in the general morphological structure and texture (the plant size, habitus, color, and shape and size of the leaves and flowers, content and chemical composition of the essential oil) and these varieties are cultivated in the World (Grayer et al., 1996; Sifola and Barbieri, 2006; Nurzynska-Wierdak, 2007; Dzida, 2010; Nurzynska-Wierdak, 2013).

Basil is an important medicinal and aromatic plant which is cultivated many part of the World. It is used for treatment of dry mouth and dental complaints, diarrhea, chronic dysentery, and respiratory disorders. It is also effective in the treatment of fungal diseases and stomach discomfort, the influential antitussive, diuretic, anthelminthic, tranquilizer and expectorant roles in medicinal approach. In addition, ceasing nasal-bleeding and preventing constipation, good for fatigue and insomnia, and uses for healing migraine headaches and incomplete paraplegia were reported (Telci et al., 2006).

Many researchers reported that morphological properties, yield and yield components of basil change in terms of plant height, fresh herb yields, dry herb yield and its components under different ecological conditions (Erşahin, 2006, Telci et al., 2006; Ekren et al., 2009; Yaldız et al., 2018a, b).

Several studies deal with grouping of basil varieties based on only one nutritional characteristics according to chemotype (De Masi et al., 2006; Telci et al., 2006).

The main objectives of our work were; to determine the essential oil, protein and mineral content of different basil genotypes, to compare local cultivar with different origin basil genotypes in terms of these properties.

2. Material and Methods

35 different genotypes which are included 8 different countries (Macedonia, US, Iran, Afghanistan, Georgia, Turkey) and 4 controls (Dino, Moonlight, Midnight and Large sweet) were used in this study (Table 1).

Table 1. Information of 35 different basil genotypes and 4 controls used in this study.

No	Genotypes/Cultivar	Accession name	Country
1	Ames 29184	GSMO 2-19	Georgia/South Ossetia
2	Ames 32309	GE.2013-21	Georgia
3	Ames 32310	GE.2013-37	Georgia
4	Ames 32311	GE.2013-43	Georgia
5	Ames 32312	GE.2013-50	Georgia
6	Ames 32314	GE.2013-78	Georgia
7	Bolu	Bolu pop.	Turkey/Bolu
8	Dino	Cultivar	Turkey
9	Large sweet	Cultivar	Turkey
10	Midnight	Cultivar	Turkey
11	Moonlight	Cultivar	Turkey
12	PI 170578	Fesligen	Turkey/Aydın
13	PI 172996	Reyhan	Turkey/Kars
14	PI 172997	Reyhan	Turkey/Kars
15	PI 173746	Reyhan	Turkey/Malatya
16	PI 174284	Reyhan	Turkey/Van
17	PI 176646	Reyhan	Turkey/Tokat
18	PI 253157	Rayhoon	Iran/Esfahan
19	PI 296390		Iran
20	PI 296391		Iran
21	PI 358466	Krsaten	Macedonia
22	PI 358468	Krupnolisten	Macedonia
23	PI 358469	Siten	Macedonia
24	PI 358471	Sitnolisten	Macedonia
25	PI 368695	Zelen	Macedonia
26	PI 368699	Edar	Macedonia
27	PI 379412	Krupen bel	Macedonia
28	PI 414193	B 49939	USA/Maryland
29	PI 414195	B 19927	USA/Maryland
30	PI 414196	B 49928	USA/Maryland
31	PI 414197	B 49929	USA/Maryland
32	PI 414198	B 49930	USA/Maryland
33	PI 414199	B 49931	USA/Maryland
34	PI 531396	1420	Hungary
35	PI 652054	Mrs. Burns Lemon Basil	USA/Mexico
36	PI 652065	Genovese	Italia/Veneto
37	PI 652071	Dark Opal	USA/Kalifornia
38	PI 211586	12832	Afghanistan/ Kondoz
39	PI 190100	1	Iran

Experiment was carried out based on Augmented Experimental Design consisting of blocks of which every block had 7 genotypes and 4 controls. Seeds of these genotypes were sown in 15 April 2018 in trial area of Agriculture (Figure 1). Before transplantation of the seedlings, 8 kg/da Diammonium phosphate (DAP) was applied to the plots as a base fertilizer and after transplantation, nitrogenous fertilizer as 6 kg/da Ammonium nitrate (AN) was applied to the plants in two splits in April and July. During the vegetation period, all required agricultural practices were carried out. The first harvest was at the beginning of flowering, and started from the first week of July until the third week of August.

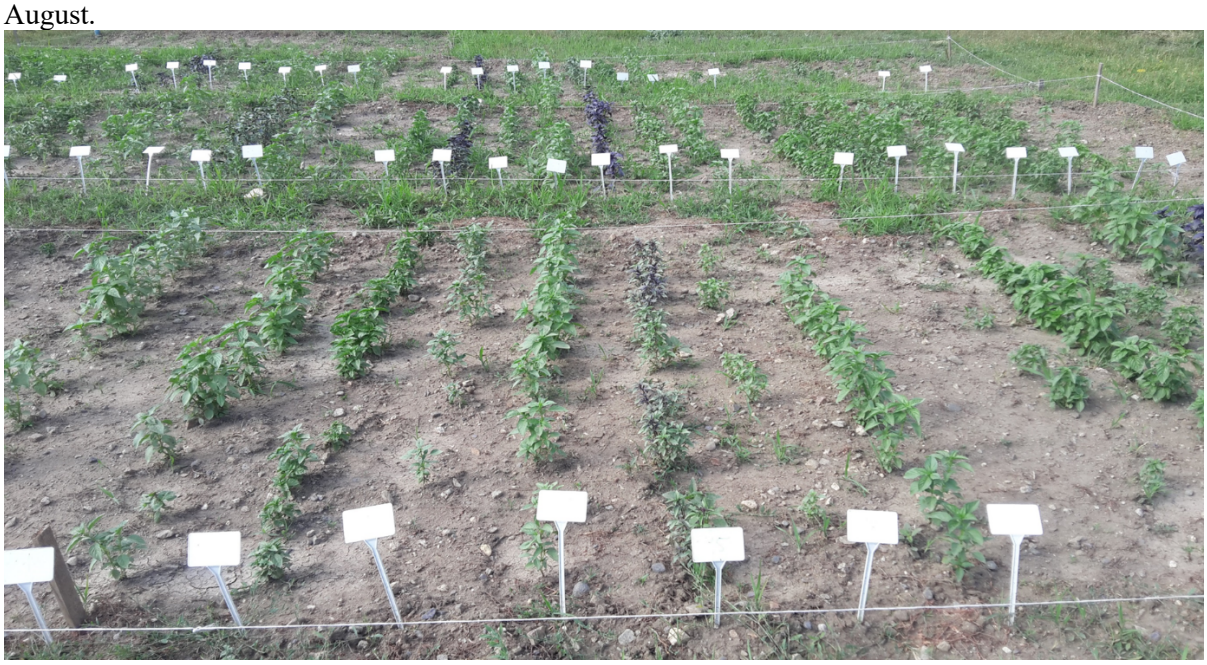

Figure 1. Image of **e**xperiment area.

A Clevenger device was used to obtain essential oil of basil genotypes and cultivars by hydro-distillation. Fifty g of basil flowering aerial parts were taken from each treatment. Afterward, they were roughly crushed and placed in a 1-L glass balloon to which 500 mL of distilled water were added. Distillation was conducted for three h to obtain the essential oil content. Once collected in a sealed glass vials, the essential oil was dehydrated anhydrous sodium sulfate and stored at 4°C in darkness until analyses.

Protein analysis was conducted with kjeldahl method and protein content was calculated as: 6.25 × N-total described by Nurzynska-Wierdak et al. (2011). Mineral content was determined as reported by Yaldız et al. (2018).

3. Results and Discussion

Essential oil content of basil is one of the most important quality parameters. Essential oil content changed from 0.10% to 1.06% among the basil genotypes and cultivars. The highest essential oil content was found from Ames32309 genotype and the lowest essential oil content was found from PI190100 genotype. Average of examined genotypes was 0.52%. 11 basil genotypes were determined higher than control means (0.66%) (Figure 2).

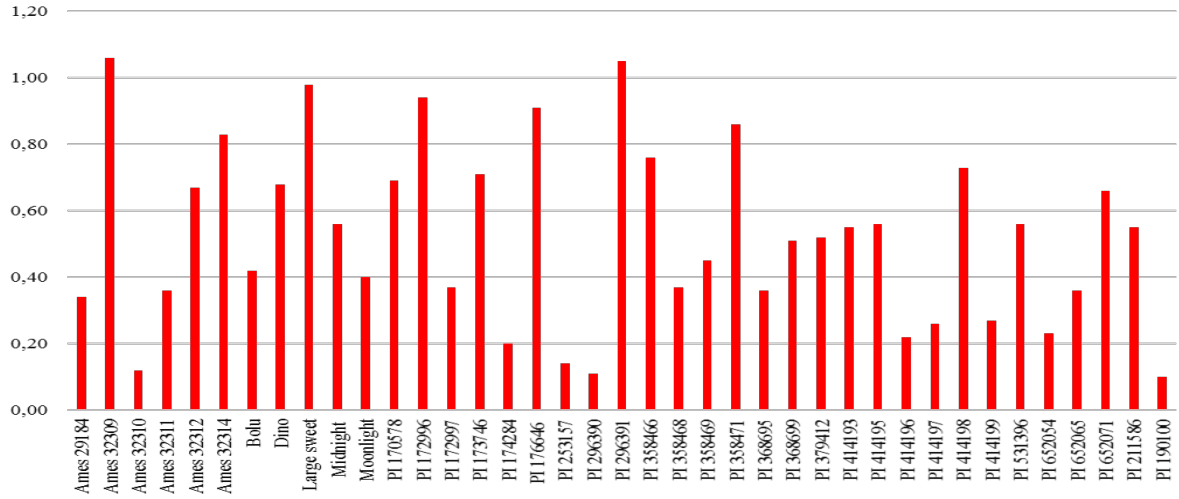

Figure 2. Essential oil content of basil genotypes and cultivars.

Protein content changed between 9.61-18.26% among basil genotypes and cultivars. The highest protein content was found from Moonlight cultivar with 18.26% and followed by Bolu genotype with 16.22%. The lowest protein content was observed in Midnight cultivar and followed by PI 170578 genotype orginated from Turkey. Total average of genotypes was 13.02%, cultivar average was 12.32%. 25 different genotypes were determined over the average of cultivars (Table 2).

Table 2. Protein content of basil genotypes.

No	Genotypes/Cultivar	Protein content (%)	No	Genotypes/Cultivar	Protein content (%)
1	Ames 29184	13.85	21	PI 358466	11.76
2	Ames 32309	12.86	22	PI 358468	13.98
3	Ames 32310	13.85	23	PI 358469	10.84
4	Ames 32311	12.98	24	PI 358471	12.52
5	Ames 32312	15.51	25	PI 368695	12.98
6	Ames 32314	15.51	26	PI 368699	10.54
7	Bolu	16.22	27	PI 379412	11.32
8	Dino	10.54	28	PI 414193	12.19
9	Large sweet	10.86	29	PI 414195	12.96
10	Midnight	9.61	30	PI 414196	12.42
11	Moonlight	18.26	31	PI 414197	13.74
12	PI 170578	10.15	32	PI 414198	10.94
13	PI 172996	12.28	33	PI 414199	11.41
14	PI 172997	12.98	34	PI 531396	14.47
15	PI 173746	15.51	35	PI 652054	11.99
16	PI 174284	13.57	36	PI 652065	14.99
17	PI 176646	15.51	37	PI 652071	15.15
18	PI 253157	10.24	38	PI 211586	14.56
19	PI 296390	13.82	39	PI 190100	10.87
20	PI 296391	13.39			

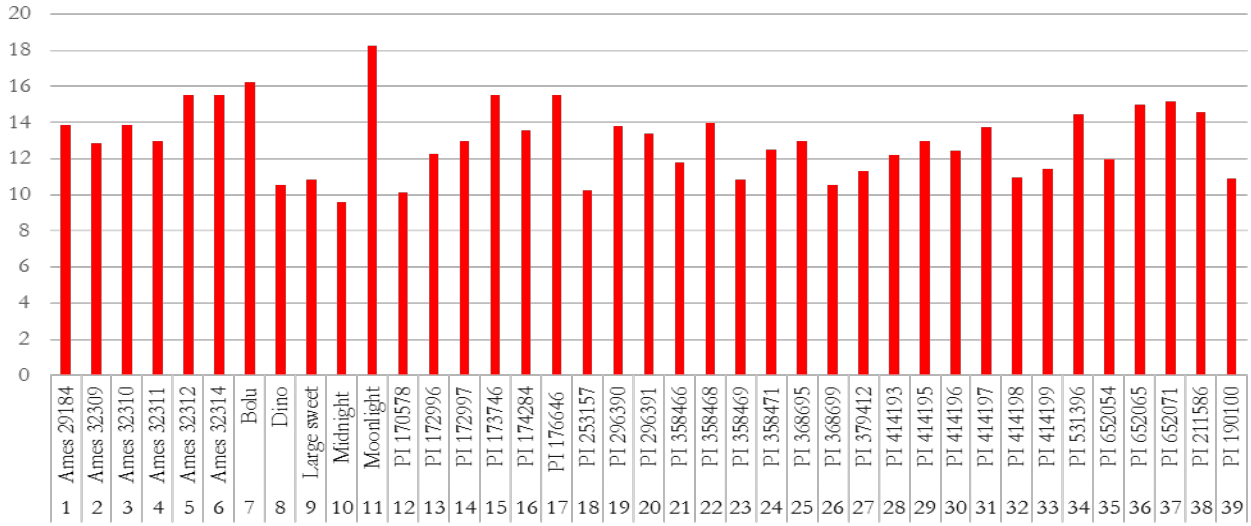

Figure 3. Protein content of basil genotypes.

Mineral contents as chlorine (Cl^-), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}), sodium Na^{2+}, ammonium (NH^{4+}), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) were determined in different selected basil genotypes based on yield properties (Table 3). Chlorine content was found as 2620.03-3850.283 mg/kg, and PI 358466 genotype had higher value than other genotype interms of chlorine content.

Phosphate ranged from 10313.56 to 6187.588 mg/kg. The highest phosphate was found from PI 358466 genotype. Sulphate changed between 675.2549-1912.825 mg/kg and sodium changed between 27.23-35.76 mg/kg between the basil genotypes. The highest and lowest sulphate and sodium values were found in different genotypes.

Ammonium was found only PI 170578 genotype as 0.69 mg/kg. Potassium content varied between 6838.30-12718.66 mg/kg in basil genotypes. The highest value was found from PI 358466 genotype. Magnesium content ranged from 1153.86 to 2182.66 mg/kg. The highest value was found from PI 358466 genotype. Calcium content of basil genotypes changed between 5037.79-6239.87 mg/kg. The highest value was found from PI 358466 genotype. Chlorine and phosphate contents were found higher in PI 170578 genotype higher than PI 358466 genotype. Other mineral contents were found opposite of these mineral contents.

Table 3. Mineral content of two basil genotypes.

Genotypes/ cultivars		Unit	Cl^-	PO_4^{3-}	SO_4^{2-}	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
	LOD	ppm	0.006434	0.020522	0.03031	0.256636	0.033089	0.640888	0.313946	0.656466
	LOQ	ppm	0.021446	0.068406	0.101033	0.855452	0.110298	2.136292	1.046486	2.188222
	R2	-	0.9967	0.9991	0.9993	0.9999	0.9946	0.9999	0.9999	0.9997
	Linearity	ppm	0.1-20	0.2-40	0.1-20	0.4-30	0.4-30	1-75	0.5-37.5	1-75
PI 170578	Fesligen	mg/kg	2620.03	6187.59	1912.83	35.76	0.69	6838.30	1153.86	5037.79
PI 358466	Krsaten	mg/kg	3850.28	10313.56	675.25	27.23	-	12718.66	2182.45	6239.87

Previous study was conducted to find protein content of basil. It was reported that protein content changed between 17.70-26.13% (Nurzynska-Wierdak et al., 2011). Essential oil content of basil genotypes had hight variatons.

Essential content was reported between 0.10-1.02 % (Egata et al., 2017) and 0.40-1.50% (Telci et al., 2006). Lavilla et al. (1999) indicated that *O. basilicum* contented Mg (7458 mg/g) and Ca (21500 mg/g).

Yamawaki et al. (1993) reported that one hundred grams of fresh basil leaves contained 250 mg of calcium, 37 mg of phosphorus, 5.5 mg of iron, and 11 mg of magnesium.

Daniel et al. (2011) reported that *O. basillicum* exhibited high potassium content (28.770 mg/kg), calcium (17.460 mg/kg) and appreciable quantity of sodium (290 mg/kg) and magnesium (266 mg/kg).

Our result was found similar interms of essential oil and found different for protien and mineral content from previous studies. The differences between our results and earlier studies may be due to the different environmental, genetic factors and applications.

4. Conclusion

The highest essential oil and protein content were found in different basil genotypes and cultivars. In this context, Ames32309 and PI296391 genotypes were recommended for high essential oil more than 1% and moonlight cultuvar and bolu genotype were suggested for protein content over 15%.

In this study, two basil genotypes were evaluated in terms of mineral contents as sulphate, amonium, magnesium and calcium. On the contrary to PI 358466 genotype, PI 170578 genotype had lower values in terms of chlorine, phosphate, potassium, magnesium and calcium content. It was also shown that sweet basil leaves are rather rich sources of K^{+}, Ca^{2+}, PO_4^{3-}, Mg^{2+} and Cl^{-} and potentially bioavailable for human consumption.

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Conflict of Interest: The authors denied the conflict of interest.

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FULL TEXT – ORAL PRESENTATION

THE EFFECTS OF DIFFERENT NITROGEN FERTILIZER APPLICATIONS ON THE ESSENTIAL OIL COMPONENTS AND CHLOROPHYLL CONTENTS OF OREGANO (*Origanum vulgare* Subsp. hirtum)

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Abstract

In this study, Origanum vulgare subsp. Hirtum plant has been studied. The experiment was planned with 4 replications according to randomized parcels. Four different doses of nitrogenous fertilizers (0, 0.5, 1 and 1.5 kg N ha^{-1}) were applied in the experiment. Plants grown in greenhouse conditions were harvested during the flowering period. At the end of the harvest, the effects of different doses of nitrogen fertilizer on the essential oil components and chlorophyll contents of O. vulgare were researched. The effects of nitrogen applications on the essential oil quantity and chlorophyll contents lead to important changes. The quantity of O. vulgare essential oil increased with nitrogen application and this increase was statistically significant ($p<0.01$). The essential oil quantity in plant were determined as 1.5 kg ha^{-1} (1.8 %), 1 kg ha^{-1} (1.69 %), 0.5 kg ha^{-1} (1.53 %) and control (1.02 %). The effect of nitrogen fertilizer applications on total chlorophyll contents and colour values of O.vulgare leaves were not found to be statistically significant but the nitrogen fertilizer applications created a difference at 5 % significance level on the chlorophyll concentrations

Keywords: Nitrogen, Origanum vulgare subsp. Hirtum, essential oil, chlorophyll

1. Introduction

Origanum vulgare subsp. Hirtum is a common species in most regions of the Mediterranean region, Europe-Siberia and the Iran-Turan region (Davis, 1965). In plant public health; it is used as a medicinal herb in the treatment of various ailments such as cough, digestive disorders, carminative, expectorant, bronchitis and asthma, and contains antioxidant, anti-inflammatory and antimicrobial properties (Lagouri and Boskou, 1996, Morshedloo et al., 2018).

O. vulgare is considered among the best essential oil plants in the world due to its rich essential oil content. The essential oil content of *O.vulgare* and its oil components are economically important. Researchers have stated that environment and growing conditons, irrigation or fertilization have influence on *O. vulgare* essential oil ration and components (Sezik et al., 1993; Russo et al. 1998; Baydar et al 2004). The essential oil amount of *O. vulgare* generally ranges from 0.12 % to 8.1%. Literature studies have shown carvacrol, thymol, cymene, sabinene, ɣ-terpinene, borneol, linalool, β-caryophyllene and β-bisabolene as the major constituents in *O. vulgare* essential oil (Azizi, Yan, & Honermeier, 2009; Verma et al., 2010; Lukas et al., 2015; Mastro et al. 2017; Morshedloo et al., 2018).

There are many studies examining the effects of nitrogen applications on the growth and yield of O. vulgare plants. In these studies, it was reported that there was an increase in essential oil yields of

plants with nitrogen applications (Karamanos and Sotiropoulou 2013; Giannoulis et al., 2020, Krol et al., 2020).

This study aims to determine the effects of four different doses of nitrogen fertilizer (0, 0.5, 1 and 1.5 kg N ha^{-1}) on the essential oil ratios, essential oil components and chlorophyll content in *O. vulgare.*

2. Material and Methods

In this study, *O. vulgare* plant which is known as Istanbul thyme was used as a plant material. Two seedlings were planted into each pot with 6 kg of a mixture consisting of 3600 g soil and 2400 g angular river sand and cultivated in a plastic greenhouse. *O. vulgare* has been cultivated for eight months in the greenhouse. The plants were harvested at the end of June, which is the flowering period.

The experiment was planned with 4 replications according to randomized parcels. Four different doses of nitrogenous fertilizers (0, 0.5, 1 and 1.5 kg N ha^{-1}) were applied in the experiment. Between November and March, 200 mL/plant water was applied once a week, and Between April and May, 200 mL/ plant water was applied at two days intervals.

a. Essential Oil Analysis

After the amount plants of moisture fell below 12% by use of toluene distillation, distillation was carried out in the Clevenger type hydro-distillation equipment for 5 hours in order to determine the essential oil amounts in plants. Then, essential oil amounts were measured as ml and their percentages ratios (v/W) were determined.

b. GC/MS Analysis

The ratios of essential oil components (%), obtained from dried plant material of O. Vulgare, were determined in Agilent 7890A 5975C GC/MS equipment.

As column, HP Innowax Capillar columns 60.0 m X 0.25 mm X 0.25 µm were used. After column temperature was incubated at 60ºC for 10 minutes, it was increased to 250ºC by 20ºC per minute and finally incubated at 250ºC for 8 minutes. Moreover, operating conditions as follows; temperature of Injection block: 250ºC, split ratio: 50:1, Injection volume: 1 µL, Carrier gas: Helium, flow rate: 1mL/min.

Identification of essential oil components are based on; data of Wiley, Nist and Flovor mass spectral library**.**

c. Chlorophyll Content and Colour Values

After determining the chlorophyll concentrations of 5 large leaf pairs starting after the top two opposing pairs of leaves at the top of each plant sample, SPAD value with SPAD-502 meter reading was done, and then the color changes in the leaves were determined (MİNOLTA CR–200, MİNOLTA Camera Co, LTD ramsey, NJ) (Banon vd 2002, Kocabaş and Kaplan 2007). For chlorophyll analysis in plants, the method specified by Witham et al. (1971) was used.

SPSS program was used for statistical analysis of all data. Statistical significance level was set at $P < 0.01$.

3. Results and discussion

The amount of O. vulgare essential oil increased with nitrogen application and this increase was statistically significant ($p<0.01$). The essential oil amount in plant were determined as 1.5 kg ha^{-1} (1.8 %), 1 kg ha^{-1} (1.69 %), 0.5 kg ha^{-1} (1.53 %) and control (1.02 %). There were many studies, showing that nitrogen fertilization increases the essential oil quantity of the plant (Sotiropoulou and Karamanos, 2010; Karamanos and Sotiropoulou, 2013; Giannoulis et al. 2020).

Table 1. Effect of the nitrogen applications in the essential oil components of *O. vulgare*

		Essential Oil Components	Different doses of nitrogen fertilizer (kg N ha^{-1})			
No	RI		Control	0.5N	1N	1.5N
1	1016	α-pinene	-	0.33	0.37	0.23
2	1019	α-thujene	-	0.63	0.69	0.40
3	1102	β-pinene	-	-	0.19	-
4	1155	myrcene	-	0.75	0.79	0.70
5	1173	α-terpinene	-	1.31	1.25	1.19
6	1192	limonene	-	0.21	0.30	0.24
7	1226	β-ocimene	-	0.53	1.06	1.04
8	1237	**ɣ-terpinene**	**0.42**	**10.45**	**11.70**	**13.90**
9	1251	3-octanone	-	0.31	0.26	0.33
10	1264	**cymene**	**1.81**	**28.67**	**28.46**	**23.20**
11	1437	1-octen-3-ol	0.42	0.55	0.59	0.64
12	1441	β-thujene	-	0.20	0.49	0.39
13	1458	trans-sabinene hydrate	*1.07*	*0.65*	*0.67*	*0.73*
14	1534	linalool	5.88	3.21	5.02	0.99
15	1543	*cis-sabinene hydrate*	*4.20*	*0.32*	*0.64*	*0.51*
16	1587	**β-caryophyllene**	**1.58**	**5.32**	**3.93**	**5.76**
17	1595	*carvacrol methyl ether*	*6.08*	*1.48*	*1.92*	*2.81*
18	1671	*α-humulene*	*3.34*	*1.33*	*1.20*	*1.58*
19	1694	*borneol*	*3.39*	*0.58*	*0.88*	*0.99*
20	1736	α-farnese	3.17	2.63	3.90	4.96
21	1995	Caryophyllene oxide	1.84	2.55	2.62	3.15
22	2223	thymol	1.59	1.09	1.20	1.63
23	***2234***	***carvacrol***	***23.62***	***29.99***	***22.94***	***24.49***

RI: Retention indice

The essential oil of *O. vulgare* shows a high content in terms of carvacrol component. Other important components in the essential oil of *O. vulgare* were cymene, ɣ-terpinene, thymol (Skoufogianni et al., 2019, Bağdat et al., 2016, Giannoulis et al. 2020). Nitrogen application were detected to be significant effect on many componenets. In the nitrogen application of *O. vulgare,* dominant components were found as carvacrol, cymene, ɣ-terpinene and β-caryophyllene. Nitrogen applications significantly increased the amount of ɣ-terpinene, cymene and β-caryophyllene components from the essential oils of the *O. vulgare*, but decreased the amount of cis-sabinene hydrate, carvacrol methyl ether, α-humulene and borneol, according to control. At the same time, the number of compounds in the essential oils of the *O. vulgare* increased with nitrogen applications. According to Table 1, carvacrol, the essential oil component of *O. vulgare* ranged from 22.94% to 29.99% in nitrogen application.

Table 2. The effects of different nitrogen fertilizer applications on the the foliar color values of *O. vulgare.*

Different doses of nitrogen fertilizer	Total chlorophyll content	Chlorophyll concentration	Colour saturation	Colour angle	Brightness
kg N ha^{-1}	mg/g	Spad value	Chroma	Hue (^{0})	L
Control	1.93	35.50	29.29	147.38	44.85
0.5	1.74	40.78	26.01	149.51	48.27
1	1.98	48.25	22.12	153.29	46.79
1.5	2.08	38.24	25.85	150.95	45.64
Significance	*N.S*	*P<0.05*	*N.S*	*N.S*	*N.S*

P: 0.05 level significant, N.S. :Not significant

The effect of nitrogen fertilizer applications on total chlorophyll contents and colour values of *O. vulgare* leaves were not found to be statistically significant but the nitrogen fertilizer applications created a difference at 5 % significance level on the chlorophyll concentrations (Table 2). In O. vulgare, the highest chlorophyll concentration with an average of 48.25 was found in 1 kg N ha^{-1} application. The chlorophyll concentrations of the nitrogen applicationed plants are higher than the chlorophyll concentration of the control plants.

In this study, the effects of nitrogen applications on the essential oil quantity, essential oil components and chlorophyll contents lead to important changes. Nitrogen application positively affected the amount of essential oil of the plants. If a production is to be made to increase the essential oil quantity of the *O. vulgare* plant, nitrogenous fertilization should be considered. To consider this situation can be guide for future studies.

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FULL TEXT – ORAL PRESENTATION

IMMUNOMODULATORY EFFECTS OF MEDICINAL PLANTS AND NATURAL PHYTOCHEMICALS IN COMBATING COVID-19

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Abstract
SARS-CoV-2 (severe acute respiratory syndrome caused by coronavirus-2), a newly discovered coronavirus, has led a worldwide pandemic named as COVID-19 by WHO. From ancient times, nature has always been attractive to scientists for the treatment of many diseases and disorders as alternative therapy. Taking into consideration that many medicinal plants and secondary metabolites with antiviral activity may shed a light on dealing with COVID-19 and effective drug development. The most effective medicinal plants known as immune system boosting are *Acanthopanax gracilistylus* W.W. Smith (Araliaceae) cortex, *Aesculus hippocastanum* L. (Hippocastanaceae), *Angelica archangelica* L. (Apiaceae), Astragalus *membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (Fabaceae) radix, *Bupleurum chinensis* DC. (Apiaceae) radix, *Cedrela sinensis* Juss. (Meliaceae), *Cimicifuga racemosa* (L.) Nutt. (Ranunculaceae), *Cinnamomum verum* J.S. Presl. (Lauraceae) cortex, *Cupressus sempervirens* subsp. *pyramidalis* (O. Targ. Tozz.) Nyman (Cupressaceae), *Forsythia suspensa* Vahl. (Oleaceae), *Geranium macrorrhizum* L. (Geraniaceae), *Glycyrrhiza uralensis* Fisch. and *Glycyrrhiza glabra* (Fabaceae), *Hyssopus officinalis* L. (Lamiaceae), *Juniperus oxycedrus* L. subsp. *oxycedrus* L., Laurus nobilis L. (Lauraceae), *Melia azedarach* L. (Meliaceae), *Melissa officinalis* L. (Lamiaceae), Mentha piperita L. (Lamiaceae), *Nepeta cataria* L. (Lamiaceae), *Nigella sativa* L. (Ranunculaceae) seeds, *Origanum vulgare* L. (Lamiaceae), *Pistacia palaestina* Boiss. (Anacardiaceae), Potentilla arguta Pursh. (Rosaceae), *Rhodiola rosea* L. (Crassulaceae) roots, *Salvia officinalis* L. (Lamiaceae), *Salvia officinalis* L. (Lamiaceae), *Sambucus nigra* L. (Adoxaceae), *Sambucus racemosa* L. (Adoxaceae), *Sanguisorba officinalis* L. (Rosaceae) radix, *Satureja thymbra* L. (Lamiaceae), *Sophora flavescens* Aiton (Fabaceae) radix, *Thuja orientalis* L. (Cupressaceae), *Thymus vulgaris* L. (Lamiaceae), *Torilis arvensis* (Huds.) Link (Apiaceae), and *Tribulus terrestris* L. (Zygophyllaceae). Most of the active compounds and molecules (e.g. quercetin, lycorine, hesperetin, emodin, glycryrrhizin, curcumin, chrysanthemum B, betulinic acid, hirsutenone, xanthoangelol E, myricitrin, licoleafol, methyl rosmarinate, calceolarioside B, glucopyranoside, amaranthine, and etc.) isolated from medicinal plants seem to be quite promising towards SARS-CoV-2 in both enhancing immune system and developing novel antiviral drugs. This presented review has emphasized that herbs and natural bioactive compounds already reported with antiviral effects against various types of viruses including coronaviruses could help to strengthen the immune system towards SARS-CoV-2 infections.

Keywords: Coronovirus, COVID-19, phytochemicals, herbs, antiviral, immune booster, phytotherapy

1. Introduction

Throughout the human history, viral infections including Cholera, Ebola, Bubonic Plague, AIDS (Acquired Immunodeficiency Syndrome), Influenza, SARS (Severe Acute Respiratory Syndrome), MERS (Middle East Respiratory Syndrome) and currently COVID-19 (causative agent SARS-CoV-2 or 2019-nCoV) have emerged and caused dramatically outbreaks almost all over the world (Bale, 2012; Gezici and Sekeroglu, 2020; Rodriguez-Morales et al., 2020).

Coronaviruses (CoVs) belong to family of Coronaviridae, Arteriviridae, Roniviridae and Mesoniviridae families. Family of Coronaviridae is the largest one and compromise subfamily of Coronavirinae, which is classified into four main genera including alpha (α)-coronavirus, beta (β)-coronavirus, gamma (γ)-coronavirus and delta (δ)-coronavirus (Walsh et al., 2013; Kanvar et al., 2017). According to the International Committee for Taxonomy of Viruses, α-coronaviruses are including FECV (Feline Enteric Coronavirus) and FIPV (Feline Infectious Peritonitis Virus), the porcine TGEV (Transmissible Gastro-Enteritis Virus), Porcine PEDV (Epidemic Diarrhea Virus), PRCoV (Porcine Respiratory Coronavirus), CCoV (Canina Coronavirus) and human coronaviruses (HCoV-229E and HCoVNL63). Murine coronavirus (MHV) and Bovine Coronavirus (BCoV) and human coronaviruses (HCoV-OC43, HCoV-HKU1, SARS-CoV, MERS-CoV and SARS-CoV-2) are of β-coronaviruses (Chan et al., 2015; Zhou et al., 2020; Sekeroglu and Gezici, 2020).

Figure 1. Pictorial Abstract of the Presented Review (Gezici and Sekeroglu, 2020)

Among these coronaviruses, SARS-CoV was determined as a global outbreak in 2003 and affected almost 8500 people in 32 countries, with the mortality of rate of 10-15%. SARS-CoV initially infected the bats and caused the disease by transmitting from bats to the palm musk cat and from there to humans (Pene et al., 2003; WHO, 2004; Pillaiyar et al., 2020). Then, approximately after ten years, another highly pathogenic coronavirus MERS-CoV emerged in Saudi Arabia and spread to other Middle Eastern countries in 2013 with the mortality rate of 39% (Park et al., 2019; WHO, 2019; Chen et al., 2020). Following this outbreak, the novel coronavirus namely 2019-nCoV or SARS-COV-2 has been led COVID-19 outbreak, which was emerged in December 2019 in Wuhan province of China. COVID-19 outbreak has spread to more than 180 countries and territories US, Spain, Italy, France, Germany, United Kingdom, China and Iran continue to be the countries hardest-hit. The World Health Organization (WHO) has declared the new coronavirus outbreak called COVID-19 a pandemic, because of its a very high mortality rate around the worldwide in a limited period (Paraskevis et al., 2020; Xu et al., 2020). As of 26 October 2020, more than 43,150,456 confirmed cases of COVID-19 have been reported in many countries, resulting in approximately 1,155,284 deaths all around the world. Furthermore, the number of infected patients with COVID-19 and death from SARS-COV-2 are alarmingly increasing day by day (WHO, 2020; GISAID, 2020).

In the current position of therapy strategies for COVID-19 patients, scientists have been racing to understand novel coronavirus disease to reveal possible therapy strategies including drug discovery, therapeutic antibodies, cytokines, nucleic acid-based therapy, vaccines with the potential for treating and preventing coronavirus infections. Among the given strategies, developing drugs against many viral diseases undertaken that medicinal plants used in traditional folk medicine have significant importance for the ongoing development of therapeutic agents and broad-spectrum drugs (Cheng et al., 2020; Sekeroglu and Gezici, 2020; Luo et al., 2020; Ni et al., 2020). Thus, possible immunmodulating medicinal plants and natural products have been summarized to search for efficient complementary and alternative medicines from different herbal formulations against emerging COVID-19 outbreak in the presented review. Boosting immune system is urgently necessary to reduce overwhelming impacts on worldwide healthcare systems. The review is summarized in the pictrorial abstract (Figure 1).

2. SARS-CoV-2 and Genomic Structure

Coronaviruses are single stranded positive sense RNA viruses encapsulated within a membrane envelopeand their genome comprises approximately 26-32 kilobases nucleotides (Gezici and Sekeroglu, 2020). In all CoVs encode five structural proteins and their functions are given below, and the structure is shown in the Figure 2.

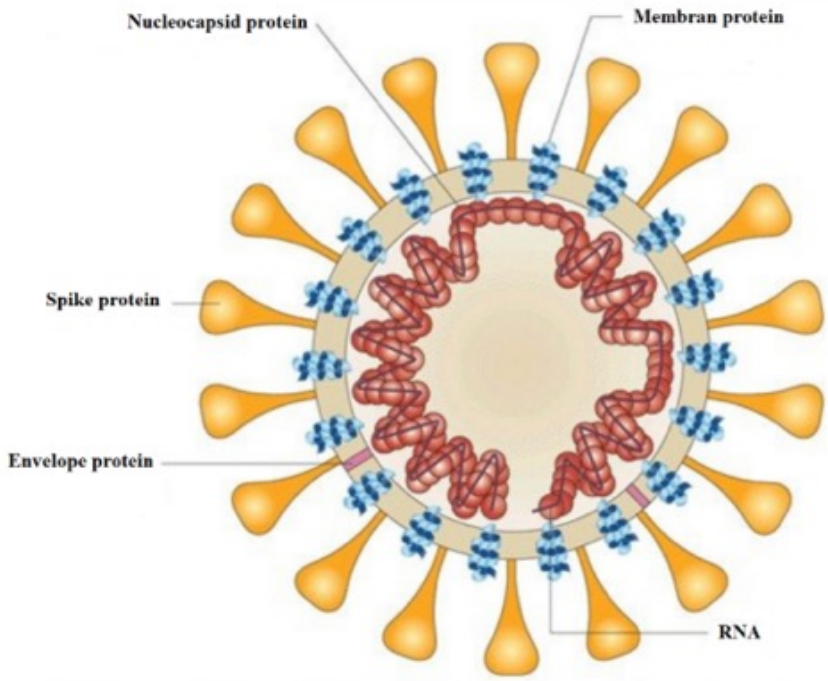

Figure 2. Structure of Coronaviruses (modified from https://www.id-hub.com/, Gezici and Sekeroglu, 2020).

(i) S proteins, located outside of the virus, are a glycoprotein that gives the typical shape for the virion. These proteins act as cell surface receptor proteins for introduce the host cell and interact with membrane proteins (Wu, 2020).

(ii) Additionally, hemagglutinin esterase (HE) proteins can be found in some beta-coronaviruses in order to strengthen invading mechanism. They help in the attachment and destruction of certain sialic acid receptors that exist on the host cell surface (Huang et al., 2015; Wu, 2020).

(iii) M proteins are glycosylated glycoproteins that are essential for regeneration in the virus cell. They are also necessary to fuse into the host celland bind to N proteins (Pillaiyar et al., 2020).

(iv) E proteins are hydrophobic small proteins, covering completely the membrane. They allow attachment to the membrane of viruses and play a key role in the combining of the viral particules in the host cell (Pillaiyar et al., 2020; Wu, 2020).

(v) N proteins are phosphoproteins that are able to bind viral genomic RNA. These proteins are important for structure, replication andtranscription mechanisms of coronaviruses through interactions with the RNA viral genome. It is also determined that these proteins give information about the pathogenesis in the host cell and virulence of CoVs (Wu et al., 2009; Bertram et al., 2013; Wu, 2020).

3. Immune-boosting Medicinal Plants and Phytochemicals against Viral Infections

No efficient antiviral therapy is currently available for emerging COVID-19, but recommended treatment strategies are available and applied to COVID-19 patienst. Generally, two potential treatment strategies have been employed for coronaviruses-related diseases: (1) Broad spectrum antiviral drugs and (2) anti-CoV drug discovery involves the de novo development. The first strategy possesses possible benefits, if pharmacokinetic and pharmacodynamic properties of the drugs are known in details. As for the second one, it is aimed to develop specific agents based on the genomic and biologic understanding of the individual CoVs (Arshad et al., 2020; Pillaiyar et al., 2020; Li et al., 2020a). To identify potential antiviral agents against novel COVID-19, current researches have been primarily tried previous drugs used for the previous CoV infections e.g. SARS- and MERS-CoV. Drug trials in the management of COVID-19 patients are still ongoing, and increasing information about novel CoVs will provide opportunities for design specific and efficient therapeutics in the nearest days (Stebbing et al., 2020; Li et al., 2020b; Ni et al., 2020).

Throughout the human history, plant-based therapy could offer an alternative treatment for virus-related infections. Therefore, it is necessary to examine natural antiviral phytochemicals used for successfully to suppress viral development and overcome viral infections (Ben-Shabat et al., 2020; Ul Qamar et al., 2020). Various medicinal plants, having diverse pharmacological antiviral properties, have been used traditionally in medicine for thousand of years. In recent years, medicinal plants, valuable sources for drug discovery and pharmaceutical industry, have been preferred for people primary healthcare. A number of plant-based phytochemicals such as alkaloids, steroids, lignans, diterpenoid lactones, aliphatics, glycosides etc. have efficient antiviral effects and are able to prevent several viral diseases. Hence, many researchers have been focused on to search for drugs obtained from medicinal plants using different herbal formulations. Reseraches from almost all around the world reveal that people have been recently tended to use medicinal plants as complementary and alternative medicines in order to enhance immune system for fighting COVID-19 (Cui et al., 2020; Latha et al., 2020, Paraskevis et al., 2020).

Accordingly, hundreds of medicinal plants and secondary metabolites have been identified and analyzed in both preclinical and clinical trials for their immune-boosting antiviral activities; however, some of them have displayed significant antiviral activity in the prevention of various viral diseases. Many medicinal plants have been recommended by individually or in combination with different formulations including decoctions, leaf powder, infusions, pastes and pills in the management of various viral infections (Dhama et al., 2018; Xu et al., 2020). In this respect, medicinal plants with a wide range of bioactive compounds, which are exhibiting antiviral activities, are able to provide possible benefits as a preventive and treatment for COVID-19. *Acanthopanax gracilistylus* W.W. Smith (Araliaceae) cortex, *Aesculus hippocastanum* L. (Hippocastanaceae), *Angelica archangelica* L. (Apiaceae), Astragalus *membranaceous* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao (Fabaceae) radix, *Bupleurum chinensis* DC. (Apiaceae) radix, *Cedrela sinensis* Juss. (Meliaceae), *Cimicifuga racemosa* (L.) Nutt. (Ranunculaceae), *Cinnamomum verum* J.S. Presl. (Lauraceae) cortex, *Cupressus sempervirens* subsp. *pyramidalis* (O. Targ. Tozz.) Nyman (Cupressaceae), *Forsythia suspensa* Vahl. (Oleaceae), *Geranium macrorrhizum* L. (Geraniaceae), *Glycyrrhiza uralensis* Fisch. and *Glycyrrhiza glabra* (Fabaceae), *Hyssopus officinalis* L. (Lamiaceae), *Juniperus oxycedrus* L. subsp. *oxycedrus* L., *Laurus nobilis* L. (Lauraceae), *Melia azedarach* L. (Meliaceae), *Melissa officinalis* L. (Lamiaceae), *Mentha piperita* L. (Lamiaceae), *Nepeta cataria* L. (Lamiaceae), *Nigella sativa* L. (Ranunculaceae) seeds, *Origanum*

vulgare L. (Lamiaceae), *Pistacia palaestina* Boiss. (Anacardiaceae), *Potentilla arguta* Pursh. (Rosaceae), *Rhodiola rosea* L. (Crassulaceae) roots, *Salvia officinalis* L. (Lamiaceae), *Salvia officinalis* L. (Lamiaceae), *Sambucus nigra* L. (Adoxaceae), *Sambucus racemosa* L. (Adoxaceae), *Sanguisorba officinalis* L. (Rosaceae) radix, *Satureja thymbra* L. (Lamiaceae), *Sophora flavescens* Aiton (Fabaceae) radix, *Thuja orientalis* L. (Cupressaceae), *Thymus vulgaris* L. (Lamiaceae), *Torilis arvensis* (Huds.) Link (Apiaceae), and *Tribulus terrestris* L. (Zygophyllaceae) are important medicinal plants having antiviral activities. Antiviral herbal medicines have been used in many historic viral diseases, the findings related to antiviral medicinal plants are collected in Table 1.

Table 1. Immune-boosting Medicinal Plants and Phytochemicals for Complementary and Alternative Therapy

Medicinal Plant	Antiviral Activity	References
Aloe vera L.	Herpes simplex virus type 2 (HSV-2)	(Moradi et al., 2016; Ben-Shabat et al., 2020)
	Hepatitis B virus	(Parvez et al., 2019).
Allium sativum L.	Novel coronavirus (SARS-CoV-2)	(Thuy et al., 2020)
Alpinia galanga L.	Novel coronavirus (SARS-CoV-2)	(Utomo et al., 2020)
Baccharis gaudichaudiana, B. spicata and *B. anomala*	Bovine herpes virus 1 and herpes simplex virus (HSV-1)	(Venturi et al., 2018)
Chamomilla recutita L. *(syn. Matricaria recutita* L.*)*	Herpes simplex virus (HSV), poliovirus	(Touraine et al., 1998; Bergner, 2005).
Eucalyptus globulus Labill.	Herpes simplex virus (HSV-1) and Influenzavirus A (H1N1)	(Brochot et al., 2017)
Cinnamomum zeylanicum Blume	Herpes simplex virus (HSV-1) and Influenza virus A (H1N1)	(Brochot et al., 2017)
Citrus sp.	Influenza A virus, hepatitis (B and C) virus, human respiratory syncytial virus (RSV), Vesicular stomatitis virus (VSV), Arenavirus Lassa virus (LASV) and novel coronavirus (SARS-CoV-2)	(Dong et al., 2014; Tang et al., 2018; Cheng et al., 2020; Hu et al., 2020; Utomo et al., 2020)
Melissa officinalis L.	Avian infectious bronchitis virus (IBV)	(Lelešius et al., 2018)
	Herpes simplex virus type 1 (HSV-1), type 2 (HSV-2) and an acyclovir-resistant strain of HSV-1 (ACVres)	(Nolkemper et al., 2006)
	Herpes simplex virus (HSV-1)	(Dimitrova et al., 1993; Geuenich et al., 2008)
	Herpes simplex virus (HSV)	(Wölbling et al., 1994; Koytchev et al., 1999)

	Avian influenza virus (AIV) subtype H9N2	(Pourghanbari et al., 2016)
	Herpes simplex virus (HSV-1 and HSV-2)	(Allahverdiyev et al., 2004; Schnitzler et al., 2008)
	Zika virus (ZIKV)	(Byler et al., 2016)
	Herpes simplex virus (HSV), vaccinia virus, Semliki Forest and Newcastle virus	(Todorov et al., 2014)
	Enterovirus 71 (EV71)	(Chen et al., 2017)
Sambucus nigra L.	Human immunodeficiency virus (HIV), feline immunodeficiency virus (FIV), influenza virus, parainfluenza virus, human rhinovirus B, coxsackievirus, adenovirus Cand respiratory syncytial virus.	(Bergner, 2005; Manganelli et al., 2005; Glatthaar et al., 2009; Porter and Bode, 2017; Akram et al., 2018)
Rosmarinus officinalis L.	Herpes simplex virus (HSV-1) and hepatitis B virus (HBV)	(Khwaza et al., 2018)
	Herpes simplex virus (HSV-1) and an acyclovir-resistant strain of HSV-1 (ACVres)	(Nolkemper et al., 2006)
	Herpes simplex virus type 1 (HSV-1), type 2 (HSV-2) Newcastle disease virus (NDV), herpes simplex, vaccinia, Semliki Forestand West Nile viruses	(Herrmann and Kucera, 1967)
	Measles, Mumps, Vesicular Stomatitis Virus (VSV) and Herpes simplex virus type-2 (HSV-2).	(El-awady et al., 2014)
	Herpes simplex virus type 1 (HSV-1), type 2 (HSV-2)	(Nasr-Eldin et al., 2017)
	Hepatitis A virus (HAV)	(Battistini et al., 2019)
	Zika virus (ZIKV)	(Todorov et al., 2014)
	Herpes simplex virus type 1 (HSV-1)	(Gavanji et al., 2015)
	Avian infectious bronchitis (IBV),	(Giraldo et al., 2017)
	Influenza A H1N1 and oral herpes simplex HSV-1	(Brochot et al., 2017)
Glycyrrhiza glabra L.	Herpes simplex, Epstein-Barr, human cytomegalovirus, hepatitis A, B and C, influenza, HIV, varicella zoster virus (VZV) and SARS coronaviruses	(Cinatl et al., 2003)
	Zika virus (ZIKV)	(Byler et al., 2016)

	Hepatitis (B and C), Human immmunodeficiency virus (HIV-1), herpes simplex virus (HSV) and SARS coronavirus	(crance et al., 2003; Fiore et al., 2008; Baltina et al., 2009; Ashfaq et al., 2011; Batiha et al., 2020)
	Herpes simplex virus (HSV-1) and influenza A virus	(Ghannad et al., 2014; Yasmin et al., 2020)
	Swine epidemic diarrhea virus	(Huan et al., 2017)
	Hepatitis A, B and C	(Mamedov et al., 2019)
	Influenza A virus (FLUAV), Rift Valley fever virus (RVFV), Human metapneumotic virus (HMPV), echovirus 1 (EV1), chikungunya virus (CHIKV), Ross River virus (RRV), Zika virus (ZIKV), hepatitis C virus (HCV), Sindbis virus (SINV), HIV-1, cytomegalovirus (CMV), hepatitis B virus (HBV) and herpes simplex virus type 1 (HSV-1),	(Ianevski et al., 2019)
	influenza virus, SARS coronavirus and Human immmunodeficiency virus (HIV)	(Cinatl et al., 2003; Mamedov et al., 2019)
	Kaposi's sarcoma herpes virus (KSHV) and hepatitis B virus	(Pastorino et al., 2018; Sun et al., 2019)
Taraxacum officinale L.	Hepatitis C virus (HCV), Influenza virus type A (H1N1)	(Rehman et al., 2016)
Vitis spp.	Simian (SA-11) and human (HCR3) rotaviruses	(Gonçalves et al., 2005)
Tamarix nilotica	Herpes simplex-1 virus (HSV) and poliomyelitis-1 virus (POLIO)	(Soltan and Zaki, 2009)
Olea europea L.	Herpes simplex, polio viruses, rhinoviruses, mycoviruses, coxsackie virus, Varicella zoster, encephalo myocarditis	(AMR, 2009)

	Herpes simplex virus (HSV-1), infectious laryngotracheitis viruses, Newcastle disease virus (NDV) and rhesus rotavirus	(Lee-Huang et al., 2003; Motamedifar et al., 2007; Zaher, 2007; Knipping et al., 2012; Salih et al, 2017)
	Rotavirus, rhinovirus, parvovirus, hepatitis, Epstein-Barr, herpes simplex, influenza, varicella zoster, cat leukemia viruses and viral hemorrhagic septicemia virus (VHSV)	(Fredrickson, 2000; Micol et al., 2005)
Salvia officinalis L.	Avian infectious bronchitis virus (IBV), Herpes simplex virus (HSV-1 and HSV-2), Measles, Mumps and Vesicular Stomatitis Virus (VSV)	(Geuenich et al., 2008; El-Awady et al., 2014, Lelešius et al., 2019)
	SARS-CoV and HSV-1	(Kalus et al., 2009)
	Zika virus (ZIKV)	(Byler et al., 2016)
Mentha piperita L.	Avian infectious bronchitis virus (IBV), respiratory syncytial virus (RSV), Herpes simplex virus type 1 (HSV-1), type 2 (HSV-2) and an acyclovir-resistant strain of HSV-1 (ACVres)	(Nolkemper et al., 2006; Geuenich et al., 2008; Li et al., 2017; Lelešius et al., 2019)
	Herpes-simplex virus (HSV-1 and HSV-2)	(Yamasaki et al., 1998)
Origanum vulgare L.	Avian infectious bronchitis virus (IBV), equine arteritis virus (EAV), feline calicivirus (FCV), canine distemper virus (CDV), canine adenovirus (CAV) and canine cororavirus (CCoV)	Blank et al., 2019; Lelešius et al., 2019)
	Respiratory syncytial virus (RSV), Coxsackievirus B3 (CVB3), herpes simplex virus type 1 (HSV-1) and nonenveloped murine norovirus (NMN)	(Gilling et al., 2014; Zhang et al., 2014)
Thymus vulgaris L.	Avian infectious bronchitis virus (IBV), Measles, Mumps, Vesicular Stomatitis Virus (VSV)and Herpes-simplex virus (HSV-1 and HSV-2)	(Yamasaki et al., 1998; El-Awady et al., 2015; Lelešius et al., 2019)
	Herpes-simplex virus (HSV-1)	(Schnitzler et al., 2007)
Thymus capitatus (L.) Hoffmans. and Link	Herpes simplex virus (HSV-1 and HSV-2), Echovirus 11 (ECV11) and Adenovirus (ADV)	(Salah-Fatnassi et al., 2010; Duran et al., 2012)
Iresine herbstii Hook.	Avian Newcastle disease virus	(Andleeb et al., 2020)
Ocimum bacilium L.	Herpes-simplex virus (HSV-1)	Yamasaki et al., 1998)
	Bovine viral diarrhoea virus (BVDV)	(Kubiça et al., 2014)

	Herpes viruses (HSV), adenoviruses (ADV), hepatitis B virus), coxsackievirus B1 (CVB1) and enterovirus 71 (EV71)	(Chiang et al., 2005)
Curcuma longa L.	Dengue virus (serotype 2), herpes simplex virus, human immunodeficiency virus type 1 (HIV-1), Zika and Chikungunya viruses	(Bergner, 2005; Mazumder et al., 1995; Mounce et al., 2017)
Rhus coriaria L.	Acyclovir resistant HSV-1 and hepatitis B virus (HBV)	(Parsania et al., 2017; Gharabolagha et al., 2018)
Solanum paniculatum L.	Herpes-simplex virus (HSV-1), murine encephalomyocarditis virus, Equine herpesvirus-1 (EHV-1)	(Valadares et al., 2009; Kaziyama et al., 2012)
Tinospora cordifolia	Hepatitis A virus, human influenza virus (HIV)	(Akhtar, 2010; Latha and Pandit, 2020)

4. Conclusions

Even though, many genetic and molecular mechanisms of the human CoVs have been explored up to date after SARS and MERS epidemic, there are some challenges about the new highly pathogenic SARS-CoV-2 and COVID-19 outbreak, since SARS-CoV-2 are able to modify the genomic structure quickly. Herewith, the identified agents, drugs or vaccines against the novel CoV are not properly evaluated for *in vitro* and *in vivo* studies. Because of this situation, complementary therapy with medicinal plants is gaining popularity around the worldwide, because of various advantages such as less expensive, more accessible, less toxicity and resistance, better patient toleranceand fewer or no side effects. Take into consideration powerful immune boosting capacities of medicinal plants and plant-derived phytochemicals, they may play as a source of antiviral drugs for pharmaceutical industry in complementary and alternative medicine.

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Conflict of Interest
Authors solely declare no conflict of interest regarding authorship and/or publication of this review.

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FULL TEXT – ORAL PRESENTATION

THE EFFECT OF SALT AND DROUGHT STRESS ON GERMINATION AND EARLY SEEDLING GROWTH OF *Lavandula stoechas* LINN. SEEDS

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Nowadays, with the increasing interest of use of organic and of herbal, aromatic and medicinal plants (HMAPs) products globally, it is important to incorporate wildering plant species, grown naturally in nature into agriculture production. Collecting medicinal and aromatic plants from nature and serving them on market is an important source of income, especially for rural people. To think that medicinal and aromatic plants will be maintained to be a source of livelihood for rural areas, as these plants are recommended to growers in the evaluation of arid, salty and sloping lands would not be wrong. In this study, seeds were collected from *Lavandula stoechas* plants, naturally growing in Kemer/Antalya Region and their potential tolerance against to salinity and drought was investigated. In order to determine the salinity impact on germination and early seedling growth characteristics, seeds were exposed to at six level NaCl concentrations treatments (0, 50, 100, 150, 200 and 250 mM). To create drought stress, PEG solution was used and seeds were germinated under at six different drought levels (0 -2, -4, -6, -8 and -9.8 MPa). The effects of stress factors were tried to be determined by being used the characters of germination ratio, radicle length and weight, plumule length, and weight. As a result of the studies, *L. stoechas* seeds germinated at 0, 50 and 100 mM NaCl concentrations and drought levels of 0, 2, 4 and 6 MPa. The effects of different salt concentrations on germination ratio, plumule and radicle length and the effects of different drought levels on germination ratio, plumule and radicle length, plumule and radicle weight were found to be significant was to be revealed.

Keywords: *Lavandula stoechas*, salt stress, drought stress, germination, seedling development

1. Introduction

The Lavandula genus, which is native to the Mediterranean Basin, from southern Europe to North and East Africa and the Middle East to southwest Asia and Southeast India includes more than 45 species and about 400 varieties, (Benabdelkader *et al,* 2011; Koulivand *et al,*2013; Mokhtarzadeh *et al,* 2013; Lis-Balchin and Lavender 2012).

In Turkey, the genus *Lavandula* L. (Lamiaceae) is represented by 3 taxa, *Lavandula stoechas* L. subsp. *stoechas*, *Lavandula pedunculata* subsp. *cariensis* (Boiss.) Upson & S. Andrewsand *L. angustifolia* Mill. subsp. *angustifolia* (Mill, 1982; Dirmenci, 2012). *L. angustifolia*, *L. pedunculata* subsp. *cariensis*, *L. stoechas* subsp. *stoechas* are locally known as "lavanta", "karan", "karabaş" in Turkish respectively (Mill, 1982; Baytop, 1984; Dirmenci, 2012).

Various species belonging to *Lavandula* genus have been widely used in folk medicine around the world. It is traditionally believed that the oils are antibacterial, antifungal, carmitative, sedative, antidepressive and effective for burns and insect bites, (Dob *et al*, 2006). In folk medicine, *L. stoechas* L. has been used as an analgesic, antiseptic, sedative, expectorant, cardiotonic and to heal eczema in various forms and preparation, (Baytop, 1984; Kırmızıbekmez vd 2009; Küçük *et al*, 2019).

Playing a major role in determining the distribution of plant species among different types of environments, important abiotic stress factors such as drought, salinity, high or low temperature to which plants are exposed, limit plant growth and development in agricultural production and cause yield losses (Janmohammadi *et al,* 2008).

Both in natural and agricultural settings, abiotic stress and its effects on plants is a topic, increasingly receiving attention, because of the potential impacts of climate change on rainfall patterns and temperature extremes, salinization of agricultural lands by excessive irrigation, and the need to main tain or increase agricultural productivity on marginal lands, (Tester and Bacic, 2005). Therefore, the development of salt and droght tolerant crops and the use of plants seem necessary, (Soroush and Gravandi 2013).

According to some researchers, about 10 % of the total area on the earth's surface consists of soils with salinity problems, and approximately half of the irrigation areas are affected by salinity (Carrow and Duncan, 1998; Marcum, 2006). In Turkey, the salinity and alkalinity problems are being experienced in approximately 1.5 million hectares. This rate corresponds to approximately 30 % of the land suitable for irrigation, (Ekmekçi *et al,* 2005).

In arid, semi-arid and coastal regions, salt stress is a major environmental problem, and its first negative effect shows at the germination stage of the plants, (Passam and Kakouriotis, 1994). Salt damage during germination causes a decrease in water intake, protein organization to be structurally affected and changes in the transport of nutrients, (Foolad and Lin, 1997).

A significant degree of climate change is occurring in the world and experts on the subject draw attention to the drought risk for large areas, included in our country too, (Rosenzweig *et al,* 2001). Drought is known as the negative impact on the field and water resources due to the amount of rainfall that is lower than the long years average (Örs and Ekinci, 2015). In this dry period, the occurrence of drought occures depending on the water holding capacity of the soil and the rate and amount of evapo-transpiration, performed by the plants, (Kalefetoğlu and Ekmekçi, 2005).

In agricultural terms, drought is not a concept the total amount of rainfall in a year, but related to the amount of water with its roots during the development period, a plant can take. In plants, suffer from water shortage in the development period, significant losses occure in terms of yield (Katerji *et al,* 1998). Worldwide, drought is effective in approximately 26 % of the areas, used for agricultural purposes. From this point of view, drought can be said yo be one of the biggest environmental stress factors (Blum, 1986; Kalefetoğlu and Ekmekçi, 2005). Even if it varies according to plant species, the most susceptible periods to drought are germination, early seedling development and flowering terms (Ahmadi *et al,* 2009).

In this study, under salinity and drought conditions, which are among the abiotic stress factors the seeds that belong to *L. stoechas* species, which spread in Antalya's flora was aimed to determine the germination and seedling growth performances. It is thought that the obtained data will guide both to similar studies and related studies to the cultural production of *L. stoechas* species.

2. Material and Methods

In the study, seeds, collected from *L. stoechas* plants in 2018 that spread naturally in Antalya Province Kemer Region were used. The trial was set up to randomized parcels trial pattern. 9 cm petri dishes were used for germination. In the study, 6 different salt doses were applied to the seeds as 0, 50, 100, 150, 200 and 250 mM NaCl. To determine the tolerance to drought stress, 6 different drought stresses were created with solutions that have water holding power 0, -2, -4, -6, -8 and -9.8 bar by being

used PEG 6000. Osmotic potentials for drought levels were adjusted to suggestion by Michel and Kaufmann (1973).

For germination, 20 seeds with similar sized were placed on double-layered germination paper in petri dishes and then 10 ml of the solutions prepared were added on the seeds. In order to prevent evaporation, round of the petri dishes was wrapped with parafilm and placed in a germination cabinet with 14 hours of light, 10 hours of darkness, 70 % humidity and 20 °C temperature conditions and observed for 15 days, (Gheidary *et al,*2017).

In the experiment, observations were made at the same time every day and seeds, radicle length exceed 2 mm were accepted as germinated. Germination percentage (%) was determined by counting the total seeds germinated at the end of the fifteenth day, (Scott *et al,* 1984). On the 15th day of germination, plumule length, radicle length, plumule wet weight and radicle wet weight were measured in each petri dish, (Çarpıcı and Erdel, 2015).

ANOVA and Duncan's multiple range test for comparisions were determined by SAS 9.3 (SAS Institute 2011).

3. Results and Discussion

The data, obtained from the study that the effects of salinity and drought on germination and seedling development of *L. stoechas* seeds was investigating, are summarized in the graphs in Figure 1 and Figure 2.

The drought stress, applied to the seeds had a significant effect on the germination rate and all other properties and reduced dramatically the germination rates of the seeds (Table 1). In the applications of -8 and -9.8 MPa among the aplied different drought levels didn't occure germination, the lowest germination was obtained with 27.50 % at -6 MPa application and the highest germination rate was obtained with 86.25% at -2 MPa application. In the control application, germination rate was determined as 81.25%.

In their study, which investigated the effects of drought (0, -2, -4 and -6 bar) and salinity (0, 0.1 and 0.5 mM salicylic acid) stresses on seed germination of lavender (*Lavandula stricta*), Sanginabadi and Khorasaninejad (2016) reported that plumula and radicle length, germination percentage and seed viability decreased significantly as drought and salinity stress increased. They stated that the stress levels of -2 and -6 bar and the applied pretreatments significantly increased all properties and that the plumule length was the most susceptible to drought and salinity stress among the studied traits. They reported that the best moisture range for germination of lavender seeds is between the range of -2 bar and stress-free conditions.

Gheidary *et al,* (2017) stated that in plant growth and development, seed germination and early seedling development were the periods, the most susceptible to environmental stressors for many plants.

The plumule length values, obtained from drought aplications, ranged from 17.85 mm (-2 MPa) to 9.39 mm (-6 MPa) and showed decreasing in significantly level in parallel with the severity of drought stress. The plumule wet weight values were significantly negtively affected by drought stress and ranged from 5.96 mg (0 MPa) to 2.21 mg (-6 MPa). In the radicle length was measured 16.52 mm in control application while Increases were determined with drought stress (31.14, 31.75 and 20.39 mm). Drought stress encouraged the radicle length up to the drought level of -4 MPa. A similar situation has been experienced in radicle wet weight values ranging from 0.43 mg to 0.76 mg, radicle wet weight values, showed the high values in -2 MPa and -4MPa applications and low values in 0 MPa and -6 MPa applications.

Wang *et al* (2016), investigated the effect of different PEG-6000 concentrations (0.5 %, 10 %, 20 % and 30 %) on seed germination and seedling growth of *Lavandula stoechas* and reported that the germination rate, germination potential and germination index generally decreased as the concentration increased and that the effects of the applications on these properties showed a statistically significant difference. They reported that root, stem and leaf area values showed similar results with seed

germination however root length of *Lavandula stoechas* increased significantly under 10% PEG-6000 stress.

Li *et al,* (2019) observed the changes in germination rate, germination potential, germination index and in catalase/amylase activities during germination after treatment with water-retaining agents to determine the effects of three water-retaining agents on germination and drought resistance of lavandula seeds. As a result, they stated that the use of a suitable water-retention agent can increase the germination rate of lavandula seeds that the best germination result was obtained by using 0.25 % water-retention agent B. For water-retaining agents (A, B, C), the best application results were reported 0.5%, 0.25%, 0.1% respectively.

Chen *et al,* investigated the changes in physiological and biochemical indexes. In order to understand the drought resistance mechanism of *Lavandula angustifolia* and *Lavandula stoechas* species, by controlling soil volumetric moisture content in periods of 0, 10, 20, 30 and 40 days with negative pressure water feeding and control devices, under mild, moderate and severe drought stress. They noted that, with increasing drought stress intensities and periods of stress, the two species showed a tendency to decrease in relative water content, under severe drought stress the amplitude of the decrease increased significantly after 40 days, while their relative water contents decreased to 30.08 % and 25.27 %, that the relative water content of *L. angustifolia* was the lowest, that the *L. stoechas* showed a stronger ability against drought stress than *L. angustifolia*.

Saxena *et al,* (1993) reported that in terms of agricultural production drought has two main effects that these were expressed as a decrease in development and yield due to the inability to achieve the desired plant output and the presence of less water than desired in the soil. According to the researchers, the irregularity, seen in the emergence may cause failure to obtain the desired plant density, irregularities in the flowering and maturation of the plants, decrease in yield and ripening irregularities at harvest.

Under the salinity stress conditions, in the experiment, conducted to determine the germination characteristics of *L. stoechas* seeds were observed that salinity stress affected significantly on germination characteristics, (Table 1). Germination did not occur at the different salt levels applications (150, 200 and 250 mM NaCl), as the salt concentration increased was observed to be a decrease in the germination rate. When Table 3 is examined, it is seen that germination rates ranged from 93.75 % (control) to 26.25 % (100 mM), and salinity stress reduced dramatically the germination rate. Similarly, plumule length values were observed to decrease from 18.11 mm (control) to 4.31 (100 mM) mm with increasing of salt concentration. The plumule wet weight values were measured as 5.39, 5.76 and 4.09 mg, at the levels of salt stress 0 mM, 50 mM and 100 mM respectively. 50 mM salt stress was seen to increase the plumule wet weight values of seeds but the difference among applications was seen not to be statistically significant. Radicle length measurements were measured as 16.59 mm, 17.80 mm and 8.48 mm, at the levels of salt stress 0, 50 and 100 mM respectively. As well as the mean value of 50 mM application was found to be higher than control and other application, control and 50 mM application took place statistically in the same group. Radicle wet weight values were found to range from 1.79 to 0.40 mg. Differences among radicle wet weight values, which decreased as the salt concentration increased, were not found to be statistically significant.

Haileselasie and Gselasie (2012) stated that radicle and plumule lengths are important parameters in terms of salt stress because the radicles contribute to the development of the stem by taking water into the plant by directly contacting the soil and water. For this reason, that radicle and plumule lengths provide the first information about the salt exposure levels of plants was reported.

Ekmekçi *et al,* (2005) stated that due to increasing salt doses, together with the toxicity of Na^+ and Cl^- ions, the decrease in the germination rate arises from the prevention of the entry of water required for germination into the seed due to the rising osmotic pressure.

On the other hand, Uyanık *et al,* (2014) and Foolad and Lin (1997) reported that some metabolic disorders and inhibition of germination regulating protein synthesis in plants, which are exposed to salt stress cause a decrease in the germination rate.

Table1. ANOVA on mean of squares of measured traits in *L. stoechas* under levels of drought and salinity

Treatments	Germination rate (%)	Plumule length (mm)	Plumule wet weight (mg)	Radicle length (mm)	Radicle wet weight (mg)
Drought levels	1441.67**	80.0786**	0.0000063**	235.18681**	0.000006015*
Salinity levels	1894.58**	86.0982**	0.0000019^{ns}	43.5502*	0.0000022^{ns}

ns, *, ** representnonsignificant, significant at 5% and 1% probabilitylevels, respectively.

Table 2. Mean comparison of main effects of drought stress levels.

Drought stres level (bar)	Germination rate (%)	Plumule length (mm)	Plumule wet weight (mg)	Radicle length (mm)	Radicle wet weight (mg)
0	81.25 a*	17.66 a	5.96 a	16.52 b	0.51 b
-2	86.25 a	17.85 a	4.74 b	31.14 a	0.76 a
-4	70.00 a	10.75 b	2.67 c	31.75 a	0.73 a
-6	27.50 b	9.39 b	2.21 c	20.39 b	0.43 b

*Means with different letter(s) in each trait is significantly different at 1% probability level according to Duncan's multiple range test

Table 3. Mean comparison of main effects of salinity stress levels.

Salinity (NaCl) level (mM)	Germination rate (%)	Plumule length (mm)	Plumule wet weight (mg)	Radicle length (mm)	Radicle wet weight (mg)
0	93,75 a*	18,11 a	5,39	16,59a	1,79
50	53,75 b	8,53b	5,76	17,80 a	0,67
100	26,25 c	4,31c	4,09	8,48 b	0,40

* Means with different letter(s) in each trait is significantly different at 5% probability level according to Duncan's multiple range test

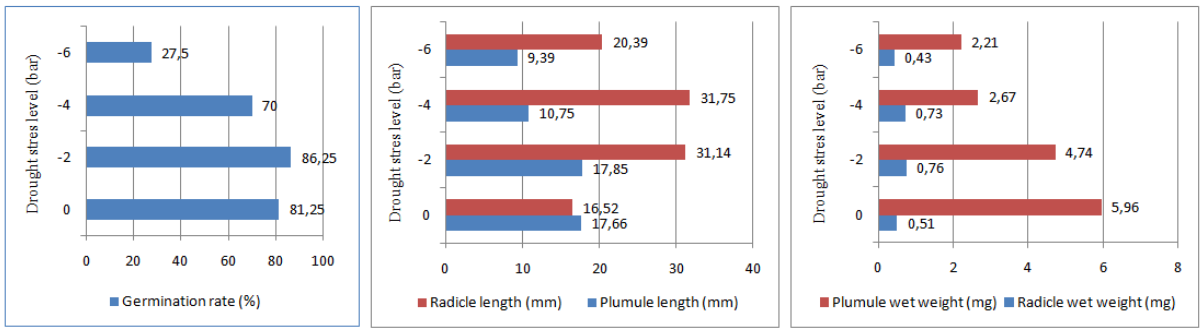

Figure 1. Germination rate, radicle-plumule length and weight values, in *Lavandula stoechas* subsp. *stoechas* seeds, subjected to drought stress

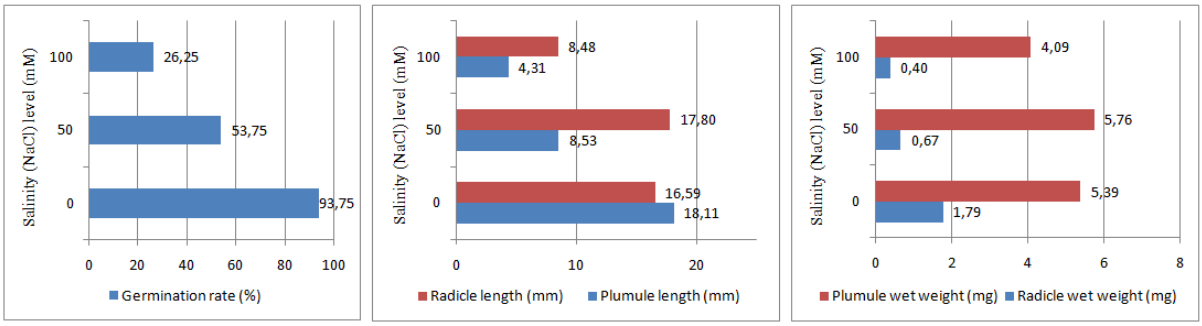

Figure 2. Germination rate, radicle-plumule length and weight values in *Lavandula stoechas* subsp. *stoechas* seeds, subjected to salinity stress

4. Conclusion

Germination and subsequently seedling development constitute the most critical steps in vegetative production. During these periods, salinity and drought stress to which seeds or seedlings are exposed have significant effects on plant development. On the other hand, as a result of climate changes, salty and arid areas are increasing day by day and to cultivate plants suitable for these areas is gaining more and more important.

This study showed that the production of *L. stoechas*, generally traded by collecting from nature can be to produce in arid areas, up to -4 bar and that drought level up to -2 bar supports germination and seedling development.

In the results of the salinity experiment, although the values of the control application is prominent, in terms of radicle length values, 50 mM NaCl application and control were in the same group. At the same time, the differences among the applications in plumule and radicle weights were not found to be statistically significant. For these reasons, in the seedling development of *L. stoechas* plant was thought to be acceptable the 50 mM NaCl level and in saline areas, that contain up to this level to be possible to grow this plant.

In near future, it is foreseen that the development of salt and drought tolerant crops and the use of plants will seem necessary therefore, considering the natural distribution areas of medicinal and aromatic plants, the availability of appropriate gene sources and sufficient variation can be perceived as an alternative and opportunity.

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FULL TEXT – ORAL PRESENTATION

INVESTIGATION OF PREPARATIONS OBTAINED FROM *Juglans regia* L. FROM THE FOLK AND TRADITIONAL MEDICINE OF REPUBLIC OF MACEDONIA

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Abstract

This study was carried out concerning ethnomedicine on preparations obtained from *Juglans regia* L. from the folk and traditional medicine of Republic of Macedonia, an area so far less frequently studied from the perspective of plant folk traditions. The district, from the ethnobotanical point of view, shows traces of the influences of the neighbouring regions. Some medicinal uses are linked to beliefs or residual forms of magic prescriptions. Amongst the more notable uses the most interesting are those of conserving of the juglandis immaturi fructus in honey and in rakiya. Walnuts in these recipes are not heat, and there is no harmful effect from heat. These useful customs are continuing to be expanded at every step by the people and valued as good old medical receipts. It should be chemically and clinically examined how vitamins are preserved in that can. In this way "sweet" (vitamin C 165.16 mg/100 g, vitamin D 7 mg/100g, sodium 142.54 mg/100g, calcium 3.01 mg/100g) and rakiya (vitamin C 6.15 mg/100 g, vitamin D 1.7 mg/100g, sodium 119.55 mg/100g, potassium 58.65 mg/100g, calcium 1.12 mg/100g) of the walnuts is our best folk can of vitamins and other medicinal ingredients.

Keywords: Honey, vitamins, rakiya, minerals, walnut

1. Introduction

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

It is interesting to mention a nice practice in Republic of Macedonia: conserving of the juglandis immaturi fructus in honey as "sweet" for service, for sick, weaker children, pale teenagers, anemic households and persons with weak stomach. One little spoon honey and one walnut are used in spring period every morning against weakness, tuberculosis and scrofula at glandural tuberculosis (Stojanova, 2009). It is used generally for immunity (Mojsoski, 2005; Stojanova, 2011). Also, juglandis immaturi fructus are stored in rakiya for 40 days on sun or in a warm place. After that the liquid is filtrated and

used according to the need of the organism, by one little spoon (Stojanova, 2010). This rakiya is used for cleanses of the stomach, liver and blood; for removing the weakness of the organism, removing bacteria from the intestine and for balances the viscous / density of the blood. To this day these medicines are prepared in some areas in Republic of Macedonia as a very efficient and irreplaceable remedy. No, literature data exist about these remedies prepared with the juglandis immaturi fructus, in our country and other countries in the world (Mez-Mangold, 1971). Because of that with our contemporarily investigations on the chemical composition of this oil extract we have tried to find an explanation of its usage for healing ear pains.

2. Material and methods

2.1. Samples

The present study comprised extract traditionally prepared by extraction of 40 juglandis immaturi fructus in 1000 g honey for 40 days. Also, 40 juglandis immaturi fructus are stored with 1 kg sugar in 1000 mL rakiya for 40 days, on sun or in a warm place. After that the liquid is filtrated.

2.2. Chemical investigation

The chemical investigation of the extract from juglandis immaturi fructus included determination of levels of 25-hydroxycholecalciferol (Vitamin D total), by the ECLIA (Electrochemiluminescence assay) on Elecsys 2010 Roche Hitachi analyzer. First, the extraction procedure was performed; by using dichloromethane (Sigma-Aldrich Co.) for extraction (600 µl of oil extract were mixed with 3 ml dichloromethane). After 10-minutes vigorous vortexing and centrifugation on 3000 rpm for 10 minutes, the supernatant was taken for 25-hydroxycholecalciferol quantitative determination, by Roche Diagnostics ECLIA tests (Dean, 1998; Roche, 2008; Michael, 2009) following the manufacturer's instruction (Roche, 2008).The lower and upper limits of measurements were 0.5 and 70 nmol/l for 25-hydroxycholecalciferol, respectively. Vitamin D (D3, D2, and metabolites) is converted to 25-hydroxy vitamin D in the liver. 25(OH) D is the major circulating form of vitamin D and its concentration in the serum or plasma is the best indicator of vitamin D nutritional status (Michael, 2009).

For the iodine assessment in the samples, a method based on the Sandell-Kolthoff reaction is used, where iodide is catalyst in the reduction of ceric ammonium sulphate to cerous form. The samples were previously diluted (1:10) to achieve the workong range of the method (0-500mcg/L) and digested with ammonium persulphate (WHO/UNICEF/ICCIDD, 2007; WHO, 2014).

Mineral composition was determined by electro thermal atomic absorption spectrometry after microwave digestion. Vitamin C content is determined by iodometric volumetric method. (Ph. Jug. IV, 1984).

2.3. Antimicrobial investigations

The in vitro activity of the extracts was examined against laboratory reference strains in the Institute of Institute of Public Health, in Skopje.

Tested microorganisms
Reference strains; *Staphylococcus aureus ATCC 6538* and *Escherichia coli ATCC 2592*

2.3.1. Agar diffusion method

Standard commercial Mueller Hinton agar plates (Oxoid) were used as medium for investigation of antimicrobial activity of extracts prepared by extraction of juglandis immaturi fructus in honey and rakiya.

2.3.2. Preparing of bacterial solutions

Microorganisms were suspended in sterile broth with turbidity corresponding to 0.5 and 1 Mc Farland. The suspensions were placed on the plates using sterile cotton swabs and after that we made holes (6 mm diameter) on each plate. The holes were filled with 100 µl of extract. The plates were incubated at 37^0C in aerobic atmosphere from 28 to 36 hours. The growth was analyzed.

3. Results and Discussion

Walnut is quite branched and solid tree, high up to 30 m, with light gray color of the bark, sometimes with longitudinal cracks. It is found both as autochthonous and cultural planting. It is wide spread plant throughout the entire territory of the Republic of Macedonia. In natural conditions, is found into the oak forests up to 1000 – 1200 m, on the habitats filled with frost and drought, regardless of the ground (Dervendzi, 1992) (Flora of Macedonia, 1993).

3.1. Local Medicinal Uses

Walnut leaves in Republic of Macedonia are used against skin illnesses: pyoderma, abscess wounds, eczema. A water infusion and decoction obtained from leaves is used as a blood cleanser, also act as digestive, aperitive and roborant (Dervendzi, 1992). Leaves are used for healing diabetes (Stojanovska et al., 2011). External the infusion and decoction are used against swollen glands, inflammation of the bone, teeth bleeding, eye catarr, inflammation of the gums, against acne, skin illnesses, rheumatism, podagra and sweating of the legs. Walnut leaves are used for bath at chronic eczema and contaminated wounds (Tucakov, 1946). On infected wounds minced fresh leaves are used. Leaves prepared as a tea are used against chronicle gastroenteritis, diarrhea, diabetes and hepatitis (Dervendzi, 1992; Mojsoski, 2005). Masculine flowers are used for preparing various preparations against bleeding, cuts, hemorroides, diarrhea and dysentery. Bark and leaves are efficacy against worms (Stojanovska et al., 2011).

3.2. Local Food Uses

Walnuts as mountainous fruits were used by Macedonians as a food long time ago (Konstantinov, 1992). From very young fruits gathered before St. John's day (on 7th July) sauce is prepared. Also, immature fruits are kept in honey or in rakiya with sugar for 40 days (Dervendzi, 1992; Mojsoski, 2005). Walnut is used as a good substitute for meat in the meal (Stojanovska et al., 2011).

3.3. Local Handicraft and Other Uses

Green fresh leaves are used as insecticide in Republic of Macedonia. Green shell is used for wood coloring (Nikolovski, 1995). In villages fresh leaves are used as insecticide (Tucakov, 1946). From the spring buds ointment is prepared against hairless (Stojanovska et al., 2011).
Old Macedonian traditional instrument gusla or kemene, in Kumanovo is manufactured from *Juglans regia* because it is firmness (Dzimrevski, 2000). Green leaves and green shell of the fruit are used for coloring of woolen fabrics (Mojsoski, 2005). Walnut oil is used in summer period for obtaining dark skin (Toplak Galle, 2005). Green leaves and green shell of the fruit are used for coloring and firming the hair (Dervendzi, 1992).

Juglans regia is a symbol of mystery, which is a walnut hidden in the shell. It is a symbol of charm, fertility, strength and tolerance. Walnut tree has a relationship with the life of man; according to this a person who falls asleep under the walnut tree will faint or get headaches. The walnut is dangerous to the person who plants it. It is believed that when walnut grows and reaches the thickness of his neck, he will die. It is also believed that it is dangerous to plant a walnut tree near the house (Vrazinovski, 2000).

In Macedonia when the illness appears people passes under the roots of a walnut located on the shore of a river or stream (Vrazinovski, 1997) Also people passes under the roots of a walnut for protection from diseases (Vrazinovski, 2000). Such medicinal walnut has the village Pogdgorci, where children pass under the roots against diseases. In the village Moshtica people used to urinate under the

walnut tree to be health. Night time people were passing and urinating under a walnut against plague in the village Dulica. The same ritual people from the village Kamenica done under the roots of walnut and during the ceremony under the walnut they prepare cookies with tar and *Artemisia* in the empty house and after that, they give each one cookie. In the village Blatec people bring cookies from wheat flour at 12 o'clock in the evening under the walnut to be healed from cough. On Blagovec (7.04) in the village Skudrinje sick people pass under the roots of walnut and give them urine from an old woman. In the village Lipec sick people pass under the walnut with baked seeds from *Papaver somniferum*, with the pronouncement "when the pope will sprout than I'll suffer from the disease" (Vrazinovski, 2002). In Shopsko-bregalnic ethnographic entity there is a custom after dinner on Badnik, each of the attendees to break the walnuts, which was previously assigned as a part to him, and according to the kind of the nut fallen to him; like that his health in the coming year will be. Also, in the same entity, swinging on swings tied up on green tree i.e. cornel, walnut etc is done on Gjurgjovden (Malinov, 2006).

Table 1. Chemical content of extracts traditionally prepared by extraction of juglandis immaturi fructus in honey and rakiya

Parameters	Extract in honey (mg/100 g)	Extract in rakiya (mg/100 g)
Vit C	165.16	6.15
Vit D	7.00	1.70
sodium	142.54	119.55
potassium	117.30	58.65
calcium	3.01	1.12
iodine	0.18	0.16

Walnuts serve for a variety of charms on Christmas, which are in the first row for health and are associated with fertility to get a good crop. In the area of Shtip after the Christmas Eve the housewife gave walnuts to the all in the house (Vrazinovski, 2000). In some villages in Pcinja glove filled with garlic, salt, walnuts, hazelnuts and chestnuts is placed under the table during the diner on Badnik, Christmas Eve. After that the items in the glove are stored as a medicine for the people and livestock throughout the whole year. In Ovce Pole only walnuts are placed under the table during the diner on Badnik, which are stored as a medicine for various diseases. In the unleavened bread which is done on a Todorica (Todorova sabota, Saturday in the first week of the Easter fasting), walnuts are added with a ritual purpose to get rid of sore throats. Walnuts are represented on the table of the badnik dinner or they are presented to the participants in the ceremonial processions on Badnik (Malinov, 2006).
With walnut twigs graves are cleaned on Duhovi in Kumanovo (Petreska, 2000) (Malinov, 2001). On Gjurgjovden children, early in the morning before the sun rises, pick up twigs from walnut, plum and apple, and put on the doors everywhere (Vrazinovski, 2002).

All the results from the extracts traditionally prepared by extraction of 40 juglandis immaturi fructus in honey and rakiya for 40 days were expressed on mg/100 g (Table 1). One little spoon honey with one walnut or small glass rakiya used in spring period every morning could improve the health conditions of the organism. With that recommended dietary intake (RDI) for iodine (150 mcg per day for adults and school age children, 220 mcg/day for pregnant women and up to 290 mcg/day for lactating women) is not exceeded.
The investigations of antimicrobial activity of extract obtained from 40 juglandis immaturi fructus in honey for 40 days in our study, have shown higher inhibition of bacterial growth only for *Escherichia coli*, and low antimicrobial action on Gram positive bacteria i.e. *Staphylococcus aureus*.

4. Conclusion

The usage of preparations obtained from *Juglans regia* L. from the folk and traditional medicine of Republic of Macedonia, from the ethnobotanical point of view, shows traces of the influences of the

previous nations who lived in these areas. Some medicinal uses are linked to beliefs or residual forms of magic prescriptions.

"sweet" and rakiya obtained by conserving of the juglandis immaturi fructus in honey and in rakiya is our best folk can of vitamins and other medicinal ingredients.

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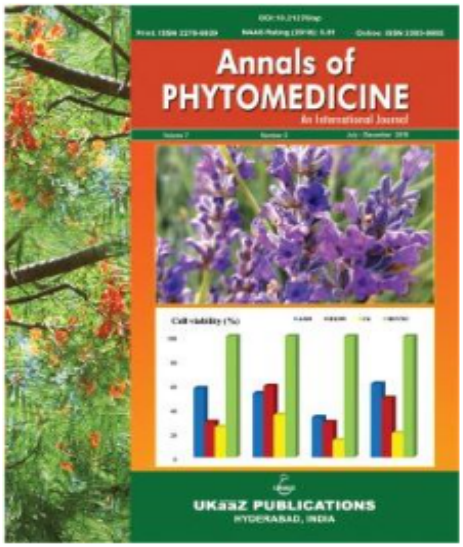

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